COMMUNICATION ORALE

Thème 1

Patrimoine oléicole méditerranéen :

Biodiversité, potentialités et perspectives de préservation et d'amélioration

S4-C1

Origines et processus de domestication chez l'olivier, *Olea europaea* L., dans le sud ouest de la méditerranée : relations génétiques entre l'olivier au Maroc et dans la méditerranée

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Résumé

Deux hypothèses sont formulées pour expliquer la diversification variétale de l'olivier au Maroc : i) une prédominance des variétés de l'est de la Méditerranée, ii) une sélection à partir des formes de l'est mais introgressées par le pool génétique local. Les relations génétiques entre 75 génotypes du Maroc, 343 accessions méditerranéennes et 43 variétés libanaises, en utilisant 12 marqueurs microsatellites et le polymorphisme de l'ADN chloroplastique, ont été étudiées. Les analyses bayesiannes à l'aide du programme « Structure » montrent que les variétés marocaines sont essentiellement classées dans deux groupes génétiques parmi les 6 identifiés au sein de l'olivier méditerranéen. Le premier groupe comprend essentiellement les variétés du sud et du centre du Maroc, alors que le second groupe correspond à des variétés du Maroc avec la «Picholine marocaine » ayant la lignée maternelle de l'est de la Méditerranée, mais également de l'Espagne. Ces résultats soutiennent l'hypothèse selon laquelle l'olivier cultivé au Maroc résulte à la fois d'une domestication primaire à partir de populations locales et d'une diversification secondaire à partir de formes de l'est de la Méditerranée introgressées par le pool génétique local.

Mots clés: *Olea europea* L., structure génétique, SSRs, polymorphisme ADN chloroplastique. Méthodes d'analyse bayésiannes.

Origins and domestication process of olive, *Olea europaea* L., in south west Mediterranean areas: A study of genetic relationships between Moroccan and Mediterranean olive

Abstract

Two hypotheses are proposed to explain olive diversification processes in Morocco: i) the most introduced varieties from the East Mediterranean, ii) selection from the introduced Eastern varieties introgressed by a local gene pool. Genetic relationships between 75 genotypes from Morocco and 343 accessions from the Mediterranean olive germplasm and 43 varieties from Lebanon were studied using 12 microsatellite loci and chloroplast DNA markers. Based on the bayesian methodology using the Structure software, varieties from Morocco are classified in two clusters among the six identified in Mediterranean olive. The first one is mainly composed by varieties from the South and the centre of Morocco. The second includes varieties from Morocco with Eastern maternal lineage and from Spain. These results support the hypothesis that cultivated olive in Morocco results from local domestication and secondary diversification from introduced Eastern varieties introgressed by a local gene pool.

Keywords: *Olea europaea* L., domestication, genetic structure, SSRs, chloroplast DNA polymorphism, bayesian model clustering analysis.

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1. Introduction

Les oliviers cultivés et leurs parents sauvages "l'oléastre" représentent deux variétés botaniques d'*Olea europaea subsp. Europaea,* var. *europaea* et var. *sylvestris,* respectivement. Les études génétiques et archéobotaniques antérieures ont montré l'existence de populations d'oléastres dans l'Est et l'Ouest méditerranéen avant le néolithique (Lumaret et Ouazzani, 2001 ; Besnard *et al.* 2002; Terral *et al.* 2004; Khadari 2005; Breton *et al.* 2006). La domestication de l'olivier aurait eu lieu dans plusieurs foyers indépendants dans le bassin méditerranéen. La lignée maternelle du *pool* génétique de l'Est est majoritaire dans les variétés méditerranéennes, résultat de migrations humaines essentiellement d'Est en Ouest (Besnard *et al.* 2002; Khadari, 2005; Breton et al., 2006).

En dépit de ces travaux, les origines et les processus de diversification à l'échelle locale demeurent méconnus. Au Maroc, Khadari et al. (2008) ont montré une diversité génétique importante des oliviers cultivés malgré la dominance d'une seule variété, « Picholine marocaine ». Sur la base du polymorphisme de l'ADN nucléaire et chloroplastique, les populations d'oléastres marocaines sont génétiquement distinctes des formes cultivées suggérant la coexistence entre des formes locales et introduites mais probablement introgressées par le pool génétique local (Khadari, 2005). Cependant, l'importance de cette introgression reste méconnue, ce qui nous amène à formuler ces deux hypothèses: i) les variétés du Maroc appartiennent aux différents groupes génétiques de l'olivier méditerranéen, ce qui traduirait des introductions du matériel allochtone sans échanges génétiques notables avec les populations locales; ii) les variétés du Maroc sont distinctes génétiquement des variétés méditerranéennes, résultat de la sélection locale des formes introduites de l'est méditerranéen introgressées par le pool génétique local.

Dans cette étude, nous présentons les résultats d'analyses génétiques de l'olivier cultivé au Maroc et des variétés méditerranéennes à l'aide des marqueurs microsatellites nucléaires et chloroplastiques selon une approche bayésienne. Nous discutons nos résultats en faveur de l'hypothèse d'une sélection locale et nous proposons un scénario sur les origines de l'olivier au Maroc.

2. Matériel et Méthodes

2.1. Matériel végétal

Les variétés méditerranéennes analysées correspondent à 343 accessions de la collection internationale implantée au domaine de Tassaout (INRA Marrakech) et à 43 variétés libanaises. Le matériel végétal marocain étudié comprend 60 génotypes précédemment identifiés par Khadari *et al.* (2008) et 31 oliviers échantillonnés dans la région d'Ouazzane et Chefchaoun (nord du Maroc). Pour ces derniers, la stratégie d'échantillonnage était ciblée sur la collecte d'oliviers distincts de la variété dominante « Picholine marocaine » en se basant sur des observations phénotypiques et des informations données par les agriculteurs locaux et les techniciens agricoles.

2.2. Analyses moléculaires

Douze locus microsatellites nucléaires ont été sélectionnés parmi les 18 proposés par Elioth-Smith (2007) et 3 marqueurs chloroplastiques de type insertion-délétion ont été utilisés (Besnard *et al.* 2005). La réaction PCR et le génotypage à l'aide d'un séquenceur semi-automatique ABI Prism 3130 XL sont décrits par Khadari *et al.* (2008). La lecture et le codage des données moléculaires ont été réalisés à l'aide du logiciel Genemapper v.3.7 (Applied Biosystems).

2.3. Analyses des données

L'analyse factorielle de correspondance a été réalisée à l'aide du logiciel Genetix 4.0 (Belkhir, 1999). Dans le but de définir des groupes génétiques sans apriori sur les origines géographiques des variétés étudiées, nous avons adopté un modèle de groupement, selon une approche bayésienne, implémenté dans le programme Structure v. 2.2 (Prichard et al. 2000). La validation du nombre de groupes K est réalisée à l'aide de la statistique d'Evanos et al. (2005).

3. Résultats et discussion

3.1. Polymorphisme nucléaire et chloroplastique

L'analyse de 477 oliviers méditerranéens et marocains à l'aide de 12 locus microsatellites a permis d'identifier 437 génotypes correspondant à un total de 176 allèles et une moyenne de 14.6 allèles par locus (Tableau 1). Le nombre total d'allèles obtenus est similaire à celui observé chez les oléastres méditerranéens (Breton *et al.,* 2006), ce qui indique que la diversité génétique de l'olivier cultivé n'est pas réduite par rapport à celle de l'oléastre.

La majorité des variétés méditerranéennes (94%) et des formes cultivés au Maroc (74%) sont caractérisées par la lignée maternelle de l'Est de la méditerranée, ce qui confirme les résultats des travaux antérieurs (Besnard *et al.,* 2002; Khadari, 2005; Breton *et al.,* 2006).

3.2. Structure génétique des variétés méditerranéennes et marocaines

L'analyse multivariable montre une structure génétique est-ouest de l'olivier méditerranéen (Figure 1). L'olivier cultivé au Maroc est relativement distinct du reste. Les analyses à l'aide du programme Structure ont été réalisées selon le modèle « No admixture » en considérant l'absence de corrélation entre les fréquences allèliques au sein des populations. La statistique d'Evanos *et al.* (2005) montrent que la structure en 6 groupes est la plus vraisemblable (Haouane et al, résultats non publiés). Sur la base d'une probabilité d'assignation à 80 %, 371 parmi les 437 génotypes définis, ont été assignés aux 6 groupes (Tableau 2).

Tableau 1. Nombre d'allèles (Nb), taille attendue et hétérozygotie pour chacun des 12 loci SSR (Hobs = hétérozygotie observée ; He = hétérozygotie attendue non biaisée)

Locus	Allèles				Fis (Wc)
	Nb	T.attendue	Hobs	He	
DCA4	29	124-193	0.6538	0.8752	0.253
DCA9	22	162-211	0.9076	0.8772	-0.035
DCA15	09	231-266	0.6748	0.6393	-0.056
DCA5	12	192-214	0.4942	0.4819	-0.025
DCA3	12	222-260	0.8951	0.8430	-0.062
UDO36	14	134-168	0.7890	0.7326	-0.077
GAP59	12	207-241	0.6683	0.6390	-0.046
DCA18	18	155-207	0.8383	0.8398	0.002
DCA8	20	125-166	0.9289	0.8239	-0.128
GAP71	13	117-166	0.9108	0.8447	-0.078
DCA11	23	126-183	0.7535	0.8201	0.081
EMO90	10	178-208	0.7586	0.6491	-0.169

Cette structure génétique montre une spécificité génétique d'une part à l'est de la méditerranée par le groupe des variétés du Liban et de la Turquie, et d'autre part dans le sud ouest. En effet, l'olivier cultivé au Maroc est classé en deux groupes : i) le premier comprend essentiellement les formes cultivées dans le sud et le centre du Maroc dont la moitié est caractérisée par les 2 lignées maternelles caractéristiques de l'ouest de la méditerranée ; ii) le deuxième groupe comprend en plus de la Picholine marocaine, des oliviers cultivés dans le nord du Maroc et des variétés espagnoles. La composition de ce deuxième groupe s'explique par les échanges du matériel entre le nord du Maroc et la péninsule ibérique depuis au moins la présence romaine au Maroc. En revanche, le premier groupe est spécifique de l'olivier marocain même si la moitié des génotypes ont une lignée maternelle de l'est.

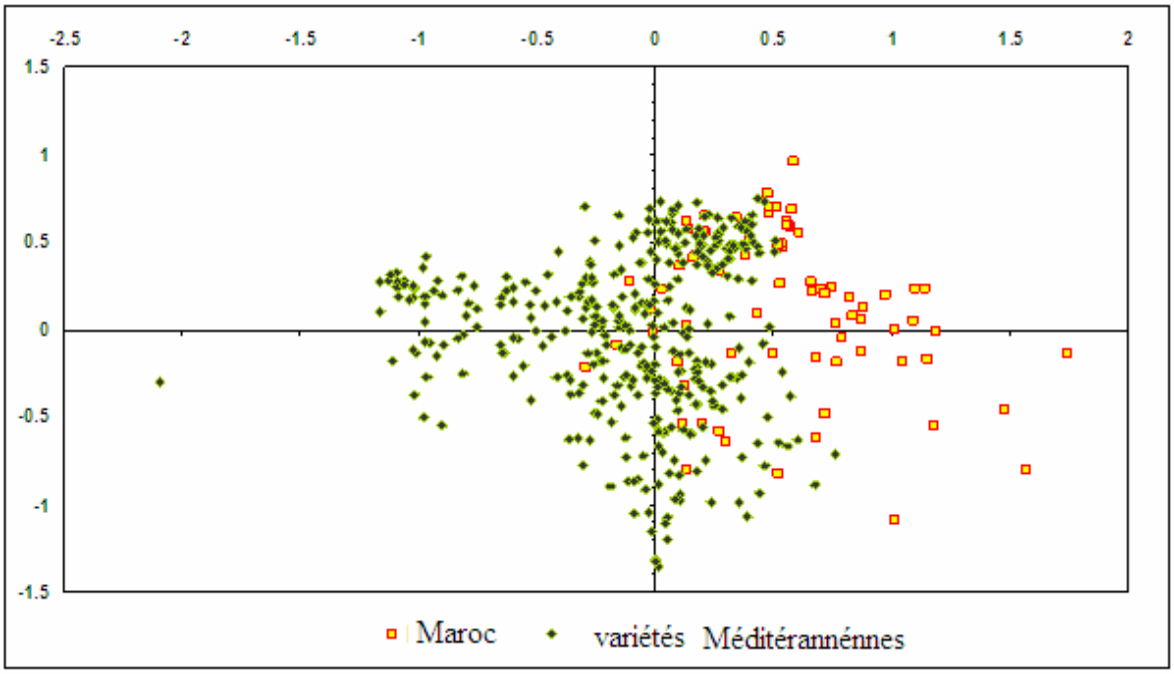

Figure 1: Analyse multivariable AFC (analyse factorielle de correspondance) basée sur les données SSR nucléaires. Comparaison des variétés marocaines et méditerranéennes.

Ce constat est probablement le résultat d'une sélection locale à partir de formes allochtones mais fortement introgressées par le pool génétique local.

Tableau 2 : Nombre de génotypes et proportion d'assignation des variétés par pays dans chacun des 6 groupes. Nombre de génotypes ; Na : nombre de génotypes assignés ; Ch-Tu : Turquie-Chypre.

Pays	N.	Na.	Groupes de cultivars					
			1	2	3	4	5	6
Maroc	69	68	40	21	2	5	2	
Italie	160	139	15	6	38	41	2	
Espagne	91	86	6	64	9	5		
Grèce	14	6	1	1	4		1	
Portugal	13	12	1	10			2	
France	13	12			2	8		
Tunisie	6	4		1	2	1	1	
Liban	23	21		1	10	2	2	7
Ch-Tu	25	23			3	2		16

4. Conclusion

Nos résultats suggèrent que l'olivier cultivé au Maroc est le résultat d'une domestication locale mais également d'une diversification secondaire. La validation de cette hypothèse nécessite d'analyser les relations génétiques entre l'olivier cultivé et l'oléastre et conduit à nous interroger sur le rapport entre la reproduction végétative et sexuée dans les processus de diversification locale de l'olivier.

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S4-C2

Olive cultivar comparisons from around the world

Paul Vossen

Abstract

Choosing the correct cultivar can make a huge difference in the profitability of an orchard. This review article shows that there are significant differences in cultivar performance based on: tree vigor, productivity, precocity, alternate bearing, oil content, fruit size, pit to pulp ratio, fatty acid composition, polyphenol content, sensory characteristics, cold hardiness, flowering and maturity dates, and disease susceptibilities. It shows how important it is to evaluate olive varieties from all over the world and to realize that there may be several cultivar choices with a potential to improve profits or create a different styled product. A review of the scientific literature from research in Spain, Italy, France, Morocco, Australia, and the USA shows that there are strong similarities in how most cultivars perform no matter where they are grown. This paper references work done in very diverse areas with a similar Mediterranean climate. The data indicates that some cultivars are consistently better than others and that many resident cultivars growing in specific geographical locations may be there only because other cultivars have not been adequately evaluated and subsequently adopted over time.

Key Words: olive oil, varieties, vigor, precocity, yield, flavor, hardiness, pollination, alternate bearing

Résumé

Choisir le bon cultivar peut faire une énorme différence dans la rentabilité d'un verger. Ce compte rendu montre qu'il existe d'importantes différences dans la performance des cultivars basée sur: la vigueur des arbres, la productivité, la précocité, l'alternance de la production, la teneur en huile, la taille des fruits, la proportion de la pulpe au noyau, la composition en acides gras, la teneur en polyphénols, les caractéristiques sensorielles, la résistance au froid, les dates de la floraison et les dates de maturité, les sensibilités aux maladies. Cela montre qu'il est important d'évaluer les variétés mondiales d'olives et de réaliser qu'il existe plusieurs cultivars avec un potentiel d'améliorer les profits ou de créer un produit de style différent. L'étude de la littérature scientifique sur les recherches en Espagne, Italie, France, Maroc, Australie et Etats-Unis montre qu'il existe de solides similitudes dans la façon dont la plupart des cultivars se comporte, peu importe où ils sont cultivés. Ce document fait référence aux travaux effectués dans plusieurs endroits différents mais avec un climat méditerranéen similaire. Les statistiques indiquent que certains cultivars performent toujours mieux que d'autres et que les cultivars autochtones qui existent dans des emplacements géographiques particuliers ne sont là seulement parce que d'autres cultivars n'ont pas été suffisamment évalués, et en conséquense n'ont pas été adoptés.

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1. Introduction

Fig. A - Ten different olive cultivars

The choice of which variety/cultivar to grow is one of the most important decisions in growing any horticultural crop, but it is especially important in tree crops that live a long time and take several years to come into full bearing. That decision could haunt the farmer for many years and a great deal of time and energy could have been lost, if it was the wrong decision. Making the right choice, however, could mean years of profitable production from quality fruit. It is particularly difficult with olives since we know that the trees can live for a thousand years. Historically, those long lived trees gave us many traditions and a passion for a certain style of oil or table fruit that was produced by our ancestors. The choice of what cultivar to plant, however, must be rational and take into consideration new information that has been developed over the last 25 years. It would be foolish to be content with what we had always done if there are better options. Previously, olive growers who were interested in planting a new orchard relied on a neighbor's advice or purchased plant material from a nursery, usually whatever the nursery had for sale. Over hundreds of years there was some selection that took place that weeded out the poor cultivars that did not have as many favorable characteristics. Much of that selection, however, was very local, without the benefit of having included other top selections from other regions or countries. Consequently, their real choices of cultivars were quite limited; see fig. A – the different look of ten cultivars from the germplasm repository in Córdoba, Spain.

Now we have many more choices due to greatly expanded worldwide information sharing and the diligent work by many researchers who have brought in cultivars from other areas and compared them side by side with their own traditional cultivars. This is a new phenomenon. Thirty years ago there were no Spanish cultivars in Italy or Greece or anywhere else outside Spain that were being compared to the regional cultivars and vice versa for Italian or Greek cultivars outside Italy or Greece. The Spanish had no idea how the Italian cultivars actually compared to their own and the Italians had never tried any Spanish cultivars. Everyone thought their own cadre of cultivars was somehow sacred. When cultivar trials were finally planted, it took a long time (20+ years) to really see how the trees performed over the long term. We are still learning from those cultivar trial plantings and many new ones have been established in the new world (California, Australia, Chile, Argentina, and South Africa). These non-traditional olive growing areas have no preconceived ideas as to which cultivars are better. Growers are simply choosing the cultivars that are the most productive, most cost efficient, and most desirable in the market.

The choice of cultivar often comes down to the bottom line, the economics of how much more profitable one might be than the other. Profitability can be influenced by how the cultivar yields and if it comes into bearing early or takes several more years while the farmer has no income. Yield is based on the raw tonnage of fresh fruit produced, but also the oil content of the cultivar and how much oil is easily extractible with the milling equipment that will be used. Oil sensory quality and keeping quality is also important. The market demand for specific oil styles and cultivars with certain characteristics can influence the choice of which cultivar to plant and grow. For table olives, producers need to be aware of fruit size, ease of hand harvest, pulp to pit ratio, ease of pit removal, appearance, and texture of the final processed product. Finally, the differences in the production costs from one cultivar to another can be extremely important since olives are a low margin crop and money wasted on unnecessary cultural costs is difficult to recover.

- Vigor
- Precocity
- Fruit Yield
- Fruit Size (table)
- Pulp to Pit Ratio (table)
- Oil Content Disease Resistance
- Salt Tolerance
- Oil Extractability
- Oil Yield
- Oil Flavor - style
- Oil Stability
- Market Demand
- Pollination Needs
- Maturity Date
- Hand Harvestibility (table)
- Machine Harvestibility
- Drought Tolerance
- Cold Hardiness
- Alternant Bearing
- Appearance table

2. Factors affecting olive yield and oil yield

The amount of oil a producer gets per hectare depends primarily on the tonnage yield of fruit per unit area, which varies by cultivar, year, fruit set, irrigation, pruning, age of trees, etc. This seems straightforward, but it must be noted that oil yield and fruit yield do not necessarily increase at the same rate. This is because olive trees have an ability to produce more oil with an increased leaf-to-fruit ratio in years with a light crop. When there is less fruit on the tree, the greater numbers of leaves per fruit boosts the fruit's oil content, which partially makes up for lower fruit tonnage. In other words, there is not a perfect correlation between fruit yield and oil yield. The amount of oil that a producer gets from a given amount of fruit depends on many factors:

- **Oil content of the fruit** – varies by moisture content, year, amount of fruit on the tree, and cultivar
- **Extractability of the oil from the fruit –** varies by year, water content, fruit maturity, and cultivar
- **Extraction process** – varies by paste fineness; use of adjuvants, malaxation time and temperature; extraction equipment type, and efficiency - speed of the machinery.

3. Yield of olive

Yields per hectare can range from less than 1 to as high as 20 tons per hectare; a good consistent yield from year to year would be about 9 metric tons per hectare. Low yields usually can be related to a lack of shoot growth the previous year from poor tree vigor. This can be caused by inadequate irrigation or inadequate rainfall for dry farmed orchards, poor weed control, disease, very low fertility, inappropriate pruning, or a very high crop load the previous year. Low yields can also be caused by poor weather conditions during bloom, lack of chilling, frost damage, or inadequate flower pollination. Olives are strongly alternate bearing, so a low crop yield one year will likely promote more shoot growth, resulting in more flowers and higher yields the following year. High yields are produced consistently only from orchards that are very well managed. These yields all depend on the factors listed in table 1.

Table 1: Orchard yield projection scenarios for oil olives

Fruit Yield	Factors Affecting Yield
2 TONS/ HA	• Medium-density orchard in the 5th – 6th year or older orchard with close spacing with shading problems. • Poor irrigation, weed control, pruning and nutrient management. • Excessively vigorous or weak growing conditions. • Poor pollination conditions from adverse weather during bloom, or inadequate pollinizer trees. • Alternate "off" year of production. • Super-high-density orchard in the 2nd year.
4 TONS/ HA	• Medium-density orchard in the 6th – 8th year or poor irrigation, weed control, pruning and nutrition. • Excessive shading. Alternate "off" year of production from very heavy production last year. • Excessively vigorous or weak growing conditions. • Poor pollination conditions from adverse weather during bloom, or inadequate pollinizer trees. • Super-high-density orchard in the 3rd year.
6 TONS/ HA	• Medium-density orchard in the 9th – 10th year without proper management. • Probable maximum yield from a coastal hillside orchard. • Some shading problems. Some poor weather during bloom or a lack of pollinizer trees. • Super-high-density orchard in the 3rd year.
9 TONS/ HA	• Medium-density orchard in the 10th year + with good irrigation, weed control, pruning and nutrition. • A great sustainable yield if everything is done right and nature cooperates. • Trees have the correct vigor and growing conditions. • Well managed super-high-density orchard in the 4th + years.
11 TONS/ HA	• Medium-density orchard in the 10th year + with excellent irrigation, weed control, pruning and nutrition. • An excellent yield especially if it can be sustained each year. • Alternate "on" year of production from a low yield last year. • Perfect growing conditions and doing everything right. • Very well managed super-high-density orchard in the 4th + years.
> 14 TONS/ HA	• Yield that cannot be sustained each year. • Medium-density orchard in the 10th year + with excellent irrigation, weed control, pruning and nutrition. • Alternate "on" year of production from a very low yield last year. • Perfect growing conditions and doing everything right. • Excellent management in a super-high-density orchard in the 5th + years.
Vossen, unpublished data.	

4. Vigor – Precocity – Productivity – Fruit Weight – Oil content – Flesh to Pit Ratio

Vigor is the term used to define the amount or relative strength of the vegetative growth of the plant. It is measured in shoot growth, trunk diameter, tree volume, and sometimes in tree form. Precocity is the term used to define the length of time until the cultivar's first significant flowering and fruit set. It is measured in number of years from planting to harvest and the quantity of fruit from the first three harvests. Productivity is the amount of fruit or oil yield produced from a particular cultivar. It is measured in the average or maximum kilos per tree, tons of fruit per hectare, kilos of oil per hectare produced per year, average per year, or cumulative for a certain number of years. Sometimes the efficiency of production is measured based on kilos of fruit per cubic meter of tree volume, number of fruits per branch, or dry weight of fruit per branch. The measure of a cultivar's tendency to bear in alternate years is called the Alternance Index. It is a numerical value indicating the amount of increased or decreased crop yield to be expected from one year to the next; the larger the number, the more alternating the cultivar. Fruit weight is a measure of fruit size given in grams per fruit. Oil content is measured as a percentage on a dry or fresh (wet) weight basis. Flesh to pit ratio is a measurement of the pit size in relation to the amount of fruit flesh or pulp.

Table 2: Yield and alternance index of the picholine marocaine olive tree population and selected clones in marrakech, morocco (11 years - large 100+ year old trees)

Cultivar	Ave. Yield (kg/tree)	Alternance Index
Haouzia	60	0.67
Menara	64	0.60
M26	88	0.50
K26	73	0.81
S19	62	0.70
P. Marocaine	20	0.90
Belkassem Boulouha. 2006. National Agriculture Research Center, Marrakech, Morocco.		

Table 3: Evaluation of newly planted cultivar selections grown in marrakech,(1987-1997)

Cultivar	Ave. Yield (kg/tree)	Max. Yield	Oil Content (% dry basis)
Menara	31	66	56
Haouzia	31	60	48
Picholine	33	70	47
Manzanillo	28	80	36
Belkassem Boulouha. 2006. National Agriculture Research Center, Marrakech, Morocco.			

Table 4: Evaluation of several newly planted mediterranean olive cultivars grown in Marrakech Morocco (1988-1996)

Cultivar	Ave. Yield (kg/tree)	Max. Yield (kg/tree)	Foliar Disease (%)	Oil content (% dry basis)
Arbequina	30	54	10	37
Manzanillo	24	56	40	42
Leccino	40	83	0	36
Carolea	33	55	80	37
P. Marocaine	25	66	70	30
Picholine	30	50	0	38
Blanqueta	30	86	20	40
Sourani	05	43	0	42
Ayvalik	04	16	20	40
Belkassem Boulouha. 2006. National Agriculture Research Center, Marrakech, Morocco.				

Field observation and research in Morocco shows that the general population of 'Picholine Marocaine' cultivars could be greatly improved through clonal selection ('Haouzia' and 'Menara') giving greater productivity and less alternate bearing. These clonal selections also have a good content of oil ranging from 48-56% on a dry weight basis to about 23% on a wet weight basis. Several other introduced selections from Spain, Italy, Greece, and France showed superior overall productivity. New cultivars that have been recommended for Morocco, based primarily on productivity are 'Leccino', 'Picholine Languedoc', 'Blanqueta', and 'Arbequina' (Tables 2-7) (Boulouha, 1995, 2006; El Antari et al., 2003; Idrissi and Ouazzani, 2003).

Table 5: Various comparisons of mediterranean cultivars grown in Marrakech, Morocco

Cultivar	Olive Fly Damage	Fruit Wt. (g)	Free Acidity (%)	Total (ppm) Polyphenols	Oleic Acid (%)
P. Marocaine	4-9%	3.78	0.37	300.2	70-73%
P. Languedoc	0	5.12	0.36	234.8	62-64%
Manzanillo	4%	6.19	0.75	254.0	64-68%
Arbequina	0	1.47	0.23	156.7	62-64%
Leccino	1-23%	2.57	0.34	131.1	70-73%
Blanquita dE	0-49%	3.45	0.36	178.8	61%
El Antari, El Moudini, and Ajana, 2003. Laboratorie de Technologie del Huiles Programme Olivier. 2003. Inst. Nat. de la Recherche Agronomique BP 533, Marrakech, Morocco					

Table 6: Recommanded new cultivar for morocco (Marrakech 2006)

Cultivar	Ave. Yield (kg/tree)	Oil content (% dry basis)	Disease Tolerance	Pollination Requirement
Leccino	40	36	T	Auto-inc.
Picholine	30	38	T	Partial
Blanqueta	30	40	T	Partial
Arbequina	29	37	T	Auto
Belkassem Boulouha. 2006. National Agriculture Research Center, Marrakech, Morocco.				

Researchers in Spain have been comparing their own local olive cultivars with introduced cultivars from Italy, Greece, France, Tunisia, Turkey, and Morocco. When comparing some Italian and Spanish cultivars grown at 204-286 trees/ha, they found differences in tree volume (vigor) with some trees being two to three times larger than others over a 9-10 year period (Tables 8-9). In Italy a comparison between several local cultivars found similar differences in overall growth and number of branches, but not in tree height or trunk diameter, which only varied by about 25-30% (Table 10) (Cimato, 2001; Del Rio et al., 2005; Tous, Romero, Plana, 2005 a).

Table 7: Olive cultivar precosity, productivity, alternance index and content in Meknes, Morocco grown at density of 178 trees/ha

Researchers in Spain have been comparing their own local olive cultivars with introduced cultivars

Cultivar	Ave. Yield* First 3 yrs. (kg/tree)	Ave. Yield Last 3 yrs. (kg/tree)	Cumulative Yield	Alternance Index	Oil content % wet wt. basis	Oil Yield Estimate kg/ha
Arbequina	6.94	29.17	122.46	0.20	23	1,040
Carolea	2.12	19.53	74.45	0.45	26	903
Frantoio	2.28	18.80	70.85	0.33	27	904
Haouzia	3.86	17.17	69.07	0.37	23	703
Koroneiki	18.14	25.19	143.60	0.39	25	1,150
Hojiblanca	5.93	33.67	131.49	0.29	20	1,080
Leccino	1.16	24.02	87.05	0.63	-	-
Manzanillo	2.17	20.22	77.99	0.52	-	-
Mastoides	11.66	16.95	97.50	0.57	30	905
Menara	5.46	18.13	75.75	0.41	23	742
P. Languedoc	5.62	26.67	103.79	0.32	22	1,000
Picual	10.23	38.60	169.61	0.21	24	1,580
* Starting three years after planting. A. Idrissi and N. Ouazzani. 2003. Département d'Arboriculture, Unité de Génétique Ecole Nationale d'Agriculture, Meknès, Maroc						

from Italy, Greece, France, Tunisia, Turkey, and Morocco. When comparing some Italian and Spanish cultivars grown at 204-286 trees/ha, they found differences in tree volume (vigor) with some trees being two to three times larger than others over a 9-10 year period (Tables 8-9). In Italy a comparison between several local cultivars found similar differences in overall growth and number of branches, but not in tree height or trunk diameter, which only varied by about 25-30% (Table 10) (Cimato, 2001; Del Rio et al., 2005; Tous, Romero, Plana, 2005 a).

Some of the same researchers measured the number of years it took for various cultivars to come into bearing, the yields from the first few years, yields once the trees were mature, and yield efficiency (kg fruit/m^3 of tree volume). In Cataluña, the cultivars 'Arbequina', 'Arbosana', 'Blanquetta', and 'Palomar' started bearing fruit in the second year, while all the other cultivars took at least a year or two longer, and some did not start fruiting until 5-6 years old. The yield efficiency for 'Arbequina', 'Arbosana', and 'Joanenca' were extremely high (4.0 to 4.8 kg fruit/m^3 of tree volume) compared to most other cultivars that were much lower (0.4 to 3.2 kg fruit/m^3). Those same cultivars also had high yields the first three years of production and maintained those higher yields into the 7-10^{th} years (Table 11). In Andalucia, the yield efficiencies ranged from 0.84 for 'Gordal' to 5.36 kg fruit/m^3 of tree volume for 'Manzanillo'. Some of the Italian cultivars had very good yields their first three years of production and into the 7-10^{th} years; especially notable was 'Leccino' though it took an average of 4.5 years to come into production (Table 12). In Australia, researchers also found 'Arbequina' and 'Leccino' to come into bearing early along with 'Picual', 'Barnea', 'Hojiblanca', 'Koroneiki', and 'Manzanillo' (Table 13) (Del Rio et al., 2005; Sweeney, 2005; Tous, Romero, Plana, 2005 a).

Table 8: Olive cultivar vigor (tree volume) in the 9^{TH} year at a density of 286 trees/ha in cataluna spain

Cultivar	Volume m^3/tree
Morrut	48.0
Changlot Real	47.0
Empeltre	42.0
Manzanilla	41.0
Blanqueta	39.0
Picual	34.0
Arbequina	34.0
Palomar	32.0
Joanenca	29.0
Santa Caterina	25.0
Arbosana	24.0
i-50	23.0
Joan Tous, Agustí Romero, and Joan Plana. Dept. d' Arboricultura Mediterránia, Centre "Mas Bové", IRTA, Reus-Constantí (Tarragona), Spain	

Table 9: Olive cultivar vigor (tree volume) in the 10^{TH} year at a density of 204 trees/ha in Andalucia Spain

Cultivar	Volume m^3/tree
Moraiolo	66.7
Maurino	43.0
Blanqueta	42.2
Leccino	40.5
Frantoio	40.3
Gordal (Sevillano)	31.0
Picudo	30.6
Empeltre	27.1
Manzanilla	24.2
Hojiblanca	22.5
Cornicabra	20.2
Coratina	16.8
Carmen del Río, Juan M. Caballero and M^a Dolores García-Fernández. CIFA "Alameda del Obispo", IFAPA, Junta de Andalucía, Córdoba, Spain	

Table 10: Olive cultivar vigor in Firenze, Italy

Cultivar	Tree height (cm)	Total vegetation (cm)	Number of branches per tree	Trunk diameter (cm)
Correggiolo	159.83	442.91	19.3	7.6
Frantoio	142.08	304.58	15.6	7.4
Grappolo	119.58	172.16	12.1	7.2
Leccino	152.30	404.00	19.0	6.5
Leccio del Corno	112.00	158.5	10.2	6.0
Maurino	130.50	299.75	22.8	7.1
Moraiolo	119.08	122,75	8.1	5.6
Pendolino	134.16	315.91	20.5	6.8
S. Caterina	121.66	199.66	9.9	6.5
San Francesco	167.41	414.41	20.1	9.4
Antonio Cimato, Istituto sulla Propagazione delle Specie Legnose Consiglio Nazionale delle Ricerche Scanticci – Firenze, Italia				

Table 11: Olive cultivar precocity and production 1991-2001 in cataluna spain

Cultivar	Years to produce	Yield first 3 yrs. kg/tree	Ave. yield 7^{th}-10^{th} yrs. kg/tree	Efficiency kg fruit/m^3 tree volume
Blanqueta	2	27.6	25.2	3.2
Arbequina	2	22.7	27.1	4.1
Arbosana	2	18.0	18.0	4.8
Palomar	2	9.0	17.2	2.6
Picual	3	24.3	19.9	2.7
Joanenca	3	23.4	20.2	4.0
Manzanilla	3	10.7	21.1	2.0
Empeltre	4	15.0	14.0	1.5
Santa Caterina	6	8.8	3.6	0.4
Joan Tous, Agustí Romero, and Joan Plana. Dept. d' Arboricultura Mediterránia, Centre "Mas Bové", IRTA, Reus-Constantí (Tarragona), Spain				

Table 12: Olive cultivar precocity, and production 1987- 1997 in Andalucia spain

Cultivar	Years to produce	Yield first 3 yrs. kg/tree	Ave. yield 7^{th}-10^{th} yrs kg/tree	Efficiency kg fruit/m^3 tree volume
Blanqueta	3	7.7	38.1	4.06
Maurino	3.5	31.0	21.5	2.88
Picudo	3.5	14.9	23.4	3.83
Manzanilla	4	21.8	26.1	5.36
Hojiblanca	4	21.5	22.2	4.71
Cornicabra	4	14.2	19.7	4.60
Coratina	4	8.1	13.1	3.74
Leccino	4.5	27.4	34.2	3.65
Frantoio	5	41.2	25.3	2.87
Empeltre	5.5	32.8	10.9	2.49
Moraiolo	5.5	30.7	15.0	0.86
Gordal (Sevillano)	6	18.6	6.4	0.84
Carmen del Río, Juan M. Caballero and M[a] Dolores García-Fernández. CIFA "Alameda del Obispo", IFAPA, Junta de Andalucía, Córdoba, Spain.				

Table 13: Cultivar comparisons for precocity in Australia

Early bearing	Picual, Barnea, Hojiblanca, Arbequina, Koroneiki, Leccino, and Manzanillo
Mid bearing	Mission, Pendolino, Columella, FS-17, and Coratina
Late bearing	Ascolano, Frantoio, Gordal, Kalamon, I-77, and Souri
Susan Sweeney, Plant Research Center, Waite Research Precinct; Rural Industries Research and Development Corporation; Hartley Grove, Urrbrae, 5064, Australia	

Additional research in Spain at the Córdoba Germplasm Repository provided more comparisons between cultivars from all over the world. 'Arbequina' and 'Arbosana' were much higher in number of fruits per branch, but not as high as 'Manzanillo' or 'Picual' in fruit dry weight per branch. Arbequina and Leccino stood out as being the most productive during years 6-9. (Tables 14-15) (Caballero et al., 2005; Ramirez and Rallo, 2005).

Table 14: Olive cultivar productivity, fruit dry weight, and weight fruit load in andalucia Spain

Cultivar	Productivity - fruit dry wt./branch	Fruit dry wt.	Fruit load fruits/branch
Manzanilla	0.53 g	1.52 g	0.35
Picual	0.51 g	1.32 g	0.41
Arbosana	0.39 g	0.61 g	0.64
Frantoio	0.39 g	1.04 g	0.38
Arbequina	0.38 g	0.53 g	0.71
Picholine	0.35 g	1.16 g	0.30
Cornicabra	0.32 g	1.02 g	0.31
Hojiblanca	0.32 g	1.28 g	0.25
Gordal (Sevillano)	0.30 g	2.93 g	0.11
P. Marocaine	0.30 g	1.13 g	0.27
Leccino	0.26 g	1.17 g	0.23
Blanqueta	0.20 g	0.57 g	0.37
Moraiolo	0.20 g	0.65 g	0.31
Koroneiki	0.19 g	0.25 g	0.75
Magdalena Ramírez de Santa Pau and Luis Rallo. 2005. Dept. de Hort., Serv. de Inv. y Desarollo Tec., J. de Extremadura, Badajoz. Dept. de Agri., U de Córdoba, Spain			

Table 15: Olive cultivar precocity, productivity, fruit weight, and oil content in cordoba, spain 1988-98

Cultivar	Yield* first 3 yrs. kg/tree	Productivity Ave. 6^{th}-9^{th} yrs. (kg/tree)	Fruit Weight g/fruit	Oil content % dry wt. basis
Arbequina	23.0	24.5	1.7	55.1
Manzanilla	7.4	10.8	3.9	51.9
Villalonga	24.9	12.8	4.6	58.4
Carolea	16.3	14.0	4.6	58.4
Koroneiki	20.5	13.3	1.1	55.7
Leccino	28.7	23.6	3.0	49.0
Picholine	21.6	10.2	3.8	56.1
P. Marocaine	16.4	15.8	3.9	53.1
Juan M. Caballero, Carmen del Río, Carlos Navarro, M[a] Dolores García-Fernández, Juan Morales, Manuel Hermoso, Luis Á del Olmo, Francisco López, Fernando Cera, and Gustavo Ruiz. CIFA "Alameda del Obispo", IFAPA, Junta de Andalucía, Córdoba; CIFA "Estación de Olivicultura y Elaiotecnia", IFAPA, Junta de Andalucía, Mengíbar (Jaén); CIFA de Cabra, IFAPA, Junta de Andalucía, Cabra (Córdoba); Delegación de Agricultura y Pesca, Junta de Andalucía, Huelva.				

Table 16: Olive cultivar precocity, productivity, fruit weight, and oil content in Cataluna, Spain 1988-01

Cultivar	Yield* first 2 yrs. kg/tree	Average Productivity 8th-11th yrs. kg/tree	Fruit Weight g/fruit	Oil content % dry wt. basis
Arbequina	23.4	29.8	1.2	50.5
Manzanilla	10.1	19.3	3.7	50.4
Villalonga	18.5	25.7	3.5	48.3
Ayvalik	1.2	5.6	3.4	52.4
Carolea	5.8	23.2	4.4	53.3
Koroneiki	4.1	8.5	0.8	49.3
Leccino	13.5	31.8	2.5	47.1
Picholine	10.2	31.5	3.8	50.7
P. Marocaine	4.9	13.5	3.6	46.9

Joan Tous, Juan F. Hermoso, Joan Plana, and Agustí Romero. dept. d'arboricultura mediterránia, Centre "Mas Bové", IRTA, Reus-Constantí (Tarragona); Estación Experimental del Ebro, IRTA, Amposta (Tarragona), Spain.

'Arbequina' was the most productive cultivar after the first two years in Cataluña, Spain and it continued to be very productive as 'Picholine' and 'Leccino' performed similarly well in years 8-11 for average productivity. (Table 16) (Tous, Romero, Plana, 2005 a).

In Australia, the highest average yield the first three years of production was from the cultivars 'Picual', 'Barnea', 'Hojiblanca', 'Arbequina', and 'Manzanilla'. The popular Italian cultivars, 'Leccino' and 'Frantoio' were poor producers very early, but 'Leccino' had the 2nd highest production after 'Hojiblanca', which was followed by the 3rd highest producing cultivar, 'Koroneiki' in the 5th year after planting. The world's most common table olive cultivars, when grown in Australia, had the following fruit weights (g/fruit): 'Gordal', 9.66; 'Ascolano', 7.06; 'Manzanilla', 7.46; and 'Kalamon', 4.53. The cultivars with the smallest fruit sizes, under 3 g/fruit, were: 'Koroneiki', 0.96; 'Arbequina', 1.89, 'Pendolino', 2.46, and 'Frantoio', 2.80. The range in oleic fatty acid content among the cultivars was from 63.4% for 'Columella' to 79.2% for 'Koroneiki'. Oil content on a dry weight basis ranged from 40.4% for 'Hojiblanca' to 61.9% for 'Arbequina', which was the highest of all the cultivars tested. (Table 17) (Sweeney, 2005).

Table 17: Cultivar comparisons for yield, oleic fatty acid content, oil content, fruit weight, and flesh to pitration in Australia (1998 - 2004).

Cultivar	Ave. yield 3 yrs. kg/tree	Yield 2004 kg/tree	Oil content % dry wt. basis	Fruit Wt. Fresh (g)	Flesh to pit ratio
Picual	7.58	11.8	52.1	4.26	8.34
Barnea	6.80	10.9	61.0	3.64	8.16
Hojiblanca	6.39	17.2	40.4	4.47	8.98
Arbequina	5.95	11.6	61.9	1.89	6.07
Manzanilla	5.42	8.7	41.5	5.46	10.86
Mission	4.41	-	48.2	4.03	7.26
Pendolino	4.39	7.8	41.4	2.46	6.19
Columella	4.11	-	56.8	4.86	8.79
Leccino	3.88	15.2	47.6	3.55	6.18
FS-17	3.31	-	61.7	3.40	11.2
Coratina	3.29	1.4	57.1	3.94	6.32
Ascolano	2.58	-	54.1	7.06	12.4
Frantoio	2.52	6.4	57.4	2.80	5.15
Gordal	2.28	5.8	54.0	9.66	11.52
Kalamon	1.81	1.5	54.1	4.53	8.45
I-77	0.85	4.4	54.4	5.13	6.57
Souri	0.00	0.0	60.2	3.34	8.11
Koroneiki	-	13.0	47.8	0.96	4.51

Susan Sweeney, Plant Research Center, Waite Research Precinct; Rural Industries Research and Development Corporation Hartley Grove, Urrbrae, 5064, Australia.

Trials in Spain that compared fruit size, pulp to pit ratio, and oil content showed that in Andalucia, the largest fruit in g/fruit was from the cultivars 'Gordal', 8.7; 'Hojiblanca', 5.0; 'Picudo' 4.9; and 'Manzanilla' 4.7. In Cataluña they were: 'Hojiblanca', 4.8 and 'Manzanilla', 4.7, while all three of the cultivars, 'Arbequina', 'Arbosana', and 'Joanenca' had the smallest fruits with an average of 1.4 g/fruit. In Andalucia, 'Manzanilla' had the highest pulp to pit ratio with 9.4, followed by 'Gordal' with 9.1, and 'Hojiblanca' with 8.2. The cultivar with the lowest pulp to pit ratio was 'Frantoio' with 4.4. In Cataluña, once again 'Manzanilla' had the highest pulp to pit ratio, which was 8.0; 'Joanenca' was the lowest with 2.4; followed by 'Arbequina' with 4.2; 'Arbosana' with 4.6, 'Blanqueta' with 4.7; and 'Picual' with 4.8 (Tables 18-19) (Del Rio et al., 2005; Tous, Romero, Díaz, 2005).

Table 18: Olive cultivar fruit weight, pulp to pit ratio, and oil content in Andalucia Spain

Cultivar	Fruit weight in grams	Pulp to pit ratio	Oil content % dry wt. basis
Coratina	3.8	6.2	53.6
Manzanilla	4.7	9.4	48.4
Gordal (Sevillano)	8.7	9.1	47.9
Moraiolo	2.3	5.7	46.3
Blanquetta	2.1	6.9	41.8
Frantoio	2.5	4.4	41.6
Cornicabra	2.4	5.4	41.3
Maurino	2.4	7.6	41.2
Picudo	4.9	7.6	40.6
Hojiblanca	5.0	8.2	39.8
Empeltre	2.4	5.2	38.0
Leccino	3.8	6.4	37.6
Carmen del Río, Juan M. Caballero and M[a] Dolores García-Fernández. CIFA "Alameda del Obispo", IFAPA, Junta de Andalucía, Córdoba, Spain.			

Table 19: Olive cultivar fruit weight, pulp to pit ratio, and oil content in Cataluna Spain

Cultivar	Fruit weight in grams	Pulp to pit ratio	Oil content % dry wt. basis
Arbosana	1.4	4.6	54.3
Blanqueta	2.0	4.7	53.8
Arbequina	1.4	4.2	52.9
Empeltre	2.9	5.5	50.5
Picual	4.1	4.8	50.1
Manzanilla	4.7	8.0	48.3
Hojiblanca	4.8	5.4	44.3
Palomar	3.0	5.0	42.2
Joanenca	1.4	2.4	37.4
Joan Tous and Agustí Romero. Dept. d' Arboricultura Mediterrània, Centre "Mas Bové", IRTA, Reus-Constantí (Tarragona), Spain			

Table 20: Yield of olives (kg/tree) from the 2nd to the 6TH year in a super-high- density system (1.35 x 3.75 m – 1,975 trees/ha) by cultivar trees planted 3/200 in Andalucia Spain

Cultivar	Year 2	Year 3	Year 4	Year 5	Year 6	Accumulated
Arbequina	0.00	8.43	8.72	10.91	3.21	31.27
Arbequina I-18	0.00	7.47	7.11	10.19	3.13	27.93
Arbosana	0.15	8.69	4.53	9.81	3.74	26.92
FS-17	0.00	2.21	1.43	4.44	0.35	8.43
Koroneiki	1.82	10.50	3.09	6.34	2.29	24.04
Raul De La Rosa, L. León, N. Guerrero, L. Rallo, and D. Barranco - CIFA – Alameda del Obispo, IFAPA – Junta de Andalucía – Córdoba, Spain and Dept. de Agronomía, Universidad de Córdoba, Spain						

Table 21: Yield of oil (kg/tree) 2ND to the 6TH year in a super-high-density system (1.35 x 3.75 m – 1,975 trees/ha) by cultivar. Trees planted 3/2000 in Andalucia Spain

Cultivar (% oil wet basis -% water)	Year 2	Year 3	Year 4	Year 5	Year 6	Accumulated
Arbequina (14.7% oil – 60.0% water)	0.00	1.26	1.25	1.54	0.65	4.70
Arbequina I-18 (15.8% oil – 59.9% water)	0.00	1.31	1.01	1.47	0.66	4.45
Arbosana (19.1% oil – 55.5% water)	0.04	1.40	0.85	2.18	0.71	5.18
FS-17 (14.7% oil – 66.2% water)	0.00	0.33	0.22	0.65	0.08	1.29
Koroneiki (18.3% oil – 54.0% water)	0.26	1.90	0.53	1.34	0.43	4.47

Raul. De La Rosa, L. León, N. Guerrero, L. Rallo, and D. Barranco - CIFA – Alameda del Obispo, IFAPA – Junta de Andalucía – Córdoba, Spain and Dept. de Agronomía, Universidad de Córdoba, Spain

Recent comparisons in Spain between cultivars grown in the super-high-density system (1,975 trees/ha) show the superior and very early productive capacities of the 'Arbequina', 'Koroneiki' and 'Arbosana' cultivars. All of the cultivars tested, except 'FS-17', indicate that they are compact growing and have a very high precocity. Of the cultivars tested, 'Koroneiki' was the most alternate bearing and 'Arbequina' was the least alternate bearing. Both had very high fruit and oil yields, but 'Arbosana' yielded the most oil in liters per hectare of all the cultivars, because it has a slightly higher oil content and consistently good yields. 'FS-17' had ¼ the production compared to the other varieties. The two clones of 'Arbequina', 'Standard' and 'I-18', were not significantly different from each other in this trial. (Tables 20-21) (De la Rosa et al., 2007). The "IRTA-i-18" clone has been shown in trials in Cataluña, however, to be more productive than standard 'Arbequina' (Tous et al., 1999; Tous, Romero, Plana, 2005 b).

A generalized comparison of the majority of the world's most widely planted table and oil cultivars is given in Table 22. While the table cultivars are primarily chosen on the basis of their fruit size, yield consistency is very important, which is why 'Manzanillo' is popular. It also has a very good pit to pulp ratio and the pit is easily extracted. The clone of 'Manzanillo' called 'Cortijo de Cuarto' has been shown to root more easily, have a larger trunk diameter, set more fruit, come into bearing earlier, produce more, and have larger fruit size with a greater pulp to pit ration than the standard 'Manzanillo' (Suárez et al., 2005). Oil cultivars are often chosen for high oil and polyphenol content, because of their greater yields of oil per hectare and long keeping quality (Vossen, 2005).

Table 22: Oil content, fruit size, and polyphenol content of selected olive cultivars

Cultivar	**% oil** (wet)	**Fruit size**	**Polyphenol content**
Arbequina	22-27	Small	Low
Aglandau	23-27	Medium	Medium
Arbosana	23-27	Small	Medium-High
Ascolano	15-22	Large	Medium
Barnea	16-26	Medium	Medium
Barouni[2]	13-18	Large	Medium
Bosana	18-28	Medium	High
Bouteillan	20-25	Medium	Medium
Chemlali	26-28	Very Small	High
Coratina	23-27	Medium	Very High
Cornicabra	23-27	Medium	Very High
Empeltre	18-25	Medium	Medium
Frantoio	23-26	Medium	Medium-High
Farga	23-27	Medium	Medium
Hojiblanca	18-26	Large	Medium
Kalamon	15-25	Large	Medium
Koroneiki	24-28	Very Small	Very High
Leccino	22-27	Medium	Medium
Manzanillo[3]	15-26	Large	High
Maurino	20-25	Medium	High
Mission[3]	19-24	Medium	High
Moraiolo	18-28	Small	Very High
Pendolino	20-25	Medium	Medium
Picudo	22-24	Large	Low
Picual	24-27	Medium	Very High
Picholine	22-25	Medium	High
Sevillano[2]	12-17	Very Large	Low
Taggiasca	22-27	Medium	Low

Paul Vossen 2005. Olive Production Manual U of California, USA

5. Tree spacing and cultivar influence on productivity

Traditional spacing of more than 7 m between trees is no longer popular anywhere in the world except for desert dry-farmed areas. In modern olive culture trees are planted either at a medium density of about 500 – 865 per hectare, or in the high-density system (also called super-high-density) where trees are planted up to 2,220 per ha. The medium-density system using trunk shakers for harvest is used: to accommodate most cultivars, achieve an aesthetic look with larger trees, in situations where the ground is too steep, or for farms that are too small for over-the-row mechanical harvesting.

Fig B. Medium-density trees in California

Medium-density plantings have been very popular over the last 40 years with hundreds of thousands of hectares planted all over the world. Common spacings usually range from (6 x 3 m), to (4 x 8 m) apart. If the trees eventually grow too close together, every other tree can be removed to reduce shading. With the medium-density system (Fig. B), inputs are much less critical, and cultural practices are not as exacting compared to the super-high density system (Fig. C). The more trees planted per hectare, up to a point, the faster the planting comes into bearing. One way to get more trees per hectare is to plant them in a hedgerow configuration in a North–South direction with less space between trees compared to between rows.

The super-high-density system is where the trees are planted from 1.5 x 4 m apart to 1 x 3 m apart in lower vigor situations. They are never allowed to exceed a size of about 2.5-3 m tall by 1.8-2 m wide through annual renewal pruning. Vigorous cultivars cannot be planted in this system, because they become too large, shade out the lower portions of the trees, and require severe pruning to keep their size down resulting in vigorous growth that is unfruitful. Only highly precocious cultivars will fruit consistently under the conditions of close spacing and heavy pruning.

The super-high-density system has only worked with three cultivars that we know of: ‘Arbequina’, ‘Arbosana’, and ‘Koroneiki’. It also helps if the orchard manager can control soil water status to moderate tree vigor. It is anticipated that this system will be more difficult to manage in excessively vigorous sites with deep soils and high rainfall or in very low vigor situations with limited capacity to irrigate the trees. Its long-term performance is not known.

Typical yields from standard cultivars <u>in the medium-density system</u>

- 1st to 4th years – insignificant
- 5th year ~ 1.0 t/h
- 6th year ~ 2.2 t/h
- 7th year ~ 4.5 t/h
- 8th year ~ 6.7 t/h
- 9th year ~ 9.0 t/h
- 10th year + ~ 11.2 t/h

Typical yields from precocious cultivars <u>in the super-high-density system</u>

- 1st and 2nd years – insignificant
- 3rd year ~ 4. 5 t/h
- 4th year ~ 6.7 t/h
- 5th year + ~ 11.2 t/h

Planting a new olive orchard is a big puzzle with many parts. Almost simultaneously, decisions need to be made regarding the type of production system, harvest method, desired style of oil, and market segment where the oil will be sold. These decisions need to fit into the suitability of the climate, the layout of the land, soil characteristics, irrigation availability, water quality, labor resources, long term viability of the orchard, and the necessity for a rapid return on the capital invested. Existing conditions and decisions made for each part of the puzzle will help lead to making good cultivar choices (Pastor et al., 2005).

Table 23 is a summary of several research trials being conducted in different countries with orchards planted to low, high, and super-high density spacings with different cultivars. There is good data to show that the three primary cultivars used in the super-high-density system ('Arbequina', 'Arbosana', and 'Koroneiki') planted at 1,975 trees per hectare provides the most rapid return on investment. In most cases these orchards are producing more tons of fruit per hectare in the third year alone compared to the cumulative production from 7-8 years with more vigorous cultivars planted at medium-density or wider tree spacings. The old wide spacing production system planted at 204 trees per hectare only produces about half of what the orchards produce at 555 trees per hectare and only a third to a quarter of the production of trees planted at 1,975 trees/ha, after ten years (Tous et al., 1999, 2003).

Table 23: Summary of early olive yield and oil yield (tons/hectare) – first ten years from different orchards in several countries 204 trees/h (7m X 7m), 555 trees/h (3m X 6m), and 1,975 trees/h (1.35m X 3.75m)

Age	trees/h	Arbequina 15-25%	Arbosana 20-22%	Koroneiki 18-25%	Leccino 18-24%	Frantoio 23-28%	Coratina 23-28%	Picual 23-28%	Manz 13-24%	P. Maroc 15-25%
Year 2	*204*	**0**	**0**	**0.4**	**0**	**0**	**0**	**0**	**0**	**0**
	555	**0.1**	**0.2**	**2.0**	**0**	**0**	**0**	**0**	**0**	**0**
	1,975	**0.2**	**0.4**	**3.6**	**-**	**-**	**-**	**-**	**-**	**-**
Year 3	*204*	**1.7**	**1.9**	**2.7**	**0**	**0**	**0**	**0**	**0**	**0**
	555	**4.5**	**4.8**	**10.4**	**0**	**0**	**0**	**0**	**0**	**0**
	1,975	**16.6**	**17.2**	**20.7**	**-**	**-**	**-**	**-**	**-**	**-**
Year 4	*204*	**1.8**	**1.0**	**3.3**	**0.1**	**0**	**0**	**0.2**	**0.1**	**0.1**
	555	**5.5**	**2.7**	**4.8**	**0.5**	**0**	**0**	**0.6**	**0.3**	**0.3**
	1,975	**17.2**	**9.0**	**6.1**	**-**	**-**	**-**	**-**	**-**	**-**
Year 5	*204*	**2.1**	**1.9**	**4.9**	**0.3**	**0.1**	**0.1**	**2.2**	**0.6**	**0.6**
	555	**6.3**	**5.2**	**6.7**	**0.7**	**0.4**	**0.3**	**5.1**	**1.1**	**1.7**
	1,975	**21.5**	**19.4**	**12.5**	**-**	**-**	**-**	**-**	**-**	**-**
Year 6	*204*	**3.0**	**3.0**	**2.8**	**4.0**	**1.0**	**0.9**	**3.9**	**2.0**	**1.9**
	555	**8.1**	**6.3**	**10.0**	**8.3**	**2.8**	**2.2**	**9.7**	**5.3**	**5.0**
	1,975	**6.3**	**7.4**	**4.5**	**-**	**-**	**-**	**-**	**-**	**-**
Year 7	*204*	**4.3**	**6.5**	**2.8**	**5.5**	**1.6**	**2.0**	**4.7**	**2.6**	**1.1**
	555	**11.6**	**9.8**	**14.2**	**5.9**	**4.3**	**4.5**	**5.9**	**5.5**	**3.0**
	1,975	**15.5**	**11.1**	**20.6**	**-**	**-**	**-**	**-**		**-**
Year 8	*204*	**8.0**	**7.4**	**1.2**	**10.5**	**1.2**	**2.9**	**5.9**	**5.5**	**5.4**
	555	**21.6**	**9.4**	**3.0**	**15.0**	**3.3**	**6.7**	**10.9**	**11.2**	**10.6**
	1,975	**14.4**	**9.5**	**7.8**	**-**	**-**	**-**	**-**	**-**	**-**
Year 9	*204*	**9.9**	**6.2**	**9.0**	**6.5**	**4.3**	**7.5**	**11.2**	**8.0**	**1.0**
	555	**10.0**	**9.6**	**12.1**	**3.1**	**11.6**	**9.0**	**8.6**	**18.2**	**2.7**
	1,975	**10.9**	**10.4**	**15.9**	**-**	**-**	**-**	**-**	**-**	**-**
Year 10	*204*	**16.0**	**11.8**	**5.2**	**12.7**	**6.1**	**6.7**	**6.5**	**6.8**	**3.4**
	555	**17.9**	**16.0**	**10.9**	**11.2**	**4.2**	**11.2**	**6.8**	**9.0**	**5.1**
	1,975	**18.3**	**17.5**	**11.5**	**-**	**-**	**-**	**-**	**-**	**-**
Yield Cum. Total	*204*	**46.8**	**39.7**	**32.3**	**39.6**	**14.3**	**20.1**	**34.6**	**25.6**	**13.5**
	555	**85.6**	**64.0**	**74.1**	**44.7**	**26.6**	**33.9**	**53.0**	**50.6**	**28.4**
	1,975	**120.9**	**101.9**	**103.2**	**-**	**-**	**-**	**-**	**-**	**-**
Yield Ave. 8-10th yrs.	*204*	**11.3**	**8.5**	**5.1**	**11.5**	**3.9**	**5.7**	**7.9**	**6.8**	**3.3**
	555	**16.5**	**11.7**	**8.7**	**10.8**	**6.4**	**9.0**	**8.7**	**12.8**	**6.1**
	1,975	**14.5**	**12.5**	**11.7**	**-**	**-**	**-**	**-**	**-**	**-**
Oil Yield Cum. Total	*204*	**9.36**	**8.34**	**6.94**	**8.31**	**3.65**	**5.12**	**8.82**	**4.73**	**2.7**
	555	**17.12**	**13.44**	**15.91**	**9.38**	**6.78**	**8.64**	**13.51**	**9.36**	**5.68**
	1,975	**24.18**	**21.39**	**22.18**	**-**	**-**	**-**	**-**	**-**	**-**

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At about ten years of age when the trees reach or approach full size in the medium-density system (555 trees/h) the orchard yields are comparable to what they are at the density of 1,975 trees/ha; it just takes much longer. Documented establishment costs for a super-high-density orchard are about twice what they are for a medium-density system, but production costs once the trees are mature, is only about half. In several cases very high yields of 10-15 tons/hectare were reached in the medium-density system and alternate bearing was quite evident. In some cases very high yields have been achieved in the super-high-density orchards of over 15-20 tons/hectare. Those orchards also are also quite alternate bearing with very low yields preceding and directly following years with very high yields. Both systems can produce consistent yields of 9-11 tons per hectare and very high quality olive oils. (De la Rosa et al. 2007; Ouazzani, 2005; Vossen et al. 2001, 2003, Vossen et al. 2004).

Super-high-density orchards need to be intensively managed and were designed for mechanical inputs like an over-the-row harvester, mechanical pruning for topping and skirting, sophisticated irrigation and fertigation systems, processing facilities at close proximity to limit fruit degradation during transport, and knowledgeable management. Short stature trees that bear heavily soon after planting, however, could also provide an advantage for small scale growers who might do most or all of the management by hand, including harvest (Vossen, 2002, 2004).

6. Oil extraction

Oil yield predictions are difficult because there are so many variables (Table 24). Oil extractability depends on the cultivar, fruit maturity, fruit moisture content, and the processing system. The quantity of oil in the fruit is a built-in genetic factor, but it can vary from year to year due to tree vigor, crop load, fruit maturity, and fruit moisture content. Oil content varies by cultivar from less than 10% to about 30% on a wet weight basis. Since oil accumulation peaks when the fruit is quite mature, delaying harvest until the fruit is ripe assures the highest yield of oil, though that will change flavor characteristics, and extractability if the weather is rainy.

In processing, the extractability of the oil from the fruit is heavily influenced by paste fineness, malaxation time and temperature, and extraction machinery type. The fruit's water content influences the percentage of oil relative to moisture, so drier fruit will have a higher percentage of oil by weight. Oil is easier to extract from low moisture fruit, conversely, it is more difficult to extract from fruit that has been over-irrigated or has received rain before harvest and if the fruit is washed (wet) just prior to crushing. High moisture fruit often forms an emulsion (watery gel) in the paste that cannot be captured, but escapes with the fruit-water or pomace solids. Oil content increases slightly and extractability usually improves as the fruit matures, becomes softer, and is easier to crush. Some cultivars give up their oil quite easily and others do not. (Vossen, unpublished).

The amount of oil that can be extracted from the crushed paste of fresh olives has been extensively studied all over the world and in the USA as long as 100 years ago (Table 25). In one trial in the Southwestern USA, the total extracted (pressed) oil varied by as much as 145% from the 'Rubra' cultivar producing 91 kg per ton from black ripe fruit to 'Mission' producing 220.6 kg per ton. When oil yield was compared between unripe green fruit or fruit just turning color from green to red with fully mature fruit that was black ripe, the amount of extracted oil increased by 53% for 'Ascolano' going from 95.5-145.2 kg per ton. For the 'Correggiolo', 'Mission', and 'Morinello' cultivars yield increased by 26%, 54%, and 55% respectively when fruit was allowed to fully ripen. For the 'Mission' cultivar, just letting the fruit ripen from green-yellow color to red blush increased yield from 142.9-150.5 kg per ton, an increase of 6%. When the 'Pendolino' cultivar was allowed to go from black ripe to shriveled black ripe in maturity the oil extraction increased by 28% from 158.4-203.5 kg per ton. Most of these increases in oil yield per ton of riper olives are believed to be due to a reduction in fruit weight due to water loss and improved extraction.

Table 24: Approximate oil yield from 1 ton of olives with different oil content & extractability (% wet weight basis)

Olive Cultivar – Water Status - Ripeness	Oil Yield
Green over-watered Sevillano	**10 gal of oil/t - 4 % = 37.9 liters per ton**
Ripe Sevillano - Green Ascolano	**15 gal of oil/t - 6 % = 56.8 liters per ton**
Very ripe Sevillano – Ripe Ascolano	**20 gal of oil/t - 8 % = 75.7 liters per ton**
Over-watered, green Arbequina or Manzanillo Very ripe deficit-irrigated Ascolano	**25 gal of oil/t - 9.5 % = 94.6 liters per ton**
Ripe over-watered Arbequina or Manzanillo Green over-watered Frantoio, Leccino	**30 gal of oil/t - 11 % = 113.5 liters per ton**
Very ripe Arbequina or Manzanillo – Green over- watered Mission - Ripe over-watered Frantoio, Leccino	**35 gal of oil/t - 13 % = 132.5 liters per ton**
Ripe Frantoio, Leccino – Green Mission Deficit-irrigated Arbequina or Manzanillo	**40 gal of oil/t - 15 % = 151.4 liters per ton**
Ripe over-watered Mission Ripe, deficit-irrigated Frantoio, Leccino	**45 gal of oil/t - 17 % = 170.3 liters per ton**
Ripe Mission, Picual	**50 gal of oil/t - 19 % = 189.3 liters per ton**
Very ripe, deficit-irrigated Mission, Picual	**55 gal of oil/t - 21 % = 208.2 liters per ton**
Paul Vossen, unpublished data.	

Research has indicated that as the season advances the oil content of the olives increases by about 1-2% as they passed from one color stage to the next providing a total increase in oil content by about 4-8% from green to black fruit. It also found that the oil content of olives grown in sandy soils was about 3-4% less than if they were grown in sandy loam or clay soil types and that in general Northern California grown olives had a higher average oil content than did those from the south central or southern growing regions by about 1-4% (Table 25) (Coit, 1909).

Another trial conducted at the UC Riverside Experiment Station showed considerable difference in oil content by cultivar and location when the trees were grown under conditions of no irrigation and no cultivation. It found that oil content on a wet basis varied by about 23% (33.7 to 11.2%), the percentage of moisture in the fruit varied by about 32% (45.0% to 76.8%), and that the oil content on a dry weight basis varied by about 21% (63.3% to 42.5%) (Table 26) (Condit, 1940, 1946).

Table 25: Oil extraction from a press as influenced by cultivar and fruit maturity

Cultivar	Maturity	Gallons of Oil/Ton of Olives	Kg oil per metric ton
Ascolano	Half ripe – beginning to turn red	25.2	95.5
Ascolano	Fully ripe – black – some shrivel	38.3	145.2
Columella	Half ripe – mostly red color	39.4	149.3
Correggiolo	Half red –half green	44.1	167.1
Correggiolo	Fully ripe – black to the pit	51.4	193.7
Manzanillo	Fully ripe – black to the pit	34.9	149.3
Mission	Green – yellow	37.7	142.9
Mission	Half ripe – red color	39.7	150.5
Mission	Fully ripe – black fruit	50.1	189.9
Mission	Fully ripe – shriveled fruit	58.2	220.6
Mission	Moldy pomace – 2nd pressing	11.2	42.4
Morinello	Unripe green	29.2	110.7
Morinello	Fully ripe – black	45.5	172.4
Nevadillo	Black ripe	44.8	169.8
Pendolino	Black ripe	41.8	158.4
Pendolino	Fully ripe – black and shriveled	53.7	203.5
Razza	Black ripe	47.8	181.2
Rubra	Black ripe	24.0	91.0
Uvaria	Fully ripe – black	31.4	119.0
J. Eliot Coit, 1909. Olive culture and oil manufacture in the arid SW - USA.			

Table 26: Oil content of olives from various California districts

Cultivar	Location	Oil % wet	Oil % dry	Water %
Correggiolo	Chico	33.7	63.3	46.8
Grappoli	Chico	33.5	60.9	45.0
Chemlali	San Fernando	28.6	52.9	46.0
Moraioli	Chico	26.9	56.5	52.4
Mission	Lindsay	25.5	56.2	54.7
Maurini	Chico	25.0	62.8	60.2
Nevadillo	Porterville	25.0	57.0	56.2
Nevadillo	San Fernando	24.5	55.8	56.1
Manzanillo	Lindsay	24.0	60.0	60.0
Lecci	Chico	22.8	58.9	61.3
Barouni	Chico	21.3	51.7	59.8
Columbella	Fallbrook	21.0	58.6	64.2
Mission	Riverside	19.0	56.7	66.5
Picholine	San Fernando	18.6	54.7	66.0
Ascolano	Riverside	16.8	60.0	72.0
Obliza	San Fernando	16.7	52.8	68.4
Barouni	Riverside	15.7	54.1	71.0
Ascolano	Lindsay	14.8	52.4	71.8
Sevillano	Lindsay	14.7	55.5	73.7
Manzanillo	Riverside	13.0	53.0	75.5
Sevillano	Corning	12.2	52.5	76.8
Chemlali	Riverside	11.2	42.5	73.7
Condit and Cruess, 1940				

7. Fatty acid composition

The triglyceride structure of olive oil varies as to the percentage of different fatty acid types by cultivar. The primary fatty acid of interest is Oleic acid (C18:1), which makes up the largest percentage of the fatty acid types in olive oil. Note that there are only slight differences in the oleic fatty acid content of the same cultivar grown in different locations. For example, the C18:1 content of 'Arbequina' oil in France is 68.3%, in Cataluña Spain it is 68.2%, in Córdoba, 65.8%, in Australia, 69.8%, and in Morocco it was measured as 70.8%. The importance of the different percentages of oleic fatty acid content by growing region is minimal since all of the cultivars meet the minimum requirement from the IOOC as extra virgin (Tables 27-31) (Moutier et al., 2004, Ouazzani, 2005; Sweeney, 2005; Tous, Romero, Díaz, 2005; Uceda et al., 2005).

Table 27: Olive cultivars percentage oleic fatty acid content in Catacluna, Spain 1993-98

Cultivar	C18:1 (%)
Picual	78.28
Arbosana	74.77
Manzanilla	71.97
Hojiblanca	73.70
Arbequina	68.20
Empeltre	68.61
Blanquetta	61.23
Tous, Romero, and Díaz IRTA, Reus-Constantí (Tarragona), Spain	

Table 28: Olive cultivars percentage age oleic fatty acid content in Meknes, Morocco

Cultivar	C18:1 (%)
Picual	80.24
Koroneiki	78.22
Leccino	76.33
P. Marocaine	75.25
Mastoides	74.99
Kalamon	74.56
Frantoio	74.23
Arbequina	70.85
Carolea	70.53
Noureddine Ouazzani, 2005. Département d'Arboriculture, Unité de Génétique Ecole Nationale d' Agriculture, Meknés, Maroc	

Table 29: Olive cultivars percentage oleic fatty acid content in France 1995-2004

Cultivar	C18:1 (%)
Aglandau	73.41
Bouteillan	68.32
Cailletier	76.34
Cayon	79.03
Grossane	68.72
Lucques	73.12
P. du Languedoc	74.35
Salonenque	64.55
Tanche	79.44
Arbequina	68.26
Cornicabra	67.55
Frantoio	75.21
Manzanillo	72.96
Natalie Moutier, Christian Pinatel, André Martre, and Jean-Paul Roger. 2004. INRA de Montpellier, AFIDOL d' Aix-en-Provence; CBNM de Porquerolles - France.	

Table 30: Olive cultivars percentage oleic fatty acid content in cordoba, Spain, Spain 1989-1997

Cultivar	C18:1 (%)
Picual	78.34
Koroneiki	77.66
P. Marocaine	76.05
Manzanilla	75.33
Hojiblanca	74.61
Kalamon	74.56
Cornicabra	74.30
Frantoio	73.91
Pendolino	71.69
Maurino	71.35
Leccino	70.82
Empeltre	69.29
Arbequina	65.83
Moraiolo	65.09
Picudo	63.29
Blanquetta	59.01
Uceda, Beltran, Jiménez. Estacion de Olivicultura y Elaiotecnia, Junta de Andalucía, Mengíbar (Jaén), Spain	

Table 31: Olive cultivars percentage oleic fatty acid content in Australia 2000-04

Cultivar	C18:1 (%)
Arbequina	68.9-70.7
Ascolano	75.8-77.8
Barnea	72.8-75.1
Blanqueta	57.8-75.2
Bouteillan	73.8-74.1
Columella	60.2-64.6
Coratina	74.6-82.7
Frantoio	72.8-74.2
FS-17	72.2-75.5
Gordal	68.3-70.2
Hojiblanca	75.3-76.9
I-77	78.3-78.4
Kalamon	76.2-81.0
Koroneiki	79.1-80.5
Leccino	77.3-77.6
Manzanillo	72.4-74.7
Mission (CA)	77.0-77.6
Pendolino	72.7-73.6
Picual	77.0 -78.7
Souri	74.1-74.4
Susan Sweeney, Plant Research Center, Waite Research Precinct; Rural Industries Research and Development Corporation Hartley Grove, Urrbrae, 5064, Australia.	

8. Polyphenol content – bitterness – stability

The total polyphenol content of olive oil, expressed in parts per million (ppm) or milligrams per liter (mg/L) based on the caffeic acid standard, is quite different by cultivar. There is most often a direct correlation between total polyphenol content, bitterness of an olive oil expressed as a spectrophotometer reading at 225 nm, and stability of an olive oil expressed as hours of oxidative stability from a Rancimat test at either 98^{o} or 120^{o} C. Table 32 shows the results of an evaluation of olive oils in Morocco ranking the oxidative stability of each cultivar's oil. Note that 'Arbequina', which is normally thought to have a low stability, actually ranked in the medium category above 'Manzanillo', 'Frantoio', 'Carolea', and 'Hojiblanca' (Ouazzani, 2005).

Table 32: Olive cultivars – oil oxidative stability in Meknes, Morocco

Cultivar	Hrs. O_2 Stab. 120^{O}C
Picual	14.0
Leccino	10.4
Koroneiki	9.22
Haouzia	8.68
Menara	8.30
P. Marocaine	8.25
P. Languedoc	6.83
Mastoides	6.68
Arbequina	6.55
Manzanillo	5.92
Frantoio	3.85
Carolea	3.35
Hojiblanca	1.58
Noureddine Ouazzani, 2005. Département d'Arboriculture, Unité de Génétique Ecole Nationale d' Agriculture, Meknés, Maroc	

The consistency in polyphenol content, oil bitterness, and oxidative stability can be noted when comparing tables 32-34 from olive oil experiments in three different growing areas. The high polyphenol and stable cultivars ('Picual' and 'Koroneiki') are consistently high and stable no matter where they are grown and vise versa for the low polyphenol cultivars ('Arbequina' and 'Hojiblanca'). Most of the other cultivars fall in the middle ranges. The Italian study (Table 35) shows the great range and variability possible in polyphenol levels that can sometimes be greater within the cultivar than between cultivars. (Cimato et al., 1996; Ouazzani, 2005; Pannelli et al., 2001; Sweeney, 2005; Tous, Romero, Díaz, 2005; Uceda et al., 2005)

The polyphenol content of olive oil and its correlated bitterness and oxidative stability have been found to be influenced by several factors. Cultivar is important as noted above as well as the level of moisture stress received by the trees, the maturity of the fruit at harvest, and elevation above sea level where the trees are grown (Cortesi et al. 2000; Fiorino, 1996; Ranalli et al. 1999; Tous et al. 1997; Tous, Romero, Díaz, 1999).

An experiment in California provides an example of how irrigation level can influence both the total polyphenol content and oxidative stability (Table 36). This experiment demonstrated that there can be as much as 3-4 times more or less total polyphenols or bitterness in a single cultivar (Arbequina) based on the amount of irrigation water the trees received (Berenguer et al. 2006).

Table 33: Poluphenol content, bitterness, and stability of selected cultivars in cataluna spain 1996-1998

Cultivar	Polyphenols ppm	K_{225} Bitterness	Hrs. O_2 Stab. 120^{O}C
Villalonga	887	0.72	10.28
Picual	509	0.42	21.45
Blanquetta	491	0.43	10.26
Joanenca	367	0.38	13.40
Manzanilla	321	0.27	15.35
Hojiblanca	273	0.27	6.25
Arbosana	269	0.24	12.90
Palomar	248	0.29	8.41
Empeltre	238	0.21	7.82
Arbequina	201	0.19	7.80
Joan Tous, Agustí Romero, and Isabel Díaz. Dept. d' Arboricultura Mediterránia, Centre "Mas Bové", IRTA, Reus-Constantí (Tarragona) and Centro Tecnología de la Carne, IRTA, Monells (Girona), Spain.			

Table 34: Polyphenol content, bitterness, and stability of selected cultivars in Andalucia Spain 1989-1997

Cultivar	Polyphenols ppm	K_{225} Bitterness	Hrs. O_2 Stab. 98^{O}C
Chetoui	1,240	0.82	75.1
P. Marocaine	787	0.52	94.8
Picual	664	0.45	140.6
Cornicabra	464	0.48	118.7
Manzanilla	461	0.2	57.8
Empeltre	420	0.22	57.5
Koroneiki	411	0.36	71.9
Frantoio	382	0.21	64.6
Maurino	334	0.21	46.1
Kalamon	332	0.27	49.3
Leccino	302	0.25	56.3
Blanquetta	293	0.25	34.8
Picudo	246	0.19	38.03
Hojiblanca	187	0.19	51.0
Arbequina	181	0.16	38.31
Moraiolo	175	0.13	36.37
Marino Uceda, Gabriel Beltrán, and Antonio Jiménez. Estacion de Olivicultura y Elaiotecnia, Junta de Andalucía, Mengíbar (Jaén), Spain.			

Table 35: Olive cultivars comparative polyphenol content

Cultivar	Region	Polyphenols ppm	C18:1 (%)
Ascolana	Marche	478.22 ± 93.78	78.94 ± 1.06
Canino	Marche	432.75 ± 58.63	76.92 ± 1.16
Frantoio	Marche	450.68 ± 75.83	77.39 ± 1.08
Leccino	Marche	308.34 ± 51.01	77.47 ± 0.79
Leccio del Corno	Marche	495.34 ± 7.83	82.12 ± 0.91
Maurino	Marche	324.76 ± 56.24	76.81 ± 2.38
Moraiolo	Marche	496.49 ± 38.68	76.97 ± 1.15
Pendolino	Marche	312.01 ± 78.22	75.94 ± 1.92
Coratina	Toscana	80.8 – 968.5	-
Frantoio	Toscana	173.0 – 501.4	79.1
Leccino	Toscana	146.0 – 354.0	79.9
Maurino	Toscana	147.0 – 290.6	76.6
Moraiolo	Toscana	190.0 – 499.0	78.9
Pendolino	Toscana	152.0 – 490.3	79.2

Girogio Pannelli, Barbara Alfei, and Alfio Santinelli. Instituo Sperimentale per l'Olivicoltura sezione di Spoleto and Agenzia Servizi Settore Agroalimentare delle Marche, Ancona, Italy. A. Cimato, Iistituto Propagazione Specie Legnose C.N.R; A. Baldini, S. Caselli, M. Marranci, and L. Marzi, Grantees, Firenze, Italy.

Table 36: Polyphenol content and oxidative stability of arbequina oils produced in 2002 and 2003 with different levels of tree irrigation in California

Irrigation level	Polyphenols (mg/L caffeic) 2002	Polyphenols (mg/L caffeic) 2003	Hrs. O_2 Stab. 120^{O}C 2002	Hrs. O_2 Stab. 120^{O}C 2003
Low 10%-34% ET	275.3-432.4 a	85.0-175.3 a	8.8-11.4 a	31.2-40.5 a
Medium 35%-59% ET	102.9-165.2 b	70.0-73.7 b	4.8-5.4 b	28.0-30.3 ab
High > 60% ET	53.3-98.2 c	73.0-97.3 b	3.2-4.6 c	23.6-28.1 b

Maria-Jose Berenguer, Paul Vossen, Steve Grattan, Joseph Connell, and Vito Polito 2006. University of California. Different letters indicate that the values are significantly different at α = 0.05.

9. Sensory characterisitics

Flavor components within each cultivar come from the water-soluble hydrocarbons, flavenoids, phenols, polyphenols, tocopherols, and esters that make up the bitter, pungent, and fruity flavor of fresh olives. Some of these compounds are naturally occurring antioxidants that extend oil shelf life by reducing rancidity; others are the source of the fruity character of the oil. High polyphenol content oils can cause a temporary burning sensation in the back of the throat when swallowed and a bitter taste noted on the back of the tongue. If the levels of some of these compounds are too high, the oils can be excessively strong and disagreeably bitter. Fruitiness can be either green (vegetative) or ripe (fruity) in character and possibly sweet (Di Giovacchino et al., 2002).

Table 37: Sensory attributes of oils from selected cultivars in Andalucia and Cataluna Spain evaluated over several years

Cultivar	Bitter		Fruity		Green		Pungent		Sweet	
	Cat	And	Cat	And	Cat	And	Cat	And	Cat	And
Manzanilla	3.56	-	2.65	-	2.51	-	2.63	-	1.09	-
Blanquetta	3.19	2.47	2.52	2.50	2.28	1.82	2.91	2.60	1.29	1.13
Picual	2.96	1.79	3.20	2.78	2.71	2.31	2.75	2.13	1.29	1.24
Frantoio	2.60	1.65	2.95	2.71	2.00	2.25	2.60	2.40	1.35	1.48
Picudo	2.06	2.50	2.28	2.94	1.67	2.52	2.44	2.73	1.50	0.70
Hojiblanca	1.98	1.37	2.90	2.77	2.67	2.02	2.09	1.92	1.58	1.36
Empeltre	1.90	-	2.66	-	2.03	-	2.31	-	1.90	-
Arbosana	1.73	-	2.45	-	1.64	-	2.05	-	1.95	-
Arbequina	1.48	1.13	2.56	2.88	1.91	1.97	1.78	1.63	2.04	2.15
P. Maroc.	-	2.64	-	2.88	-	2.35	-	2.42	-	0.42
Koroneiki	-	2.01	-	2.86	-	2.35	-	2.53	-	0.97
Kalamon	-	2.22	-	3.22	-	2.36	-	2.93	-	1.29

Agustí Romero, Joan Tous, and Luis Guerrero. Dept. d' Arboricultura Mediterránia, Centre "Mas Bové", IRTA, Reus-Constantí (Tarragona) and Centro Tecnología de la Carne, IRTA, Monells (Girona. Marino Uceda and Ma Paz Aguiler. Estacion de Olivicultura y Elaiotecnia, Junta de Andalucía, Mengíbar, Jaén), Spain.

Table 38: Sensory attributes of oils from selected cultivars in Australia 2002-03

Cultivar	Fruitiness		Bitterness		Pungency	
	2002	2003	2002	2003	2002	2003
Arbequina	5.0	2.8	4.2	3.6	4.7	5.2
Barnea	4.8	-	4.7	-	4.7	-
Coratina	5.4	3.7	6.7	4.5	7.0	5.4
Paragon	4.6	-	3.8	-	4.9	-
Pendolino	-	3.1	-	5.3	-	5.7
Picual	5.1	3.5	4.7	2.8	4.6	3.8

Richard Gawel, P. Cox, and S. Sweeney. Recognose Pty Ltd P.O. Box 487, Unley, South Australia, 5061; Plant Research Center, Waite Research Precinct, Hartley Grove, Urrbrae, 5064; Rural Ind. R and D Corporation

Table 39: Intensity of sensory characteristics single cultivar Tuscan oils evaluated over several years

Cultivar	Fruity	Green	Bitter	Pungent	Sweet
Frantoio	*****	**	**	****	**
Moraiolo	****	**	****	***	**
Leccino	***	*	*	**	*****
Pendolino	****	*	***	**	***
Maurino	****	**	**	***	****

A. Cimato, Baldini, Caselli, Marrunci, & Marzi; Firenze, Italia. 1996

Tables 37-39 show numerical sensory ratings for oil from different cultivars grown in Spain, Australia, and Italy. In Spain the differences in sensory attributes are sometimes quite evident from one region to the next for the same cultivar, plus there are clear differences between cultivars. In Australia, the sensory ratings were much higher one year compared to the next and again there are clear differences between cultivars. The Italian example of five Tuscan cultivars provides a relative comparison of their flavor attributes. All of these sensory characterizations by cultivar indicate that different styles of oils can be made based on cultivar. (Cimato et al., 1996; Gawel et al., 2005; Romero et al., 2005).

Though the intensity of an olive oil's flavor characteristics may be influenced by the location where the trees are grown, experience has shown us that all of the typical varietal flavors are part of the genetic make-up of the cultivar. Oil made from a specific cultivar will taste like that cultivar regardless of where it was grown. It has been demonstrated that the amount of irrigation water given to trees can have a profound effect on oil flavor intensities, which might be more important than growing region. In table 40 the fruitiness, bitterness, and pungency were shown to be greatly reduced as the olive trees were given more irrigation water (Angerosa, 2002; Berenguer et al., 2006; Tous et al., 1997; Tura et al., 2000).

Table 40: Sensory attributes of california arbequina oils produced with different levels of tree irrigation

Irrigation level	Fruitiness	Bitterness	Pungency	Description
Low 10%-34% ET	3.2-4.1	1.9-6.0	1.9-4.9	Very fruity, some bitterness and pungency. Balanced. Herbaceous, grassy, green apple, nettle, and artichoke.
Medium 35%-59% ET	2.7-3.2	0.7-1.7	1.1-1.2	Very fruity, slightly bitter and pungent. Herbaceous, ripe fruits, and complex.
High > 60% ET	1.7-2.0	0.2-0.3	0.2-0.6	Mildly fruity mild ripe fruit flavors. Not bitter or pungent. Unbalanced.
Maria-Jose Berenguer, Paul Vossen, Steve Grattan, Joseph Connell, and Vito Polito 2006. University of California. Different letters indicate that the values are significantly different at α = 0.01.				

10. Flowering – fruit maturity – pollenizers – cold hardiness

The selection of cultivars was thought to depend on when they bloom relative to each other for pollination. However, the range in full bloom for 18 selected cultivars listed in table 41 is only 8-9 days, 'Lucques' being the earliest and 'Pendolino' the latest. In table 42, Australian researchers rated the cultivars by early, mid, and late flowering. In most cases, the time of full bloom is preceded and followed by several days of light bloom, indicating adequate overlap for all cultivars in all locations. Proper selection of pollenizer cultivars known to improve set in the main cultivars is very important. The most commonly recommended pollenizer cultivars are given in table 44. Since olives are wind pollinated, pollenizers should be placed upwind within 60 m of the main cultivar. (Barranco and Rallo, 2005; Griggs et al., 1975; Sweeney, 2005; Vossen 2005).
The time of fruit ripening can help spread out the harvest season and is a consideration used to avoid frost, rain, or other weather related problems. In Andalucia, Spain the range in fruit maturity calculated over several years went from October 3 to January 5, which is a span of three months. Within a cultivar the variability could be as much as 2.5 months depending on the year and crop load. Heavy crops delay fruit ripening (Table 41) (Barranco and Rallo, 2005).

Table 41: Olive cultivar flowering and maturity dates in Andalucia Spain

Cultivar	Full Bloom	Maturity
Lucques	5/1-4	10/3-11/20
Picholine	5/3-6	10/23-1/1
Frantoio	5/4-7	10/9-12/16
Empeltre	5/4-7	10/8-11/26
Gordal (Sevillano)	5/5-8	10/15-11/24
Tanche	5/5-8	10/15-12/10
Picudo	5/5-9	10/11-12/12
Manzanilla	5/5-9	10/10-12/24
Arbequina	5/6-9	10/15-12/25
P. Marocaine	5/6-9	10/11-12/22
Aglandau	5/7-10	10/4-12/24
Picual	5/7-11	10/17-10/21
Blanquetta	5/8-11	10/21-1/5
Hojiblanca	5/8-11	10/25-12/18
Leccino	5/8-11	10/3-11/20
Moraiolo	5/8-11	10/7-11/25
Kalamon	5/8-11	11/6-12/25
Pendolino	5/9-12	10/11-12/4
Diego Barranco and Luis Rallo. Dept. de Agron, U de Córdoba, Spain		

Table 42: Cultivar comparisons for time of flowering in Australia

Early flowering	Pendolino
Mid flowering	Leccino, Mission, Manzanilla, Gordal, Barnea, Frantoio, Hojiblanca, and Kalamon
Late flowering	Arbequina, Aglandau, Coratina, Columella, Koroneiki, and Picual
Susan Sweeney, Plant Research Center, Waite Research Precinct, Rural Industries R and D Corporation Hartley Grove, Urrbrae, Australia.	

Cold hardiness of olive cultivars is very important in many locations because of occasional freezing temperatures that can defoliate trees, kill branches in larger trees, kill entire young trees, and eliminate fruit production. Various rating systems have been developed to evaluate the cold hardiness of olive trees. Table 43 provides a subjective rating based on one cold event in California in 1990 (Denney et al., 1993). Many books list the relative cold hardiness of cultivars, which has been done by rating cultivars exposed many times to freezing temperatures over many decades (Table 44) (Bandino et al., 2001; Barranco et al. 2000; Cimato et al., 1997; Moutier et al., 2004; Pannelli et al., 2001; Vossen 2005)

Table 43: Subjective rating of december 1990 cold (lowest temperature: 15ºF [-9.4ºC]) damage to olive cultivars in the U.S. dept. Of agriculture germplasm repository in winters, California

RESISTANT
'Ascolano Dura', 'Asioloni', 'Chitori', 'Grappolo', 'Grossane', 'Karydolia', 'Mavrela', 'Merhavya', 'Mission', 'Sir George Grey's Spanish (Nevadillo)', No. 63, No.65A, 'Redondillo de Lomgrona', 'Rigali', 'Saiali Magloue', 'Souri', 'Touffahl', 'Vassilika', 'Yuaca', 'Yullutt'

SUSCEPTIBLE
'Arbequina', 'Ascolano-Robinson', 'Azapa', 'Bidh el Hamman', 'Calamata', 'Columello', 'Cordovil', 'Gaidourelia', 'Galega', 'Grossa di Spagna', 'Hamid', 'Leiva', 'Memeli', 'Moraioli', 'Morcal', 'Nabali', 'Nuevo di Sicrone', 'Ogliarola', Piconia', 'Picual', 'Redding Picholine', 'Rouget', 'Salome', 'Sigoise', 'Syroglyolia', 'Verdale', 'Zitoum', 'Zoragi', '355-117'

VERY SUSCEPTIBLE
'Ascolano – El Toro', 'Balady', 'Barouni', 'Bouteillon', 'Conservolia', ' Coratina', 'Criolla', 'Cucci', 'Dwarf-D', 'Ferruginea', 'Franklin – Coming', 'Frantoio', 'Gairaffa', 'Gigante de Cerignola', 'Grosse Aberkan', K18, 'Kadesh', 'Kalamon', 'Karolia Aghizi', 'Koroneiki', 'Liguria', 'Manzanillo-Hoag', 'Manzanillo-Sharpe', 'Maruini', 'Meski', 'Mission-Davis', 'Nevadilio', 'Nieland Banger', 'Ouslati', 'Picholine de Languedoc', 'Rubra', 'Sevillano-Stralock', 'UC52-24-1'

Visual field rating based on shoot tip dieback, defoliation and bark splitting. Combined numerical scores (0-5) as follows: none or light, "resistant" (0, 1); moderate, "susceptible" (2, 3); and heavy, "very susceptible" (4, 5).

Denney J. O., G. C. Martin, R. Kammereck, D. O. Ketchie, J. H. Connell, W. H. Krueger, J. W. Osgood, G. S. Sibbett, and G. A. Nour. 1993. University of California, USA.

Table 44: Cold hardiness, & recommended pollenizers for selected olive cultivars

Cultivar	Cold Hardiness	Pollenizer cultivars[1]
Arbequina	Hardy	Arbosana - Koroneiki
Aglandau	Hardy	Bouteillan - Cayon
Arbosana	Hardy	Arbequina - Koroneiki
Ascolano	Hardy	Manzanillo - Mission
Barnea	Sensitive	Manzanillo - Picholine
Barouni[2]	Hardy	Manzanillo - Ascolano - Mission
Bosana	Hardy	Tondo de Cagliari - Pizzé Carroga
Bouteillan	Hardy	Aglandau - Melanger Verdale
Chemlali	Hardy	Chetoui - Gerboui
Coratina	Sensitive	Cellina di Nardo - Ogliarola
Cornicabra	Hardy	Verdial - Manzanilla
Empeltre	Sensitive	Arbequina - Blanqueta
Frantoio	Sensitive	Pendolino - Moraiolo - Leccino
Farga	Hardy	Arbequina - Empeltre
Hojiblanca	Hardy	Picual – Picudo - Arbequina
Kalamon	Moderate	Koroneiki - Mastoides
Koroneiki	Sensitive	Mastoides - Arbequina
Leccino	Hardy	Frantoio - Pendolino - Moraiolo
Manzanillo[3]	Sensitive	Sevillano - Ascolano
Maurino	Hardy	Lazzero - Grappolo
Mission[3]	Hardy	Sevillano - Ascolano
Moraiolo	Sensitive	Pendolino - Maurino
Pendolino	Hardy	Moraiolo - Frantoio - Leccino
Picudo	Hardy	Hojiblanca - Picual - Ocal
Picual	Hardy	Picudo – Hojiblanca
Picholine	Moderate	Aglandau - Bouteillan
Sevillano[2]	Hardy	Manzanillo - Mission - Ascolano
Taggiasca	Sensitive	Leccino

Most olive cultivars are somewhat self-incompatible. They will usually set a better crop with cross-pollination especially under adverse weather conditions. Leccino, Pendolino, Moraiolo, and Maurino are self-sterile and require a pollen source from another cultivar.

Barouni and Sevillano are not compatible cross pollenizers for each other.

Manzanillo and Mission are not compatible cross pollenizers for each other.

Paul Vossen, University of California Cooperative Extension. 2005.

Cultivar	Full Bloom	Maturity
Lucques	5/1-4	10/3-11/20
Picholine	5/3-6	10/23-1/1
Frantoio	5/4-7	10/9-12/16
Empeltre	5/4-7	10/8-11/26
Gordal (Sevillano)	5/5-8	10/15-11/24
Tanche	5/5-8	10/15-12/10
Picudo	5/5-9	10/11-12/12
Manzanilla	5/5-9	10/10-12/24
Arbequina	5/6-9	10/15-12/25
P. Marocaine	5/6-9	10/11-12/22
Aglandau	5/7-10	10/4-12/24
Picual	5/7-11	10/17-10/21
Blanquetta	5/8-11	10/21-1/5
Hojiblanca	5/8-11	10/25-12/18
Leccino	5/8-11	10/3-11/20
Moraiolo	5/8-11	10/7-11/25
Kalamon	5/8-11	11/6-12/25
Pendolino	5/9-12	10/11-12/4

Diego Barranco and Luis Rallo. Dept. de Agron, U de Córdoba, Spain

11. Disease susceptibility

Choosing olive cultivars based on disease susceptibility is another consideration depending on the level of management in the orchard. The three major diseases of olive are Peacock Spot (***Spilocaea oleaginae)***, Verticillium Wilt (***Verticillium dahliae),*** and Olive Knot (***Pseudomonas savastanoi).*** Several researchers in different parts of the world have been evaluating the relative resistance of olive cultivars to these diseases. The Spilocaea and Pseudomonas organisms are only problems in wet cold climates and can be controlled with well timed applications of fixed copper sprays. Strong Verticillium resistance has only been shown in two cultivars (Frantoio and Empeltre). (Table 45) (Blanco et al., 2005; Civantos, 1999; Krueger and Vossen 2005; Peñalver et al., 2005; Trapero and López-Doncel, 2005; Vossen et al., unpublished).

Table 45: Olive cultivar susceptibility to *Spilocaea oleagina*, *Verticillium dahliae*, and *Pseudomonas savastanoi*

Cultivar	*Spilocaea oleagina*	*Verticillium dahliae*	*Pseudomonas savastanoi*
Aglandau	S	R	-
Arbequina	S	VS	VS
Arbosana	VR	-	-
Ascolano	S	R	R
Blanqueta	VS	-	R
Bouteillan	R	-	-
Cayon	R	-	-
Coratina	R	-	-
Cornicabra	VS	VS	-
Empeltre	VS	VR	S
Frantoio	VR	VR	R
Gordal Sevillana	S	S	S
Hojiblanca	S	VS	S
Koroneiki	VR	R	S
Kalamon	S	S	R
Leccino	R	S	R
Manzanilla	S	S	VS
Maurino	R	-	-
Mission	VS	S	R
Moraiolo	R	S	S
Pendolino	S	S	S
Picholine	S	S	-
P. Marocaine	VS	-	R
Picual	VS	VS	S
Picudo	S	VS	VS
VR = Very Resistant, R = Resistant, S = Susceptible, and VS = Very Susceptible			
Paul Vossen, William Krueger, Rachel Elkins, Ed Weber, Ken Churches, and Alexandra Kicenik-Devarenne, University of California, USA; Blanco-López, M. A. and F. J. López-Escudero. 2005. U de Córdoba, Spain; Civantos López-Villalta, M. 1999, U of Jaén; Peñalver, R., A. Garcia, J. Pérez-Panadés, C. Del Rio, J. M. Caballero, J. Pinochet, J. Piquer, E. A. Carbonell, and M. Milagros López. 2005; Trapero, A. and L. M. López-Doncel. 2005			

12. Conclusions

Olive cultivars are not created equal. The perfect cultivar does not exist. It is even more difficult to expect a cultivar to be both excellent for table fruit and oil production (dual-purpose). But, no matter where they are grown, some cultivars are far superior to others, based on their vigor, productivity, regular bearing, oil content, fruit size, pit to pulp ratio, fatty acid composition, polyphenol content, sensory characteristics, cold hardiness, flowering and maturity dates, pollenizer needs, and disease susceptibilities. The basic characteristics of the cultivar are inherent in the genetics. Cultivars with high or low polyphenol content, certain specific flavor characteristics, big or small fruit size, high or low oil content, or whatever percent of each fatty acid type, tend to retain those same characteristics beyond the influence of the country in which they were grown or how they were grown. All of the data comes from countries with a Mediterranean-type climate. Manipulations such as tree water stress, or

fruit maturity can have a significant influence on the ultimate characteristics of the product, but the oil, or fruit still retain the basic characteristics of the cultivar.

Some cultivars consistently seem to come into bearing before others (more precocious) and tend to produce heavier yields each year. Some have more oil content, more polyphenol content, are more cold hardy, and have greater resistance to some diseases. This does not seem to be based on local adaptation or natural selection process, but more likely a provincial adoption process. Until now many regions thought they had the best cultivar in the world, but only because they had never tried anything else. Therefore, it behooves researchers and industry to take a close look at the potential benefits of introducing superior producing cultivars or cultivars that have superior quality characteristics that would likely lead to greater efficiency and profit. New world producers beyond the Mediterranean region have planted most of the world's well known cultivars and are still in the process of evaluating the real potential of each one. There are many others, however, that still need to be tried and have not been simply because they have not yet been introduced or propagated.

Early production (precocity) is very important economically and it is difficult or impossible to make up for with cultivars that come into bearing much later. For similar reasons, regular bearing is extremely important; cultivar choice can reduce the problem of alternate bearing, but it is also very heavily influenced by cultural practices, particularly the availability of water. In managed orchards, cultivars that have consistently shown superior productivity are: 'Arbequina', 'Arbosana', and 'Koroneiki', followed by 'Barnea', 'Blanqueta', 'Coratina', 'Leccino', 'Hojiblanca', 'Manzanillo', and 'Picual'.

Of course, the choice of cultivar does not end with yield performance. Oil content and oil extractability can be an extremely important economic factor in cultivar choice. Cultural practices that influence fruit water content, fruit ripeness, and processing systems all have an influence on oil yield. Choices need to be made that fit in with the prevailing production and processing methods. Cultivars with superior oil yield are: 'Coratina', 'Koroneiki', and 'Picual' – followed by 'Arbequina', 'Arbosana', 'Empeltre', 'Frantoio', 'Haouzia', 'Hojiblanca', 'Leccino', 'Manzanillo', 'Menara', 'Mission', 'Picudo', and 'Taggiasca'.

Market characteristics such as fruit size, texture, flavor, and appearance are very important for table fruit, though table olives can be made from any cultivar. Oil processors look for superior stability, flavor, and style, again any olive can be made into oil. The table cultivars with consistently recognized quality are 'Ascolana', 'Chalkidiki', 'Gordal-Sevillano', 'Kalamon', 'Manzanilla', 'Picholine du Languedoc', and 'Santa Caterina'. The oil cultivars with consistently recognized flavor and style characteristics are: 'Arbequina', 'Bosana', 'Frantoio', 'Koroneiki', and 'Picudo' – followed by 'Ayvalik', 'Chetoui', 'Cornicabra', 'Coratina', 'Hojiblanca', 'Leccino', 'Moraiolo', and 'Picholine Marocaine'. The main cultivars with consistently superior oxidative stability are 'Coratina', 'Picual', 'Koroneiki', and 'Mission'. The fatty acid profile of a cultivar's oil is not normally used as a selection criterion, but could be if they produced fatty acid levels that were outside the accepted range of the IOC standard for olive oil.

Extremely important is how a cultivar will fit into the climate and site where it is to be grown and how it will be managed. Choosing cultivars based on high vigor, rustic hardiness, and ability to withstand drought is a step backwards in terms of modern world productive competitiveness. Dry-farmed or rain-fed orchard sites should only be chosen if they have adequate winter rainfall and deep enough soils to hold the moisture. Dry-farming can be successful, but it is certainly a management system that puts the farmer at a strong competitive disadvantage. Irrigation water is really necessary in order to assure no plant stress, so that adequate shoot growth can provide the foundation for fruit production in the following years. Good cultural practices must be followed in order to prevent weed competition, shading within the tree, alternate bearing, nutrient deficiencies, or disease outbreaks.

Pollenizer cultivars should be chosen based on their traditionally observed value in the field and on their documented pollen viability. They should be placed within 60 m of the principal cultivar to assure adequate cross pollination in years with weather problems during bloom. There are not enough differences in bloom dates to choose cultivars based when they bloom. It would also be wise to plant several cultivars that mature early to late in order to spread out the harvest season and make the best use of labor.

Cultivars with superior cold hardiness are ‘Arbequina’, ‘Arbosana’, ‘Leccino’, ‘Hojiblanca’, and ‘Picual’. The most disease resistant cultivars are ‘Arbosana’, ‘Blanqueta’, ‘Bouteillan’, ‘Frantoio’, ‘Koroneiki’, and ‘Leccino’. Disease resistance cultivar choices should be based on the past severity of specific diseases in the growing region.
Admittedly there are many gaps in the data for cultivar evaluation. Much of what is presented here is based on good comparative trials in two different regions of Spain that was published and widely available. It was combined with limited information from Italy, France, Morocco, Australia, and the USA. Many varieties were missing from the comparison trials and some of the trials have only measured results for a few years. Unfortunately, this research is very costly and very time consuming work. It is very important, however, for each growing region to make these comparisons – otherwise cultivar selection becomes haphazard and based on non-scientific criteria.

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S4-C3

Morphological and pomological characteristics of Jordanian olive cultivars

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Abstract

Morphological and pomological characterization was performed for 13 local olive cultivars held in the national germplasm collection in Jordan.
Results of the study indicated the presence of significant differences between the olive cultivars regarding the studied morphological characteristics; Shami cultivar characterized by the highest fruit weight, length, width and stone weight. Nabali Muhasan gave the highest leaf length. Kfari Romi and Nabali Muhasan cultivars characterized by the highest inflorescence length and number of flowers per inflorescence.
Pomological characterization showed significant differences between the cultivars. Fruit oil content was the highest for Nabali Baladi, Kfari Romi, Kfari Baladi and Kanabisi cultivars. Regarding olive oil fatty acid composition, oleic acid content was the highest in Nabali Baladi, Kfari Romi and Kfari Baladi cultivars. Palmitic acid was the highest in Nasouhi Jaba and Souri cultivars. Linoleic acid was the highest in Souri cultivar. Stearic acid was the highest in Bathni and Kfari Romi cultivars. Total polyphenol content was the highest in Kfari Romi, Nabali Baladi and Souri cultivars. The data obtained can be used for construction of a database that could form the basis for the national design of breeding programs.

Key words: *Olea europaea*, genetic resources, local cultivars, morphological characteristics, pomological characteristics.

Caractéristiques morphologiques et pomologiques des cultivars d'oliviers en Jordanie

Résumé

La caractérisation morphologique et pomologique ont été réalisées pour 13 cultivars locaux d'olive détenue dans la collection de germoplasme nationale en Jordanie. Les résultats de l'étude ont révélé la présence de différences significatives entre les cultivars d'olive sur les caractéristiques morphologiques étudiés; Shami cultivar caractérisé par le plus haut le poids des fruits, longueur, largeur et le poids de la pierre. Nabali Muhasan accordait la plus grande longueur de la feuille. Kfari Romi et Nabali Muhasan cultivars caractérisés par la plus grande longueur de l'inflorescence et le nombre de fleurs par inflorescence.

La caractérisation pomologique a montré des différences significatives entre les cultivars. Fruit teneur en huile est le plus élevé pour Nabali Baladi, Kfari Romi, Kfari Baladi et cultivars Kanabisi. En ce qui concerne l'huile d'olive composition en acides gras, teneur en acide oléique est le plus élevé Nabali Baladi, Kfari Romi et Kfari cultivars Baladi. L'acide palmitique est le plus élevé Nasouhi Jaba et cultivars Souri. L'acide linoléique est le plus élevé cultivar Souri. L'acide stéarique est le plus élevé Bathni et Kfari cultivars Romi.

Teneur en polyphénols totaux est le plus élevé Kfari Romi, Nabali Baladi et cultivars Souri. Les données obtenues peuvent être utilisées pour la construction d'une base de données qui pourrait servir de base à la conception nationale de programmes de sélection.

Mots-clés: Olea europaea, les ressources génétiques, les cultivars locaux, les caractéristiques morphologiques, les caractéristiques pomologique.

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1. Introduction

The olive, Olea europaea L. is adapted to the Mediterranean climate and produces table olives and olive oil, both of which are important commodities in word markets. The cultivation of olive trees started in the Mediterranean basin some 6000 years ago.

Jordan can be considered one of the homelands and natural habitats of cultivated olives. The olive tree has nutritional, social and economic importance in the life of the Jordanian people. Olive plantation makes up about 70% of the orchards of Jordan. Approximately 128000 hectares are planted with around 17 million olive tree, making it the country's top agricultural product. In 2008, the total production of olive oil was 18000 tons (Anonymous, 2008).

The original center of olive cultivation is Palestine, Syria and Cyprus (Loukas and Krimbas, 1983). The Olea europaea L. species include the wild olive var. Oleaster distributed in various areas in the Middle East and the cultivated olive var. sativa. The cultivated olive trees were selected from the wild. The propagation of the selected types had already been performed vegetatively, thousands of years ago (Zohary and Spiegel, 1975). Thus, specific types and clones were preserved locally in each area or village. Many of these old clones were lost over the generations but due to the high viability potential of the olive, many of these trees survived and were grafted with some common cultivars.

Many olive cultivars are grown in Jordan since centuries; the main autochthonous ones are 'Nabali Baladi','Rasei', 'Shami', 'Kanabisi' and 'Nasouhi Jaba'. Other cultivars have been introduced to Jordan many years ago from various olive growing regions of the world. The most common local olive cultivars grown in Jordan are Nabali Baladi and Rasei. However, several clones of these cultivars are widely spread in different areas of Jordan and several synonyms are used for these cultivars depending on the region they were grown.

It is of great importance to evaluate and characterize the existing genetic diversity of the crop species, mainly for those, such as the case of olive, which still have well preserved a great cultivar patrimony, in spite of the disturbance of the environments were they are cultivated. Within the olive species, more than 2000 cultivars have been already described for the Mediterranean area as a whole (Bartolini et al., 1998). The problem of olive germplasm classification is not only complicated by the richness of its genetic patrimony, but also by the absence of reference standards and by the confusion on the cultivar names, with numerous cases of homonymy and synonymy (Bartolini et al., 1998). The identification of olive tree cultivars has been carried out using methods based on morphological, agronomical or biochemical traits (Cantini, et al., 1999; Barranco et al., 2000; Fabbri et al., 1995). Morphological approach has traditionally been used for olive cultivar identification and continues to be the initial main step for the description and classification of olive germplasm (Rotondi et al., 2003).

Currently, according to our knowledge, no studies have been implemented in Jordan regarding the morphological or pomological characterization of local olive cultivars. Therefore the objective of this study was to evaluate the morphological and pomological characters of the local olive cultivars present in Jordan.

2. Materials and methods

2.1. Field conditions and plant material

This study was carried out in 2007-2008. Plant material were collected from 13 olive cultivars conserved at the National collection which was established in 1996 at Al-Mushaqar Research Station (Altitude: 790 m above sea level, longitude: 35º 47` East and latitude: 31º 46` North), located 28 Km to the South West of Amman.

The national collection consists of 47 olive cultivars, including 34 foreign cultivars and 13 autochthonous. Trees were planted in rows of 10 trees of each cultivar. Tree spacing was at 6 meters within rows and 7 meters between rows. The olive trees were trained with minimum pruning and grown under rain-fed conditions and standard cultural practices. These cultivars are grown in different areas of Jordan including Jerash, Ajloun, Irbid, Balqa, Tafeleh, and Kerak. Passport data were recorded for the 13 local olive cultivars held in the national collection as shown in table 1.

Table 1: Passport data of the autochthonous olive cultivars held in the national collection.

N°	Common name	Synonyms	Origin	Distribution	Purpose
1	Nabali Baladi	Romi, Souri	Jordan, Palestine	Widespread in rain-fed areas of Jordan	Dual-purpose
2	**Rasei**	Muhasan	South of Jordan	**Widespread in rain-fed and irrigated areas**	Dual-purpose
3	Nabali Muhasan	None	Jordan	**Rain-fed and irrigated areas**	Table olive
4	Nasouhi Jaba	None	Jordan, Palestine	Few plantations in the irrigated areas	Black table olive
5	Shami	None	Jordan	Few plantations	Table olive
6	Souri	None	Jordan	Few plantations in the rain-fed areas	Olive oil
7	Kfari Romi	Romi	Jordan	North of Jordan / Irbid	Dual-purpose
8	Kfari Baladi	Baladi	Jordan	North of Jordan / Irbid	Dual-purpose
9	Kanabisi	None	Jordan	North of Jordan / Ajloun and Jerash	Olive oil
10	Arabi Altafila	None	Jordan	South of Jordan / Tafeleh	Table olive
11	Ketat	None	Jordan	South of Jordan / Tafeleh	Table olive
12	Bathni	None	Jordan	South of Jordan / Tafeleh	Table olive
13	Rosai	None	Jordan	South of Jordan / Tafeleh	Table olive

2.2. Morphological characteristics

Primary characterization of the Jordanian olive cultivars was carried out for the 13 olive cultivars held in the national collection. Morphological characterization was performed according to the "methodology for the primary characterization of olive varieties" adapted by the International Olive Council (IOOC, 1997).

Each individual tree was characterized by primary descriptors relating to leaf characters (Shape, length, width, longitudinal curvature of the blade), inflorescence (length, number of flowers/ inflorescence), fruit (weight, shape, symmetry, position of maximum transverse diameter, apex, base, nipple, presence of lenticels, size of lenticels, location of start of color change, colour at full maturity) and endocarp characters (weight, shape, symmetry, position of maximum transverse diameter, apex, base, surface, number of grooves, distribution of the grooves, termination of the apex) (IOOC, 1997).
Representative samples of leaves, shoots, inflorescences and fruits were taken from each of four plants. 10 random samples were evaluated for all characters. Leaf and flower samples were collected from the mid-shoot portion of the current year growth, while 1-year-old shoots were taken at random from fruiting branches following a rotation around the tree at approximately 1.5m from the ground. At harvest time (mid November), 100 fruits were picked from each group of four plants and studied for fruit and pit characters.

2.3. Agronomical and pomological characteristics

Rooting ability of leafy stem cuttings of each cultivar was studied during March 2008. Percentage of rooted cuttings, average number of roots per cutting and average length of roots was reported after 60 days under mist. The cuttings were prepared from the middle section of vigorous shoots taken from trees on an "off" year and were treated with 3000 ppm indolebutyric acid (IBA) for 5 seconds (Caballero, 1981).

Study of pomological characteristics includes flesh/stone ratio of the fruit, percentage of moisture and percentage of oil in the fruit. The fruits were characterized using homogeneous samples of black olives that were harvested when the Maturity Index reached 4. Average fresh weight of the fruit and average fresh weight of the stone was calculated from two sub-samples of 50 olive fruits. Percentage of moisture in the fruit was calculated from two sub-samples of 50g of crushed fruits, dried in the oven at 105℃ for 24 hours. Percentage of the oil in the fruit was calculated from two sub-samples using soxhlet extraction method (AOAC, 1980).

For each olive cultivar under study, olive fruits were harvested and sent directly to small- scale olive mill (Mini-Olomio) for oil extraction. The extracted olive oil samples were stored in the refrigerator until analysis. Olive oil fatty acid composition was determined according to the method reported by

European Committee (2003). Total polyphenols content was determined using caffeic acid as standard (Gutierrez, et al., 2001).

2.4. Statistical analysis

Statistical data analysis was performed by analysis of variance (ANOVA). Duncan's Multiple-Range Test was carried out to test significance of differences between treatment means (cultivars) using the SPSS program for Windows, version 17.0.

3. Results and Discussion

Results of the morphological characterization indicating the presence of significant differences between olive cultivars under study (Table 2 and 3). Shami cultivar characterized by the highest fruit weight, length, width and stone weight. However, Nabali Baladi, Nabali Muhasan, Souri and Kfari Romi were the lowest in fruit weight (Table 2). The highest leaf length was recorded for Nabali Muhasan and Souri cultivars, and the highest leaf width was recorded for Shami, Rosai, Nabali Baladi, Nabali Muhasan, Souri and Kfari Romi cultivars (Table 3). Higher internode length was recorded for Kfari Romi as compared to other cultivars. The highest inflorescence length was recorded for Kfari Romi and Nabali Muhasan cultivars and the highest number of flowers per inflorescence was recorded for Nabali Muhasan cultivar (Table 3).

Table 2: Average mean values of the parameters used for morphological characterization and description of Jordanian olive cultivars.

No.	Olive Cultivar	Fruit Weight (g)	Fruit Length (cm)	Fruit Width (cm)	Stone Weight (g)	Stone Length (cm)	Stone Width (cm)
1	Nabali Baladi	2.78 ef*	2.17 ef	1.52 fg	0.54 c	1.67 def	0.75 b
2	Rasei	3.95 c	2.24 cde	1.71 de	0.51 c	1.51 a	0.79 b
3	Nabali Muhasan	2.48 ef	2.13 ef	1.41 h	0.79 b	1.82 bc	0.85 b
4	Nasouhi Jaba	4.62 c	2.81 b	1.68 e	0.81 b	2.11 a	0.80 b
5	Shami	7.04 a	3.00 a	2.13 a	0.92 a	1.94 b	0.95 ab
6	Souri	2.34 ef	2.06 fg	1.44 gh	0.52 c	1.62 defg	0.71 b
7	Kfari Romi	3.13 e	2.27 cde	1.58 f	0.51 c	1.66 def	0.72 b
8	Kfari Baladi	4.20 cd	2.41 c	1.82 c	0.61 c	1.72 cd	0.78 b
9	Kanabisi	4.32 cd	2.46 c	1.79 cd	0.76 b	1.69 cde	0.88 b
10	Arabi Altafila	3.98 c	2.21 cde	1.85 c	0.51 c	1.49 a	1.58 a
11	Ketat	4.14 cd	2.26 cde	1.81 c	0.55 c	1.57 efg	0.80 b
12	Bathni	4.23 cd	1.99 g	1.88 c	0.72 b	1.28 h	0.95 ab
13	Rosai	5.33 b	2.33 cd	2.00 b	0.80 b	1.54 fg	0.96 ab

Table 3: Average mean values of the parameters used for morphological characterization and description of Jordanian olive cultivars.

N°	Olive Cultivar	Leaf Length (cm)	Leaf Width (cm)	Inter-Node (cm)	Inflores. Length (cm)	No. of Flowers per Inflorescence
1	Nabali Baladi	5.00 bc*	1.14 ab	2.09 b	1.79 de	17.0 b
2	Rasei	4.79 bcd	1.03 bc	1.45 def	2.63 b	16.2 bc
3	Nabali Muhasan	5.61 a	1.10 abc	1.57 f	2.93 a	28.2 a
4	Nasouhi Jaba	5.10 b	0.88 d	1.37 ef	1.61 e	10.5 e
5	Shami	5.16 b	1.20 a	2.10 b	1.92 d	12.9 d
6	Souri	5.61 a	1.12 abc	1.81 bcd	2.52 b	16.1 bc
7	Kfari Romi	4.81 bcd	1.10 abc	2.57 a	2.95 a	17.1 b
8	Kfari Baladi	4.64 cde	1.05 bc	2.04 bc	2.57 b	10.8 e
9	Kanabisi	4.31 ef	0.80 d	1.75 bcde	1.43 f	6.5 f
10	Arabi Altafila	4.12 fg	0.88 d	2.02 bc	1.75 de	9.8 e
11	Ketat	4.49 de	0.81 d	1.40 ef	2.18 c	14.5 cd
12	Bathni	3.69 h	1.02 c	1.92 bc	1.31 f	6.4 f
13	Rosai	3.84 gh	1.17 a	1.68 bcdef	2.57 b	9.5 e

* Means within each column having the same letters are not significantly different at 5% probability level according to Duncan's Multiple-Range Test.

Rooting ability study indicated that Rasei cultivar gave significantly the highest values regarding rooting percentage (85.2%) and average number of roots per cutting (10.7) compared to the other cultivars. On the other hand, Nasouhi Jaba gave the lowest rooting percentage (15.3%) without being significantly different from Nabal Baladi, Ketat and Kfari Baladi (Table 4). Regarding average length of roots, it was the highest for Arabi Altafila (9.2cm) without significant differences from Rasei, Nasouhi Jaba, Souri, Kfari Baladi, Kfari Romi, Kanabisi and Ketat cultivars (Table 4). It was reported that some olive cultivars are easy-to-root, while others are difficult (Fouad *et al*., 1990). Rasei cultivar is easy-to-root, however, Nabali Baladi is difficult-to-root and has a low rooting percentage of cuttings (Qrunfleh *et al*., 1994; Ayoub and Qrunfleh, 2006).

Table 4: Rooting ability of leafy stem cuttings for 13 olive cultivars rooted during March 2008.

Cultivar	Rooting %	Average number of roots per cutting	Average length of roots (cm)
Nabali Baladi	19.3 ef *	3.5 bc	4.3 b
Rasei	85.2 a	10.7 a	6.2 ab
Nabali Muhasan	53.6 b	6.1 b	4.5 b
Nasouhi Jaba	15.3 f	2.6 c	7.0 ab
Shami	25.5 de	3.5 bc	5.6 b
Souri	30.3 cd	3.2 bc	6.4 ab
Kfari Romi	25.4 de	5.4 bc	7.2 ab
Kfari Baladi	21.2 ef	2.5 c	7.5 ab
Kanabisi	32.4 c	3.7 bc	6.3 ab
Arabi Altafila	56.3 b	5.4 bc	9.2 a
Ketat	18.5 f	2.4 c	7.2 ab
Bathni	32.9 c	6.5 b	4.6 b
Rosai	58.7 b	5.7 bc	4.5 b

Results of pomological parameters showed higher values regarding flesh-stone ratio of the fruit for Arabi Altafila, Rasei, Shami and Ketat cultivars compared to the other cultivars. However, the lowest values were recorded for Nabali Muhasan and Souri cultivars (Table 5). These results indicated that cultivars with higher flesh-stone ratio are more suitable to be used for table olive purposes than those with low fresh-stone ratio. Percentage of moisture in the fruit was the highest for Rasei and Nabali Muhasan cultivars. Percentage of oil in the fruit was the highest for Nabali Baladi, Kfari Romi, Kfari Baladi and Kanabisi cultivars (Table 5). It was reported by Said *et al*. (2005) that percentage of oil in the fruit for the local Nabali Muhasan cultivar was 45% based on dry weight bases, which is similar to our finding in this study. There are no reports available regarding oil content for the other local cultivars.

Table 5: Average values of the parameters used for pomological characterization of Jordanian olive cultivars.

N°	Olive cultivar	Flesh-stone ratio of the fruit	Percentage of moisture in the fruit (%)	Percentage of oil in the fruit (dry wt.) (%)
1	Nabali Baladi	4.2 cd*	45.0 cd	60.6 a
2	Rasei	6.7 a	60.5 a	46.4 bc
3	Nabali Muhasan	2.1 e	60.1 ab	36.6 cd
4	Nasouhi Jaba	4.7 cd	52.0 abcd	35.4 d
5	Shami	6.6 a	50.3 bcd	45.5 bcd
6	Souri	3.5 de	49.4 cd	47.6 b
7	Kfari Romi	5.1 abcd	46.8 cd	63.2 a
8	Kfari Baladi	5.9 abc	43.8 d	65.7 a
9	Kanabisi	4.7 cd	42.0 d	59.1 a
10	Arabi Altafila	6.8 a	45.9 cd	35.4 d
11	Ketat	6.5 ab	50.8 abcd	43.6 bcd
12	Bathni	4.9 bcd	43.6 d	44.7 bcd
13	Rosai	5.7 abc	54.5 abc	37.9 bcd

* Means within each column having the same letters are not significantly different at 5% probability level according to Duncan's Multiple-Range Test.

The composition of fatty acids in olive oil is shown in table 10. Oleic acid content was the highest in Nabali Baladi (71.4%) without significant differences from Kfari Romi or Kfari Baladi cultivars. However, the significantly lowest oleic acid content was recorded for Nasouhi Jaba cultivar (56.4%) (Table 6). Palmitic acid was the highest in Nasouhi Jaba and Souri cultivars. Linoleic acid was the significantly the highest in Souri cultivar (19.4%). Stearic acid was the highest in Bathni and Kfari Romi cultivars (Table 6). The results show that olive oil fatty acid content in all studied cultivars were within the normal reference values and legal limits as established by International Olive Oil Council (IOOC, 2006). In a study on olive oil fatty acid content of two local olive cultivars, Tawalbeh (2005) reported that oleic acid content in olive oil obtained from Nabali Baladi and Romi cultivars were 75.7% and 76.5%, respectively. Al-Rousan (2004) reported that oleic acid content in olive oil extracted from Nabali Muhasan cultivar grown in the north of Jordan contains 69.9% oleic acid, 14.1% palmitic acid, 2.8% Stearic acid and 8.7% lenoleic acid.

Table 6: Fatty acid composition of olive oil for the 13 olive cultivars.

N°.	Cultivar	Oleic acid (C18:1) %	Palmitic acid (C16:0) %	Linoleic acid (C18:2) %	Stearic acid (18:0) %
1	Nabali Baladi	71.42 a*	10.87 de	11.40 f	3.83 abc
2	Rasei	68.69 cd	12.41 c	13.51 bc	2.94 cde
3	Nabali Muhasan	68.26 d	11.07 de	14.34 b	3.75 abc
4	Nasouhi Jaba	56.35 g	15.41 a	12.31 def	2.24 e
5	Shami	67.13 e	14.24 b	13.14 cde	2.37 e
6	Souri	60.13 f	14.39 ab	19.42 a	2.66 de
7	Kfari Romi	71.48 a	10.10 e	11.82 f	4.23 ab
8	Kfari Baladi	71.21 a	11.00 de	12.07 f	3.24 bcde
9	Kanabisi	69.08 cd	12.33 c	13.24 cd	2.57 de
10	Arabi Altafila	69.61 bc	12.57 c	11.92 f	3.39 bcd
11	Ketat	70.18 b	11.75 cd	12.25 ef	3.41 bcd
12	Bathni	68.25 d	11.12 de	13.55 bc	4.56 a
13	Rosai	66.78 e	13.65 b	13.73 bc	2.88 cde

With regard to olive oil polyphenols content, results of this study showed that the highest values were reported for olive oil obtained from Kfari Romi (323 mg/kg) and Nabali Baladi (308 mg/kg) cultivars. On the other hand, the lowest value (187mg/kg) of total polyphenols content was found in olive oil obtained from Rosai cultivar (Table 7). Similar finding was reported by Tawalbeh (2005) who found that total polyphenols content in olive oil obtained from Nabali Baladi and Romi cultivars were 372 mg/kg and 322 mg/kg, respectively.

Table 7: Total polyphenols content in olive oil for the 13 olive cultivars

N°	Olive cultivar	Total phenols content mg/kg
1	Nabali Baladi	308 ab*
2	Rasei	252 def
3	Nabali Muhasan	273 cd
4	Nasouhi Jaba	235 efg
5	Shami	210 ghi
6	Souri	294 abc
7	Kfari Romi	323 a
8	Kfari Baladi	285 bcd
9	Kanabisi	264 cde
10	Arabi Altafila	226 fgh
11	Ketat	198 hi
12	Bathni	268 cd
13	Rosai	187 i

*Means having the same letters are not significantly different at 5% probability level according to Duncan's Multiple-Range Test.

The differences in morphological and pomological characteristics among olive cultivars under this study could be attributed to genetic factors of these cultivars since they were grown under the same ecological conditions. In conclusion and based on the results of this study, it is possible to find cultivars with valuable morphological, agronomical and/or pomological traits that can be immediately distributed to the farmers or employed in breeding programs.

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S4-C4

Comportement de six variétés d'olivier à huile dans le biotope de Taous (Sfax.Tunisie) : Résultats de 4 campagnes de suivi

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Résumé

Le suivi du comportement de six variétés d'olivier à huile (Chemlali de Sfax, Chemchali, Oueslati, Chetoui, Arbéquina et Koroneiki) plantées à Taous (Sfax, Tunisie) en 2002 à raison de 204 arbres par hectare durant quatre campagnes agricoles (2005-2008), nous a permis de mettre en évidence les qualités de chacune de ces variétés dans ce biotope particulier. En ce qui concerne la production moyenne en olives, ce sont les variétés Chemlali de Sfax et Arbéquina qui se distingue par des productions moyennes par arbre et par an dépassant les 14 Kg. La teneur en matière grasse par rapport au poids frais est variable de 22 à 35.5% selon le stade de maturité et la variété, cependant c'est la variété Oueslati qui enregistre le taux le plus élevé (35.5%) pour des olives cueillies au stade de fin véraison. Quant aux phénologies de la croissance végétative et de la floraison, elles présentent certaines variations d'une campagne à une autre.

Mots clés: Olive à huile, croissance, floraison, taux d'huile, production

Abstract

Monitoring the behaviour of six olive oil varieties (Chemlali Sfax, Chemchali, Oueslati, Chetoui, Arbequina and Koroneiki) planted at Taous (Sfax, Tunisia) in 2002 at the rate of 204 trees per hectare for four crop years (2005 -2008), allowed us that Chemlali and Arbequina are characterized by an average of olive production per tree per year exceeding 13 Kg. The fat content compared to fresh weight varies from 19 to33% depending mainly on variety, however, Oueslati and Chemchali varieties recorded the highest rate. As to the penology of vegetative growth and flowering, there are some variations from one cultivar to another and from one year to another.

1. Introduction

La Tunisie, malgré ces 66 millions d'oliviers souffre de la fluctuation de sa production annuelle qui engendrerait des répercussions très néfastes pour l'économie nationale. Plusieurs facteurs sont à l'origine de cette situation dont les conditions climatiques caractérisées par l'irrégularité et parfois l'insuffisance des précipitations (Trigui, 1993). Pour remédier à cette situation, de multiples mesures ont été préconisées dont l'encouragement de l'installation de vergers intensifs (Chaari et al, 2006 et même hyper intensifs (Larbi et al, 2006) qui, étant donné leur relative indépendance des conditions climatiques, assureraient une production annuelle minimale permettant à la Tunisie, entre autres, d'honorer ces engagements envers ces partenaires. Or, le comportement en intensif des variétés tunisiennes d'olivier les plus cultivées (Chétoui et Chemlali) est peu connu à travers la Tunisie et ce en dépit de leur parfaite adaptation aux conditions de culture tunisiennes. En plus, des variétés comme Oueslati ou Chemchali Gafsa qui sont très performantes aussi bien sur le plan agronomique que technologique (Kammoun et Khlif 2001 ; Gheriani, 2006), se sont très peu propagées à travers le pays et leur étude en collection pourrait aboutir à des bonnes découvertes. Les vertus des variétés étrangères comme l'Arbéquina ou encore Koroneiki les présentent comme de sérieux concurrents ou compléments pour les variétés locales.

Le travail objet de ce papier traite de l'évaluation de quelques performances agronomique et technologique de ces six variétés d'oliviers à huile (Chemlali de Sfax, l'Arbéquina, Koroneiki, Chétoui, Oueslati El Alla et Chemchali Gafsa) dans le biotope de Taous (Situé à 28 Km au nord de la ville de Sfax-Tunisie).

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2. Matériel et méthodes

2.1. Le verger d'étude

Le verger d'étude est situé dans la ferme expérimentale de l'institut d'olivier, situé à 28 Km du Nord ouest de la ville de Sfax (centre de la Tunisie). Les oliviers ont été plantés au mois de Février 2002 avec une densité de 204 pieds à l'hectare et un espacement de 7 m/7 m, irriguée au goutte à goutte. Au cours des années d'étude, l'apport en eau et en éléments nutritifs a été calculé selon les besoins annuels d'oliviers.

2.2. Matériel végétal

- *Chemlali de Sfax* : C'est une variété tunisienne à huile qui occupe 60% de la surface oléicole. Sa floraison est précoce. Ses fruits mûrissent tard, sont petits de poids moyen de 1gramme. Le rendement moyen en huile est de 20 % (Grati- Kammoun et Khlif, 2002).

- *Chetoui* : C'est une variété tunisienne appelée aussi« Zayati ». Son entrée en production est précoce et son époque de floraison est tardive (Trigui et Msallem, 2002).Les fruits sont nombreux et d'un poids moyen de 2grammes. Le rendement en huile est généralement de 28 %.C'est la principale variété à huile du Nord tunisien.

- *Chemchali Gafsa* : Appelée encore « Zeitoun el Ouaha», c'est la variété la plus répondue dans la région de Gafsa, aussi bien en sec qu'en irrigué dans les oasis où elle est plus productive. Son entrée en production est moyennement précoce et son époque de floraison est précoce. Le poids moyen de ses fruits est de 2.26grammes et son rendement en huile est de 28 %.

- *Oueslati* : C'est une variété tunisienne appelée encore « El Leguim ». Peuplant avec « El Horr » les plantations ancestrales de la zone traditionnelle de Oueslatia. Son entrée en production et son époque de floraison sont précoces. Ces fruits mûrissent tard, sont nombreux et petits de poids moyen de 2.5 grammes. Le taux moyen en huile de ses fruits est de 31 % (Grati- Kammoun et Khlif, 2002).

- *Koroneiki* : C'est la principale variété à huile de Grèce. Les arbres fleurissent précocement et produisent un pollen abondant. La maturation des fruits est précoce à moyenne. Sa productivité est élevée. Le fruit pèse en moyenne 1.8 gramme et son rendement à huile est élevé.

- *Arbequina* : C'est une variété des provinces de Lerida et Taragane (en Espagne), elle est très productive. Les arbres sont généralement de vigueur moyenne, les fruits sont petits de poids moyen 1 à 2 grammes avec un rendement en huile de 17 à 20 %.

2.3. Evaluation de la croissance végétative

Pendant la période de croissance, l'évolution de l'allongement végétatif est suivie sur huit arbres de chaque variété. Sur chaque arbre on suit la croissance de 4 rameaux disposés dans les quatre orientations de l'arbre, ces rameaux sont généralement représentatifs de l'état de végétation de l'arbre. Les mesures effectuées concernent : la longueur total du rameau, son diamètre mesuré à l'aide d'un pied à coulisse à 5 cm du point de son insertion et le nombre de nœuds. On rapporte les accroissements relatifs moyens pour ces 3 paramètres c'est-à-dire : (Valeur Finale- Valeur Initiale) du paramètre/Valeur Initiale.

2.4. Evaluation des potentialités reproductives des variétés étudiées

Il s'agit de l'étude de la biologie florale qui consiste à déterminer les dates du début de la floraison (qui correspond à 10% des fleurs ouvertes), de la pleine floraison quand on a 50% des fleurs qui sont ouvertes et de la fin de la floraison correspondant au début de la chute des pétales.
On s'est également intéressé à l'évaluation de la production annuelle en Kg de fruits par arbre et par variété**.**

2.5. Technologie oléicole :

- Caractérisation pomologique des olives :

- Le poids moyen d'une olive est déterminé systématiquement pour chaque échantillon étudié par la pesée de 3x100 fruits frais.

- Rapport pulpe/noyau : On procède au dénoyautage de 100 fruits préalablement pesés. Après nettoyage et pesée des noyaux obtenus, le rapport pulpe/noyau est calculé selon la formule suivante :

$$\text{Pulpe/Noyau} = \frac{\text{Poids de 100 fruits - Poids de 100 noyaux}}{\text{Poids de 100 noyaux}}$$

- Teneur en matière grasse

Elle est déterminée par la technique de spectrométrie de résonance magnétique nucléaire (R.M.N) à l'aide d'un spectromètre du type « OXFORD 4000 ».

La détermination de ce paramètre est réalisée sur des prises de 50 fruits frais préalablement pesé et séché à 105℃. Le poids sec des olives est communiqué à l'appareil à l'aide d'une balance électronique. L'appareil est programmé pour effectuer 3 mesures successives et affiche une valeur moyenne. La valeur affichée par l'appareil représente la teneur en matière grasse par rapport au poids sec. Celle exprimée par rapport au poids frais est déterminée par le calcul suivant : MG/poids frais = (MG/poids sec) x (poids sec)

- Composition acidique de l'huile

L'extraction de l'huile à partir des olives fraîches (2,5 Kg environ pour chaque échantillon) a été effectuée à l'aide d'un oléo doseur au laboratoire d'Oléotechnie de l'Institut d'Olivier à Sfax. La technique d'extraction de l'huile comporte quatre étapes : le broyage**,** le malaxage, la centrifugation et enfin la décantation naturelle basée sur la différence de densité entre les deux liquides, l'huile (0.915 à 0.920) et les margines (1.015 à 1.086) suite à laquelle la phase supérieure constituée d'huile est recueillie pour déterminer sa composition en acides gras.

3. Résultats et discussions

3.1. Caractéristiques agronomiques

3.1.1. Production en olives

Alors qu'en 2004, seul 20 % des arbres ont donné des fruits, l'année 2005 a enregistré l'entrée en production de la quasi totalité des oliviers (85%). La Chemlali, l'Arbéquina et la Koroneiki, après 4 années de production, présentent les moyennes les plus élevées (Tableau 1). Une alternance franche caractérise la Oueslati. La production moyenne à l'hectare est variable de 1,5 Tonne/ha (Oueslati) à 3 Tonnes à l'hectare (Chemlali et Arbéquina). In faut noter que dans les mêmes conditions de culture, l'Arbéquina peut produire jusqu'à 6 T/ha (Lopez-Villalta, 1997).

Tableau 1: Production en olives en Kg par arbre par variété et par année.

Variété	2005	2006	2007	2008	Moyenne
Arbéquina	4,1	9,69	14	24,7	13,1225
Chemchali	5,78	2,32	4,9	18,8	7,95
Koroneiki	5,39	4,17	19	18,18	11,685
Chemlali	11,46	8,12	21,5	18,58	14,915
Chétoui	7,72	4,06	5,45	12,7	7,4825
Oueslati	7,72	0,1	22,2	0,1	7,53
Moyenne	7,03	4,74	14,51	15,51	10,45

3.1.2 Floraison

Le suivi de la floraison des 6 variétés laisse apparaître une légère variation d'une année à une autre (Tableau 2). Ainsi pour les années 2005, 2006 et 2009, la pleine floraison coïncide avec le début du mois de Mai, alors que pour 2007 et 2008, elle se passe vers la fin du mois d'Avril. Les conditions climatiques peuvent être à l'origine de ces fluctuations (Lavee, 1997).

Une autre remarque à faire est le comportement presque identique de toutes les variétés quant à l'apparition des fleurs. Ce ramassage dans le temps de la floraison ne pourrait qu'avoir un effet bénéfique sur la qualité et la quantité des fruits puisqu'il permettrait d'éviter certaines malformations et imperfections des drupes (Lavee, 1997).

Tableau 2: Date du début de la floraison (DF), pleine floraison (PF) et fin de la floraison (FF).

		2005	2006	2007	2008	2009
Arbéquina	DF	25/4	18/4	10/4	16/4	30/4
	PF	2/5	2/5	20/4	22/4	3/5
	FF	15/5	15/5	5/5	2/5	9/5
Chemchali	DF	28/4	27/4	12/4	18/4	30/4
	PF	5/5	2/5	20/4	28/4	7/5
	FF	15/5	12/5	5/5	5/5	12/5
Koroneiki	DF	25/4	27/4	12/4	16/4	30/4
	PF	2/5	2/5	20/4	22/4	3/5
	FF	15/5	17/5	5/5	2/5	9/5
Chemlali	DF	25/4	18/4	10/4	16/4	30/4
	PF	2/5	2/5	20/4	22/4	7/5
	FF	15/5	17/5	5/5	2/5	12/5
Chétoui	DF	25/4	18/4	10/4	18/4	30/4
	PF	2/5	2/5	2/5	25/4	7/5
	FF	15/5	17/5	7/5	5/5	12/5
Oueslati	DF	25/4	27/4	12/4	18/4	30/4
	PF	2/5	2/5	20/4	25/4	7/5
	FF	15/5	17/5	7/5	5/5	12/5

3.1.3. Croissance végétative

Les variétés Chemlali de Sfax, Arbéquina, Chemchali et Chétoui s'adaptent relativement bien à ce système de culture puisqu'elles présentent des croissances relatives assez importantes (Tableau 3).

La Koroneiki se distingue surtout par un accroissement relativement important du diamètre moyen et un allongement des rameaux plutôt faible avec un nombre moyen de nœuds élevé ce qui explique le port naturellement ramassé de cette variété et la conservation de cette caractéristique dans le biotope de Taous (Tableau 3).

La variété Oueslati présente des accroissements faibles ce qui pourrait être le résultat du fait que les arbres sont issus de boutures semi ligneuses donc franc de pieds alors que cette variété est généralement cultivée greffée dans son site d'origine.

Les années à venir nous permettront de vérifier toutes ces hypothèses et de se prononcer sur le degré d'adaptabilité de ces variétés aux conditions édaphoclimatiques de la région sfaxienne.

Tableau 3: Allongement Relatif Moyen des Rameaux (ARR), Diamètre Relatif Moyen des rameaux à 5 cm du point de leur insertion (DRR) et le Nombre de Nœuds relatif Moyen des mêmes rameaux (NRN) par variété et par campagne.

Variété	Paramètre	2005/06	2006/07	2007/08
Arbéquina	ARR	0.62	0.32	0.71
	DRR	0.90	0.52	1.29
	NRN	0.87	0.41	0.86
Chemchali	ARR	0.35	0.51	0.73
	DRR	0.64	0.82	0.69
	NRN	0.86	0.62	0.94
Koroneiki	ARR	0.50	0.28	0.31
	DRR	1.19	0.61	0.53
	NRN	0.83	0.34	0.65
Chemlali	ARR	0.57	0.76	0.68
	DRR	0.86	0.96	0.80
	NRN	1.59	0.78	0.97
Chétoui	ARR	0.49	0.46	0.92
	DRR	0.75	0.56	0.62
	NRN	0.69	0.38	1.26
Oueslati	ARR	0.56	0.61	0.37
	DRR	1.02	0.70	0.37
	NRN	1.03	0.59	0.70

3.2. Caractéristiques technologiques

3.2.1. Variation du Poids Moyen d'un Fruit (PMF) et du rapport pulpe/noyau (P/N).

Le PMF est, pour presque toutes les variétés, variable d'une saison à une autre (Tableau 4). On constate que pour Chemlali et Koroneiki, le PMF est inférieur par rapport à celui de leur site d'origine (SO) (Kotsaftakis et al, 2000 ; Grati Kammoun,2007). Alors que pour les autres variétés, il est supérieur ou égal au PMF dans le SO (Grati Kammoun, 2007) .Quant aux rapports P/N, ils sont variables durant les 2 campagnes 2005 et 2007, mais en 2008, on peut remarquer que les valeurs sont proches de ceux des sites d'origine sauf pour la Koroneiki dont le P/N demeure inférieur à sa valeur (5) dans le SO (Kotsaftakis et al, 2000).

Tableau 4: Le PMF et le P/N par variété et par année.

	2005		2007		2008	
	PMF	P/N	PMF	P/N	PMF	P/N
Oueslati	1,78	6,215	1,4	4,47	-	-
Arbéquina	1,98	5,565	1,75	6,19	1,11	4,71
Chemlali	0,82	3,995	0,94	5,09	0,94	5,34
Chemchali	1,86	5,575	2,45	5,6	2,23	8,27
Koroneiki	0,94	4,31	0,93	3,36	0,88	4,91
Chétoui	2,52	7,075	2,38	7,89	1,89	6,74

3.2.2. Teneur en matière grasse par rapport au poids frais (MGF) et au poids sec (MGS).

Tableau 5: La MGF et La MGS par variété et par année

	2005	2007		2008	
	MGF	MGS	MGF	MGS	MGF
Arbéquina	24,05	51,81	26,37	39,65	19
Chemchali	23,99	59,34	33,6	58,87	33
Koroneiki	24,92	50,05	27,2	47,49	23
Chemlali	22,28	49,82	27,13	44,66	19,9
Chétoui	27,04	53	26,2	46,88	21,5
Oueslati	27,92	55,67	32,77	--	--

Les taux de matière grasse par rapport au poids frais varient de 19 à 33 % (Tableau 5). Ils sont très variables d'une campagne à une autre. Il est à remarquer aussi la distinction de la Chemchali par des taux élevés (2007 et 2008) et une diminution de ces taux en 2008 pour le reste des variétés. La stabilisation de ce paramètre pourrait avoir lieu dans les années à venir.

3.2.3. Variation du pourcentage moyen des principaux acides gras

L'examen de la composition en acide gras majeurs de l'huile d'olive (Acide Palmitique, Acide oléique et Acide linoléique, Tableau 6) des 6 variétés nous permet de constater que seule la Chemlali Sfax a présenté des taux meilleurs en acide oléique en comparaison avec celui du site d'origine estimé à 57%. Toutes les autres variétés ont vu leur taux en acide oléique diminuer, par rapport aux valeurs des sites d'origines, en faveur des taux des acides Palmitique (Arbequina) et surtout du linoléique (Arbequina, Chetoui, Chemchali et Koroneiki), ce qui pourrait altérer la qualité de ces huiles. Ces paramètres devraient être suivis les années à venir pour pouvoir se prononcer sur l'utilité de ces introductions dans la région de Sfax.

Tableau 6: Variation du pourcentage moyen des principaux acides gras par variété et par année.

Variétés	Acide Palmitique			Acide oléique			Acide linoléique		
Année	2005	2007	2008	2005	2007	2008	2005	2007	2008
Oueslati	12,92	12,5	-	69,25	68,98	-	14,02	15,8	-
Arbéquina	18,70	16,75	18,74	59,17	62,56	57,83	15,99	16,62	16,02
Chemlali	18,11	16,67	17,94	61,56	62,13	59,1	14,34	16,94	16,94
Chemchali	16,65	16,12	16,49	66,40	62,81	62,7	12,02	17,6	15,,97
Koroneiki	12,34	12,24	12,4	77,78	76,74	73,7	5,57	8,1	9,82
Chétoui	14,51	12,54	13,21	62,79	61,47	67,33	18,30	22,93	15,57

Enfin, cette étude préliminaire laisse supposer que la Chemchali, la Chemlali et la Chetoui s'adaptent de mieux en mieux à ce système de culture car leurs performances en 2008 sont relativement meilleures qu'en 2006 (Gheriani, 2006).L'Arbequina par contre, malgré une plus ou moins importante production en fruits, régénère un faible taux d'huile (40%) inférieur à la moyenne habituelle dans son site d'origine.

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S4-C5

Interaction between some environmental parameters and plant behavior of different varieties of olive cultivars in Tunisia

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Abstract

Phenology refers to events that are periodic in the plant-life cycle. Our biannual survey (2008-2009) covered three olive varieties: Chetoui, Chemlali, Meski of Institut de l'Olivier germoplasm collection in Tunis. Our goal was to study the plant-environment interaction in terms of accumulation of cold (CUmodified), accumulation of heat (GDD) and phenological variability. Specific optimal thresholds temperatures have been carried out as far as the starting accumulation dates for CUm and GDD in each cultivars. In 2008, Meski's requirements for cold and heat are quiet larger then Chetoui and Chemlali moreover, for all cultivars, the C and D phenological stages respectively for floral clusters formation and corolla differentiation coincide with the GDD and CUm accumulations. On the contrary during D stage (buds swelling), a significant difference has been calculated. A phenological comparison with the main olive South-Italy cultivars allowed us to evaluate behavior's differences.

Key words: *Olea europaea* L., Phenology, Chilling Unit, Growing Degree Day, Threshold Temperature.

Interaction entre certains paramètres environnementaux et le comportement de plantes d'oliviers de différents cultivars en Tunisie

Résumé

La phénologie étudie des événements périodiques du cycle de la plante. Notre étude biennale (2008-2009) a concerné trois variétés d'oliviers : Chetoui, Chemlali, Meski de la collection du germoplasme de l'Institut de l'Olivier de Tunis. Notre but était d'étudier l'interaction plante-environnement en terme d'accumulation de froid (CU modifié), d'accumulation de chaleur (GDD) et de variabilité phénologique. Les résultats étaient la détermination de températures seuils optimales et spécifiques ainsi que les dates de début d'accumulation aussi bien des Cum que des GDD. De plus en 2008, les exigences en froid et en chaleur de la Meski ce sont révèles supérieures à ceux de Chetoui et Chemlali. Enfin, les phases phénologique C et D, correspondant respectivement à la formation des grappes florales et à la différenciation de la corolle, coïncident en terme d'accumulation de Cum et de GDD au contraire, la phase D (gonflement des boutons floraux) reflète une différence significative. Une comparaison phénologique avec les principaux cultivars du Sud d'Italie à permis d'estimer les différences de comportement et d'adaptation au climat.

Mots clés : *Olea europaea* L., Phénologie, Unité de Froid, Degré Jour, Température Seuil

1. Introduction

Phenology is the discipline that studies biological phenomena that occur periodically with obvious changes in the appearance of organisms describing in details the different development phases and correlating them with the environmental variables. The succession of different stages of the plant-life cycle is regulated and influenced not only by biotic forces (biorhythms) that are regulated by the genetic constitution of the species (Orlandi et *al*., 2007), but also by abiotic forces depending on climatic conditions among which the most important is the air temperature (Orlandi et *al*., 2004), soil moisture and finally the photoperiod (Galan et *al*., 2005). A detailed knowledge of crops phenology allows both to monitor the impact of the environment on different species and to make predictions about the impact of weather events more or less favorable or climate change and consequently to define the most appropriate strategies for cultivation considering the real productive potentials.

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Nowadays, phenology has taken an increasing importance in various disciplines and vegetative species, among these the olive (*Olea europaea* L.) is one of the more studied tree crop. The olive phenology has been utilized in bioclimatic models that have carried out interesting results in measuring the extent of climate change (Galan et *al*., 2005). Generally, olive pollen is considered one of the most important factors for allergy in the Mediterranean area. Determining flowering date and its extending is very important to establish preventive measures anti-allergy. In agriculture, bioclimatic models based on phenology have proved to be valuable tools for predicting olive yield (Fornaciari et *al*., 2005; Galan et *al*., 2008). The study of quantitative relationship between crop phenology and meteorological parameters has been addressed for many species of agricultural interest to guide management decisions in a rational crop management and for the determination of the vocation of cultivation areas. One of the principal factors that influence the growth and development of organisms is the temperature that is recorded in a certain stage of development when a definite amount of "heat" is accumulated. Other researchers have been interested by the olive which is one of the main crop in the Mediterranean area with the aim to quantify the effects of both high and cold temperatures on the plant cycle considering that olive is characterized by a late flowering period compared to other species of its range of cultivation and which depends strongly on the temperature during the months preceding anthesis. By a bioclimatic point of view it is very important to estimate the olive chill requirements and assess its accumulations. Some authors have reported the importance of chilling requirements evaluation in olive underlining its crucial role in breaking dormancy in previously-initiated buds.

Recently a new flowering indicator (modified chilling units: CUm) has been proposed in order to forecast the flowering timing in olive thanks to phenological and meteorological data. Moreover, in consideration of spring heat requirements we have to consider that for the olive species the thresholds for heat calculation vary from one region to another according to the bio-geographical characteristics and according to the altitude (Galan et *al*., 2005). The aim of this study was to evaluate the plant-environment interaction in a Tunisian olive growing area in terms of consecutive accumulation of chilling and heating units in reference to the recent research findings (Orlandi et *al*., 2006). Finally, a comparison between Italian and Tunisian olive growing areas and cultivars was realized regarding the amounts of classic Growth Degree Days (GDD) and their relative calculation thresholds.

2. Materials and Methods

The phenological study was conducted during two study years (2008-2009) on three olive (*Olea europaea* L.) cultivars: two oil cultivars (Chemlali and Chetoui) and one table olive cultivar (Meski) selected in the olive germoplasm bank of the Institut de l'Olivier at Tunis (IO). These cultivars were chosen on the base of their diffusion in the Tunisian olive-growing landscape and of their economic importance. Operationally the survey was made by IO technical staff and biological and meteorological data were sent to phenology laboratory of the Department of Applied Biology (University of Perugia) for statistical processing.

In order to obtain objective results we selected four plants for each cultivar in good phytopathological and nutritional status. The phenological surveys have been conducted every 7 days beginning on the season of awakening to post-anthesis buds along the scale Maillard (1975). During full bloom the measurements were conducted every two days. The phenological stages were represented by different letters (B to G) in a phenogramme and every stage represents the average expression of a twig bearing type for each of the four plants chosen and monitored for each cultivar. The biometeorological analysis was based on the calculation of thermal amounts required by each phenological stage, moreover the meteorological variables (max, min temperatures and rainfall) were considered as 7-day averages according to the phenological frequency. In addition, the temperature amounts were drawn utilizing various mathematical formulas that refer respectively to the calculation of modified Chilling Units (CUm) and Growth Degree Day (GDD) (Orlandi et *al*. 2006; Fornaciari et *al*. 2000 a, b). The CUm were calculated by introducing different threshold temperatures beyond which a value of -1 is accumulated, so the chilling and heating effects were considered contemporary by the modified formula and they were calculated consecutively from the winter to the spring periods. Three threshold temperatures (TT) were taken into consideration (8-9-10°C). CUm = -1 for hourly temperature > TT above which negative values (CU= -1, chilling negation) were recorded and interpreted as heat amounts. The chill unit (CUm) summations were calculated from three different start dates (1st December, 1st January and 1st February) till the beginning of each phenological stage determined by the precedent phenological survey. The GDD method considered the maximum and minimum daily temperatures using five threshold temperatures (9-10-11-12-13°C). The summations of

GDD were calculated considering two start dates: from 1st January and from 1st February to the beginning of each phenological stage. Statistical analysis considered the calculation of coefficients of variation (CV) of chill and heat amounts for the assessment of lowest variability between the two study years for each phenological stages and for the three cultivars. Finally, the tunisian spring temperature amounts (GDD) calculated to full flowering dates were compared with those calculated in 16 olive growing areas in central-south Italy where different olive cultivars are present (representative areas of different Italian Provinces: Perugia, Avellino, Benevento, Salerno, Bari, Brindisi, Foggia, Taranto, Lecce, Cosenza Catanzaro, Reggio C., Messina, Palermo, Trapani and Agrigento). The comparison was realized to evaluate the differences between Mediterranean olive growing areas in terms of GDD thresholds and temperature requirements to develop reproductive structures.

3. Results

Thermopluviometric graph shows no anomalies during 2008 compared to 2009 where we noticed more relevant climatic anomalies specially during the second week of January and April, where peaks of high rainfall led to a reduction of the maximum temperatures. Comparing the phenology of the three cultivars with reference to the cumulated rainfall recorded during 2008, we noted that the olive-oil cultivars reacted positively to rainfall from the first week of March till the fourth week more than the table-olive cultivar which showed a marked delay. Later in the season, Chetoui and Chemlali tended to homogenize their behavior specially during C, D, E and F1 Maillard phenological stages. In 2009 the exceptional rainfall event during the first week of April has prompted a slowdown in the development of the cultivars. The phenological survey during this year revealed that chemlali (olive-oil cultivar) was distinguished by an advanced cycle followed by table-olive cultivar Meski finally by Chetoui. The temperature trend affected the hole phenological cycle. Definitely the analysis of maximum temperature graph revealed that 2009 was pretty cool year compared to 2008 resulting in a delay of phenological-cycle beginning of the three cultivars (third week of March) rather than by the third week of February. This fact is also confirmed by the CUm accumulation values. In fact, during 2008 the CUm accumulations reached approximately, at the B phase date, 875 units on the contrary, in 2009, CUm amounts reached 950 units in the wake of the low temperatures recorded even during March that increased the positive chilling units. Moreover, the temperatures also affect the progress of phenological stages. Indeed, during 2008 we see that the minimum temperatures at the beginning of the cycle had very low impact on the olive phenology compared to maximum temperatures which since the first week of March till the third raised the differences between olive-oil and olive-table cultivars (Fig. 1 A-B; 2 A-B). The analysis of variance (CV) realized considering the CUm amounts between the two study years evidenced the lowest variability during the pre-flowering and flowering phases (E, F, F1). In correspondence of these phases all the three cultivars showed CUm summations very similar manifesting that the olive trees in the two years accumulated, in different manners, chilling and heating amounts arriving however to similar final amounts. The variance analysis carried out with GDD amounts obtained for the three cultivars evidenced for the B and C phenological stages a temperature of 9°C as optimal threshold. Further mo re we noted that the three cultivars registered the least variability, in the two years and considering all the thresholds, during F and F1 stages (full flowering); however the maximum homogeneity in this phase was obtained with the threshold of 12°C.

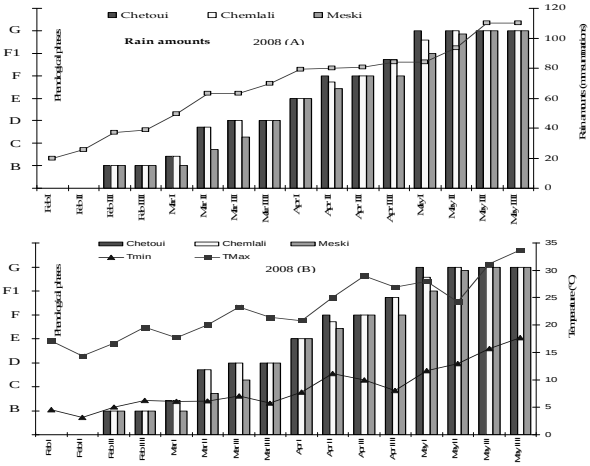

Figure 1 (A, B): Thermopluviometric graphs (2008**) .**

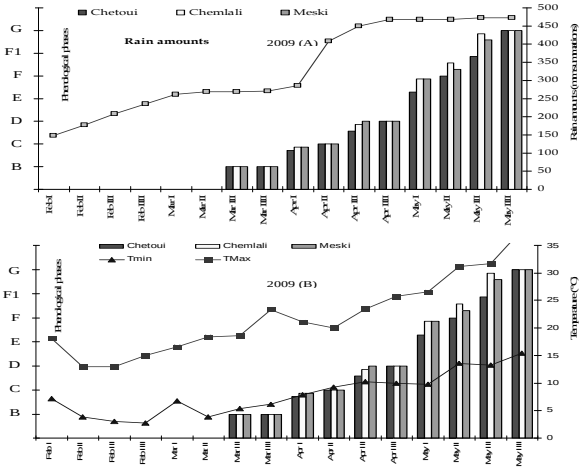

Figure 2 (A, B): Thermopluviometric graphs (2009) **.**

Meski cultivar, in particular, manifested the least variability for the accumulation of GDD for all the thresholds. The specific investigation at full flowering phase show that the three tunisian cultivars evidenced the best threshold temperature (which minimize the variance of GDD amounts) at 12℃; moreover, comparing the GDD requirements of the cultivars under study with Italian southern ones the same threshold values were recorded in Catanzaro, Palermo, Taranto, Messina provinces (Fig. 3). The average of GDD summation for each Tunisian cultivar is almost equal to that of Sicilian ones. In fact, the tunisian cultivars accumulate around 250 and 280 GDD (Fig. 4) in comparison to those of 320 and 350 GDD related to the cultivars growing at Messina, Agrigento and Trapani (the last two characterized by a threshold of 11℃).

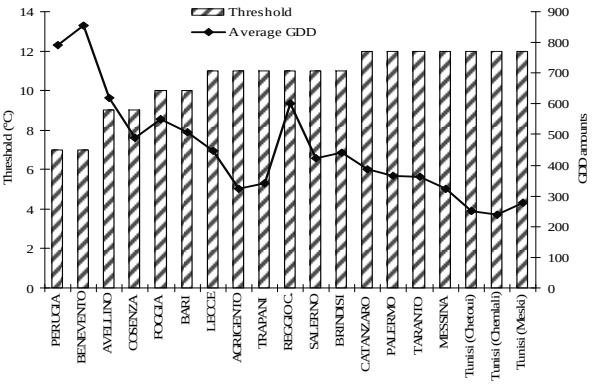

Figure 3: Optimal threshold temperatures of Tunisian and Italian cultivars.

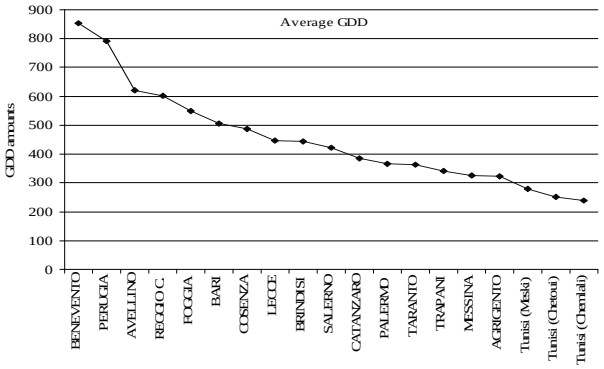

Figure 4: Average GDD amounts of Tunisian and Italian olive growing areas.

4. Discussion

The present bio-meteorological analysis permitted to evidence the principal differences of vegetative/reproductive phases between olive cultivar grown under the same climatic conditions.

Temperatures affect the hole plant cycle and can cause variation in some phenological stages in fact, maximum temperatures excursions hold up the phenological advance of olive-oil cultivars compared to table-olive one instead, the minimum temperatures excursions tend to homogenize them. Meski cultivar presents chilling requirements superior then olive-oil cultivars Chetoui and Chemlali. However Chetoui showed some sensitivity to cold in fact, its phenological cycle was delayed in 2009 compared to 2008 where the same cultivar had an advanced cycle. Meski was the least affected by the cold accumulating in any case enough GDD to continue its phenological cycle. Finally, during the two years survey, chemlali confirmed its intermediate behavior. The best threshold temperature during pre-anthesis stages for the three cultivar was 9℃. Mor eover during full blooming (F, F1), the best threshold was 12℃ in accordance with the southern Italy cultivars growing specially in Sicily.

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S4-C6

Latitude and altitude effect on flowering phenology of olive tree in two Mediterranean countries Tunisia and Spain

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Abstract

Latitude and altitude are two of the main factors influencing plant phenology Orlandi et al. (2005). The present study shows the effect of these geographical parameters on olive flowering phenology in two Mediterranean countries Tunisia (Mornag (36° 39'N 1 0°16E) and Chaal (34° 19', 10°04'E) and Spain (Cordoda (37°50'N 4º45'W) and Baena (37°37'N 4º20'W) during 2006-08. The floral phenology was studied by using the Hirst type volumetric spore trap, as an aerobiological monitoring method. The pollen emission trends showed a close relationship between phenological phases and some geographical parameters, i.e. altitude and latitude. Flowering start occurred before in Tunisia (Chaal and Mornag, 35m and 45m respectively) than in Spain (Cordoba and Baena, 118m and 420m respectively). Concerning the latitude, except 2008 there is a high correlation between the flowering start and the pick day with the latitude (R^2= 0.9).

Keywords: pollen emission, geographic parameters, phenology,*Olea europaea*

Effet de l'altitude et la latitude sur la phénologie florale de l'olivier (*Olea europaea* L.) dans deux pays méditerranéens la Tunisie et l'Espagne

Résumé

La phénologie de la plante est très influencée par les paramètres géographiques à savoir la latitude et l'altitude Orlandi et al., (2005). Ce présent travail montre l'effet de la latitude et l'altitude sur la phénologie florale de l'olivier dans deux pays méditerranéens la Tunisie (Mornag (36° 39'N 10° 16E) et Chaal (34° 19' 10° 04'E)) et l'Espagne (Cordoda (37°50'N 4º45'W) et Baena (37°37'N 4º20'W)) durant 2006-08. La phénologie florale a été déterminée en utilisant le capteur volumétrique de pollen de type Hirst installé au milieu de l'oliveraie. Les résultats obtenus ont montré que les paramètres phénologiques sont en étroite relation avec l'altitude et la latitude. En effet, le début de la floraison en Tunisie (Chaal et Mornag, avec une altitude 35m et 45m respectivement), est plus précoce par rapport à l'Espagne (Corboda et Baena à altitudes respectives de 118m et 420m respectivement). De même la période de floraison varie proportionnellement selon l'altitude. Concernant la latitude, excepté l'an 2008, une forte corrélation (R^2= 0.9) existe aussi bien entre la latitude et le début de la floraison ainsi qu'avec le pic de la floraison.

Mots clefs: Emission pollinique, paramètres géographiques, phénologie, *Olea europaea*.

1. Introduction

The Mediterranean region is the main area in the world devoted to the olive tree crop (*Olea europaea* L.), where it is one of the most important agricultural activities COI (1996). i.e. In Tunisia the olive-tree occupied 1.7 million hectares whereas in Spain this species occupied 2.3 million hectares and this surface is continually in growing. The region of Andalusia accounts for 80% of total Spanish surface.
Knowledge of olive tree phenology could greatly enhance a grower's ability to plan management practices in relation to the events occurring within the tree. Olive aerobiological data have been commonly used as a pheonological tool for predicting the beginning of the pollination period, for crop forecasting Galán et al. (2008) and even as a bio-indicator of global climatic changes, Galán et al. (2005) .

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Field phenology on plants allows to describe the flowering period taking into account different developing phases, manifesting the percentage of opened flowers with respect to the total tree canopy Maillard (1975). Likewise, several periods indicates the main phase of the olive reproductive cycle Galàn et al. (2001). In fact, it has been demonstrated by different researchers a good relationship between the olive pollen season and the main flowering phenological phases Fornaciari et al. (2000) and Galán et al. (2001). Several factors affect plant development, temperature and photoperiod exert the strongest effect on vegetation development and especially on flowering Cenci et al. (1998). The variation in temperature and photoperiod due to altitude and latitude affect the spring generative phenophases that differ geographically Wielkerson (1999). i.e. a high temperature induce an advance in flowering start commonly with a short flowering phonological phases, however a low temperature cause a delay of flowering with a large period.

Two large cultivated areas were chosen because of their importance in olive oil production, the province of Córdoba in Spain and Tunisia. Aerobiological studies carried out previously reflect that pollen curve represents pollen grains released by different cultivars and, in some cases, point out the different crop flowering contributions. For that reason, phenological investigations were carried out during three years in those areas trying to verify the behaviour of pattern diversity within this species.
The aim of this work was to study the spatial and temporal differences observed in the airborne *Olea europaea* pollen distribution in 4 sites placed at different latitude and altitude of two Mediterranean countries.

2. Material and methods

Phenological data were collected along a latitudinal gradient, from Chaal (34° 19’ 10° 04’E), with an altitude of 35 m above sea level and situated in the southern part of Tunisia up to Cordoba (37°50’N 4º45’W), with an altitude of 118 m above sea level in the middle part of Andalusia.

On the other hand, other two olive cultivated areas were considered, taking into account also the different altitude Mornag (36° 39’N 10° 16E) at 45 m above sea level in Tunisia, and Baena in Spain (37°37’N 4º20’W) at 420 m above sea level.

Cordoba and Baena climate is sub-continental, average annual rainfall is 536 mm and average annual temperature is 17.6℃. Chaal and Mornag climate is, however, sub-arid with 200 mm and 350 mm as average annual rainfall respectively, the average annual temperature is 18.5℃ in both sites.

Different olive cultivars were cultivated in the monitored areas. In the two Tunisian areas the principal cultivars are Chemlali in the south (Chaal) and Chetoui in the north (Mornag); in Andalusia the cultivars Picual, Hojiblanca and Arbequina are the most abundant cultivars in the investigated areas.

The floral phenology was determined by using the Hirst type volumetric spore trap Hirst (1952). Pollen concentration in the atmosphere was expressed as the daily average of pollen grains per cube meter of air (pollen grains/m^3). The pollen trap in the forth sites was placed average 15 meters above ground level. The study was carried out in the years 2006, 2007 and 2008. The aerobiological data were collected in every monitoring station offering us information about the main phases of the reproductive cycle of olive tree. The phenological phases were defined as follows: SF: Start of Flowering, defined as the day in which one pollen grain/m3 was reached wherever five subsequent days contained one or more pollen grains/m3 Galán et al. (2001); PP: Pollen Peak day, maximum of flowering, defined as the day of maximum pollen concentration; EF: End of Flowering, defined as the first day with 0 pollen grains and at least 3 consecutive days with 0 pollen grains).

3. Results

Variations of the annual pollen concentration, expressed by the amplitude of the curves of pollen emission for the 4 sampling stations are shown in Figure 1 which shows the trends of pollen concentration during 2006, 2007 and 2008 in 4 sites.

As regards *Olea* spatial distribution, the start of the pollination period and concentrations varied depending on the study year and different sites. *Olea* pollen is generally present in the atmosphere of Tunisia from beginning of April to middle of May lasting, on average 42 days through the country. However, in Andalusia *Olea* pollen lasting on average 72 days, from beginning of April to end of June.

The day of maximum pollen concentration varied according the year and the geographical site. Nevertheless, the maximum of pollen emission was recorded in Tunisia on average 117th Julian day pass to 131st in the province of Cordoba (Figure 1).

Figure1 also shows a variation of pollen concentration, from year to year at different sites. Maximum of pollen concentration in Baena passes from the daily average of 3000 p/m^3 in 2007 to 1400 p/m^3 in 2008 whereas in 2006 the pollen concentration does not exceed the 2000 p/m^3. In Cordoba variation of pollen concentration was registered from the daily average 630 p/m^3 in 2006 at 1400 p/m^3 in 2008.

In Tunisia a great variability of pollen concentrations between the two stations was recorded, indeed, the Chaal site presented during the three years the higher pollen concentration considering the abundance of the olive-tree in this area, whereas, the inter-annual variation is very weak. In Chaal the daily average of pollen concentration passes from 950 p/m^3 in 2006 to 1250 p/m^3 in 2008 However in Mornag the concentration passes from 200 p/m^3 in 2006 to 570 p/m^3 in 2008.

The curves of Figure 1 show a prior flowering period in Tunisian stations comparatively with those in Andalusia, this precocity is expressed by a beginning of flowering as well as pollen peak earlier in Chaal and Mornag sites comparatively with those in Baena and in Cordoba. In Chaal site the flowering start took place on average 97th Julien days, whereas in Cordoba it took place on average 115th julien days. However the pollen peak took place in Chaal on average 111th Julien days, and in Baena it took place on average 133rd Julien days that means 22 days precocity due to the difference in altitude and latitude between the two sites. The numerical explication of this difference summaries in a delay of 6.43 days each degree of latitude and one day each 17.5 meters of altitude.

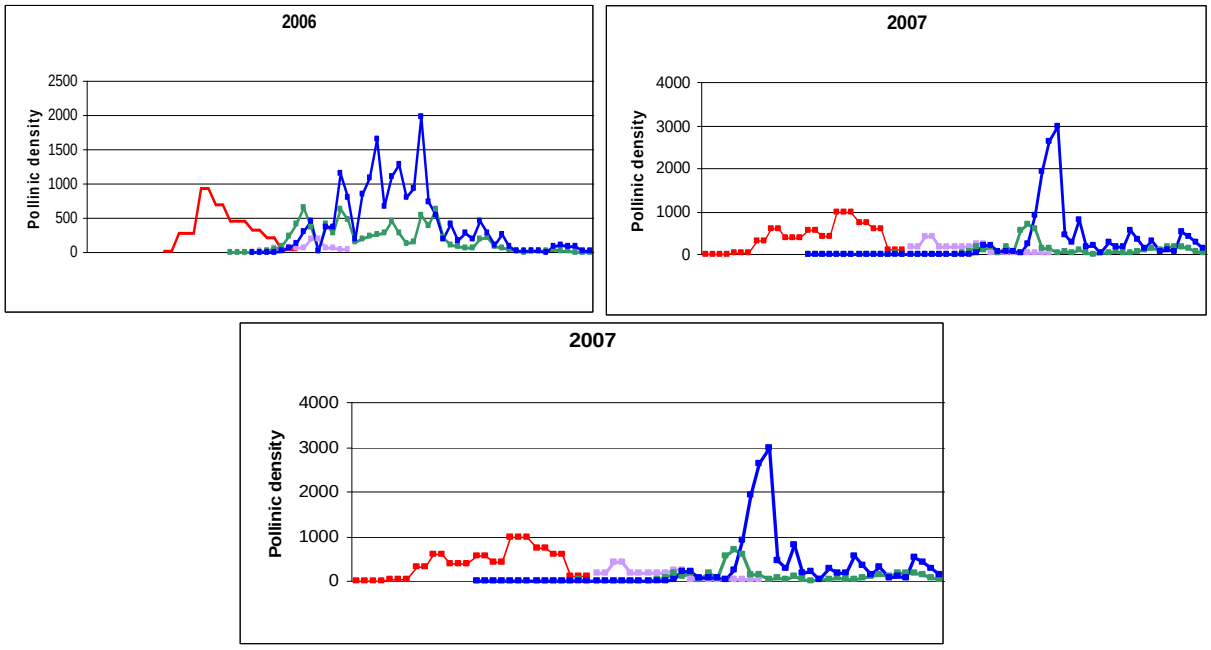

Figure 1: Comparison between the trends of pollen concentration during 2006, 2007 and 2008 in Chaal, Mornag, Baena and Cordoba.

In Tunisia the graphs show a prior flowering start in Chaal region on average 14 days comparatively with Mornag station, this precocity finds its explication in the low level in altitude of 10 meters and latitude of one degree of Chaal region comparatively of Mornag's. The combined effect of these two parameters makes that flowering in Mornag is two weeks later than in Chaal.

In Andalusia, the great difference in altitude between the two localities, Cordoba and Baena, (302 meters) made that pollen peak in Baena is earlier than in Cordoba in spite of the last site is located at higher latitudinal level.

Figure 2 represents the relationship between full flowering dates and geographic variables (altitude and latitude), explicated by linear regressions with R-Square 0.73 and 0.67 respectively,. On the other hand, good statistical correlation was found between the geographic variables and the flowering

period with R-Square 0.82 and 0.65 respectively altitude and latitude. The statistical results evidenced the high positive influence of latitude and altitude on flowering dates. Indeed a low altitude and latitude is often accompanied with advance in flowering start and a long period season. However a high altitude and latitude is accompanied with a delay in flowering start and a short period season.

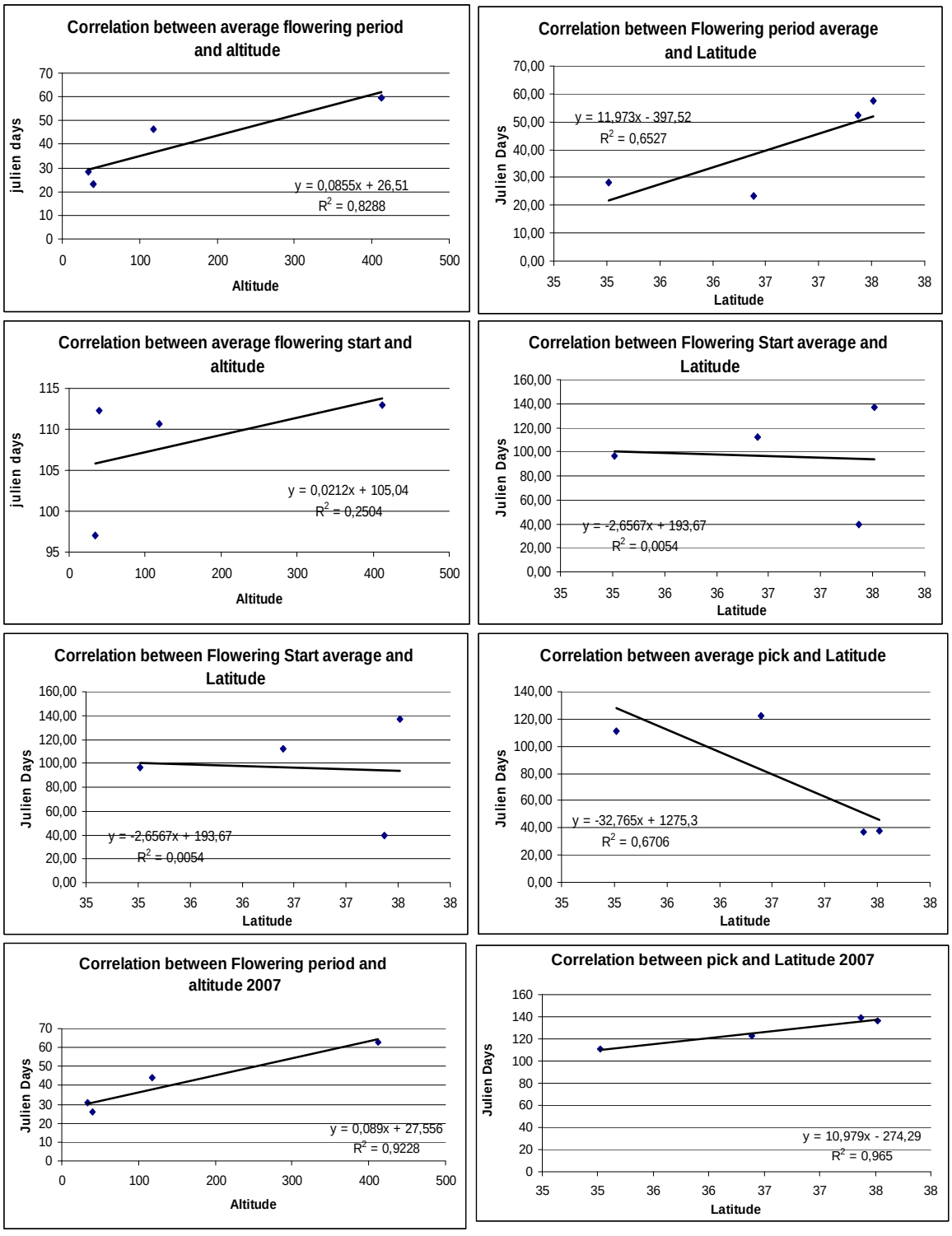

Figure 2: Linear regressions between flowering phenophases and geographical parameters of all the monitoring.

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S5-C1

Functional genomics in *Olea europaea*: identification and annotation of differentially expressed transcripts in developing olive fruit

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Abstract

Differently from other fruit tree species, a few sequences of genes and gene products are available for olive in the NCBI databases. This study deals with the identification and annotation of differentially expressed genes in developing olive fruit. Total RNA from fruit of the cv. Leccino sampled at three different developmental stages [i.e., 30 days after full bloom (DAF) (A), 90 DAF (pit hardening, B) and 130 DAF (veraison, C)] was used for the identification of differentially expressed genes putatively involved in main processes characterizing fruit growth, development and ripening. In order to isolate up- and down-regulated genes, four subtractive hybridization libraries were constructed: forward and reverse between A and B, forward and reverse between B and C. mRNA was isolated from total RNA using oligo-dT cellulose resin and forward and reverse subtractive cDNA libraries were constructed using a combination of Clontech's PCR-based subtraction kit and Rx Biosciences unique procedure. About 300 clones were isolated and sequenced for each library.
All sequences were preliminary analyzed through BlastX against non-redundant NCBI databases and about 60% of them showed similarity to known proteins. Library-specific cDNA repertories were annotated according to the three main vocabularies of the gene ontology (GO): cellular component, biological process and molecular function, respectively. BlastX analysis, GO terms mapping and annotation analysis were performed using the Blast2GO software, a research tool designed with the main purpose of enabling GO based data mining on sequence sets for which no GO annotation is yet available. Furthermore, the olive fruit-specific transcriptome dataset was used to query all known KEGG (Kyoto Encyclopaedia of Genes and Genomes) metabolic pathways for characterizing and positioning retrieved EST records within the drupe development. On the whole, our approach led to the identification of differentially expressed sequences that proved to be significantly differentiated in terms of GO category sequence amounts among the three developmental stages. Similarly, the integration of the olive sequence datasets within the MapMan platform for microarray analysis allowed us to display them into pathways along with metabolic and regulation diagrams useful for the definition of key functional categories in time course analyses for gene groups. Such characterization is a first step toward both, functional genomics and systems biology research in olive, especially for understanding gene regulatory networks and metabolic pathways in fruit growth, development and ripening.

Génomique fonctionnelle dans *Olea europaea*: identification et annotation des transcriptions, exprimées différemment, concernant le développement du fruit d'olive

Résumé

Différemment des autres espèces fruitières, seulement peu de séquences géniques de l'olivier sont disponibles dans le *database* NCBI. Cette étude s'occupe de l'identification et annotation des gènes différentiellement exprimés dans le développement du fruit de l'olivier. Les fruits de la cultivar Leccino ont été cueillis en trois différent stades de développement (i.e., 30, 90 et 130 jours après la floraison) et l'ARN total des fruits a été utilisé pour l'identification des putatifs gènes impliqués dans la pousse, le développement et la maturation du fruit. Quatre bibliothèques SSH (*Suppression Subtractive Hybridization*) ont été construites et 300 clones environ ont été isolés de chaque bibliothèque. Les séquences ont été annotées avec les programmes informatiques BlastX et Blast2GO. En suite les séquences ont été analysées en utilisant le KEGG (*Kyoto Encyclopaedia of Genes and Genomes*) pour identifier les voies métaboliques dans lesquelles se place le transcriptome du fruit de l'olivier étudié dans ce travail.

Mot-clés: Bibliothèque SSH, expression génétique, qualité de l'huile.

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1. Introduction

Olive (*Olea europaea* L.) is an evergreen species, widely cultivated in the Mediterranean Basin and its oil is a predominant component of the worldwide known 'Mediterranean diet'. Health benefits and cancer-protective properties have been demonstrated for of olive oil (Pérez-Jiménez et al. 2007) and these beneficial attributes are closely related to composition and concentration in olive oil biological active molecules resulting from catabolic and anabolic processes during olive fruit development. The oil content of olives can reach up to 30% on a fresh weight basis at full ripening (Sánchez 1994) and accumulates mainly in the mesocarp. Oil accumulation in the pulp increases slowly, reaching the plateau after veraison and a marked tryacylglycerol accumulation in seed and pulp occurs after endocarp lignification. Olive oil fatty acid profile is important in relation to its nutritional properties and it varies during maturation and ripening, according to cultivars and environmental conditions (Zarrouk et al. 1996; Ayton et al. 2001). Other metabolites affecting olive oil quality and its technological and nutritional properties accumulate throughout olive fruit development and include polyphenols, carotenoids, chlorophylls, sterols and terpenoids.

The making of a fruit is a genetically programmed developmental process and is largely influenced by environmental factors. To identify and characterize these genes, different genomic approaches (ESTs, large-scale microarrays, deep transcriptome profiling, etc.) have been used (Seymour et al. 2008) and the information concerning transcriptional networks and regulatory circuits involved in fruit physiological and developmental processes have increased tremendously. However, the information concerning genetic regulation of fruit metabolic processes in olive is still limited. Among different strategies available for identifying differentially expressed genes, suppression subtractive hybridization (SSH) libraries have been successfully used in fruit science. This article deals with the identification via SSH of large repertories of differentially expressed genes in developing olive fruits, and their computational annotation by means of different bioinformatic software. The identification and characterization of gene regulatory networks and key metabolic pathways during fruit growth and development represent a relevant step for improving olive oil quality and its health-related properties.

2. Material and Methods

Olive (*Olea europaea* L., cv Leccino) fruits growth was determined by monitoring fresh weight accumulation and samples were collected at 30 days after flowering (DAF) (initial fruit set, stage 1), 90 DAF (completed pit hardening, stage 2) and 130 DAF (veraison, stage 3). Total RNA was extracted from pericarp of about 16 fruits for each sampling date, using the RNeasy Plant Mini Kit (Qiagen). The RNA was quantified by both, spectrophotometer and gel electrophoresis on a denaturing agarose gel and mRNA was isolated from about 400 µg of total RNA for each sample using oligo dT cellulose resin.

The first strand cDNA was synthesized with reverse transcriptase using oligo dT primer, modified for a tail containing RsaI restriction site. The second strand was synthesized by incubating the first strand cDNA with DNA polymerase I, RNAse H and DNA ligase at 16℃ for 2.5 hrs. Subtracted and reverse subtracted cDNA libraries were constructed using a combination of a PCR-based subtraction kit (Clontech) and a unique subtraction procedure (Rx Biosciences). Four subtracted cDNA libraries have been constructed to compare samples collected at 30 and 90 DAF (libraries A and B) and samples at 90 and 130 DAF (libraries C and D). For the two forward libraries A and C, cDNA from samples collected at 30 and 90 DAF, respectively, were used as tester and cDNA from 90 and 130 DAF, respectively, were used as drivers, vice versa for the reverse subtractive libraries B and D.

The DNA sequencing was performed using ABI3700 automatic DNA sequencers using 25-50 ng of DNA template according to manufactures protocol. Nucleotide sequences retrieved by SSH were screened for vector contamination by using a home made BioPearl script. Once cleaned by vector residuals, all sequences were used for contig assembly by using a web interface of the CAP3 software (CAP3, http://deepc2.psi.iastate.edu/aat/cap/cap.html) and redundancy within and among libraries were calculated as ratio of sequences belonging to a contig out of the total sequences considered. Computational annotation of the four olive EST datasets was performed using the Blast2GO software v1.3.3 (http://www.blast2go.org). Annotation distribution among originated libraries was represented by Venn diagrams by computing all retrieved annotation with the VennMaster software. Enzyme mapping of annotated sequences was done by direct GO to Enzyme annotation and used to query the Kyoto Encyclopaedia of Genes and Genomes (KEGG - http://www.genome.jp/kegg) to define the main

metabolic pathways involved. MapMan (http://gabi.rzpd.de/projects/MapMan/) analysis was done using the olive dataset properly rearranged as input files. Quantitative Real-Time PCR experiments were carried out to validate some of the genes isolated by SSH and characterized by GO. Among the whole dataset of non-redundant sequences, 89 gene sequences belonging to key biosynthetic and metabolic pathways were selected.

3. Results

The preparation of cDNA libraries using SSH represents an efficient strategy to isolate genes with an antagonist expression pattern and enable to identify transcripts of genes differentially expressed among the three developmental stages of olive fruit studied. The number of differentially expressed sequences randomly chosen varied from a minimum of 236 to a maximum of 317 per library, with a total number of clones equal to 1,132 (Table 1).

Table 1: Library characteristics - clone singlets and contigs abundance and mean size.

Library	Clones		Singlets		Contigs	
	No.	Mean (bp)	No.	Mean (bp)	No.	Mean (bp)
A	293	522	139	464	40	767
B	317	637	132	583	35	963
C	286	694	114	639	43	835
D	236	517	127	506	64	558

The average length of the cDNA clones was 597 bp, ranging from 48 up to 1,283 bp. Within each single library the redundancy was relatively low, ranging between 1.7% and 5.3%. Querying with cDNA sequences the non-redundant NCBI databases allowed the attribution of a BLAST hit and around 75% of the BLAST hits of the olive fruit cDNA sequences were homologous to coding sequences present in the rice, *Arabidopsis* and grapevine genomes, with more than 1,000 hits per species (Table 2).

Table 2: Number of BLAST hits retrieved from NCBI databases and their distribution among different plant species and different organisms.

Organism Name	BLAST hits
Oryza sativa	1471
Arabidopsis thaliana	1142
Vitis vinifera	1083
Popolus trichocarpa	146
Nicotiana tabacum	108
Glycine max	83
Solanum tuberosum	77
Mus musculus	74
Zea mays	68
Picea **sitchensis**	67
Homo sapiens	53
Medicago truncatula	51
Olea europaea	47
Gossypium hirsutum	47
Triticum aestivum	44
Shigella flexneri	43
Lycopersicum esculentum	39

The computational analysis of the whole EST collection using the software Blast2GO allowed the annotation of the expressed sequences according to the terms of the three main Gene Ontology (GO) vocabularies, i.e. cellular compartment, molecular function and biological process. For cellular compartments, the most represented are plastids and mitochondria, with more than 50% of the total annotations, followed by cytosol, plasma membrane, endoplasmic reticulum and nucleoplasm, whereas other cellular compartments were represented at a much lower scale. Regarding the molecular function, the most represented categories were those of nucleotide binding proteins,

followed by proteins with transport, kinase and enzymatic activities. The other molecular functions were represented at a lower extent and concerning the biological process vocabulary more than 30 categories were found: carbohydrate metabolism, response to biotic and environmental stresses, generation of precursors, metabolites and energy, and catabolic processes, secondary metabolites, metabolism of lipids, synthesis of amino acids and derivatives, metabolites and their precursors, and protein modification process.

A number of olive fruit stage-specific GO terms were identified and among the 296 and 464 GO terms found in the A and B libraries, 75 and 101 were associated to down- and up-regulated genes, respectively. The most significant GO terms were encoding elements of hormone biosynthesis and signal transduction mediated by ethylene, jasmonic acid, salicylic acid, and abscisic acid, as well as biosynthesis of secondary metabolites, such as terpenoids. In the C and D libraries, a total of 375 and 549 GO terms were recovered, 78 and 183 of which were related to down and up-regulated genes, respectively. Among these, there are GO terms associated to environmental stress responses, catabolism of secondary metabolites, and auxin signal transduction. Quantitative Real-Time PCR experiments were carried out to corroborate the expression patterns of a subset of sequences and validated the results from SSH experiments for 42 out of 61 genes. The Kyoto Encyclopaedia of Genes and Genomes (KEGG) was queried for sequences encoding enzymes and the deduced gene products were associated to specific metabolic and/or biosynthetic pathways related to carbohydrates, fatty acid and secondary metabolism. As expected, several genes encoding enzymes related to carbohydrate and fatty acid compounds were transcriptionally up- or down-regulated during olive fruit development. The integration of the olive sequence datasets within the MapMan platform for microarray analysis allowed the identification of specific biosynthetic pathways useful for the definition of key functional categories in time course analyses for gene groups. Extensive data analysis can be found in Galla et al., (2009).

In conclusion the generation of the SSH library throughout olive drupe development and the bioinformatic annotation of all gene sequences recovered was useful to highlight the metabolic pathways and transcriptional aspects related to carbohydrates, fatty acids, secondary metabolites, transcription factors and hormones as well as response to biotic and abiotic stresses. These results are a first step toward functional genomics and systems biology research aimed to understanding the gene functions and regulatory networks in olive fruit growth and ripening.

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S5-C2

Transcriptomic analysis and computational annotation of genes involved in olive flower development

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Abstract

Little is known on the physiological and molecular basis of olive ovule abortion and flower abscission, both influencing plant yield. Recent findings suggest that both processes might be under genetic control and are influenced by both nutritional and environmental factors. Two different approaches have been carried out to unravel the molecular networks. The first deals with the identification of large sets of differentially expressed genes trough subtractive hybridization libraries (SSH), in order to isolate up- and down-regulated genes, between two selected flower developmental stages in the cv Leccino. SSH approach originated a total of 1,127 clones which were analysed and computationally annotated. The second deals with expression studies of genes modulated during olive flowering on two cultivar: Dolce Agogia (abundant flowering and low fruit set) and Leccino (fruit set capacity).

Key words: *Olea europaea;* flower development; SSH; gene expression, Gene Ontology.

Analyse transcriptomique et annotation microinformatique des genes integers dans le développement de la fleur de l'olivier

Résumé

Les connaissances des bases physiologiques et moléculaires de l'avortement de l'ovule et de l'abscission florale chez l'olivier ne sont pas abondantes, et toutes ces deux caractéristiques influencent le rendement. Deux approches différentes ont été effectuées pour dénouer le réseau moléculaire responsable de leur contrôle génétique. La première traite l'identification d'un vaste ensemble de gènes exprimés diversement, à travers une librairie de l'hybridation soustractive (SSH) pour pouvoir isoler des gènes régularisés positivement et négativement entre deux phases développementales de floraison du cultivar Leccino. L'approche SSH a résulté en un total de 1127 clones qui ont été analysés et annotées bio-informatiquement. La deuxième approche traite des études de l'expression de gènes modulés pendant la floraison de l'olivier, en deux cultivars : Dolce Agogia (floraison abondante et nouaison basse) et Leccino (capacité élevée de nouaison).

1. Introduction

In olive inflorescence hermaphrodite flowers are paired to male flowers in which androecium is well conformed and ginoecium is not completely differentiated or necrotic. Fruit set is correlated to the percent of perfect flowers. Ovule abortion and flower abscission are influenced by genetic background, since different cultivars behave in a different way, and by nutritional and environmental conditions. Studying the flower development, it was found that Dolce Agogia had the highest percentage of aborted ovaries and flowers, while Leccino had the lowest percentage of aborted ovaries and flowers (Reale et al 2006). Cyto-histological studies show that pistil development in staminate flowers is interrupted after the differentiation of the megaspore mother cell and microspore resulted enveloped by a callose wall (Reale et al 2009), suggesting that, also in olive, callose has an important role in the programmed cell death (PCD) of the non functional microspores (Angenent et al 1996). At the same stage of development, starch grains were only detected in the ovary, style and stigma of hermaphrodite flowers. No starch was observed in the pistils of the staminate flowers. It is known that carbohydrate supply is of fundamental importance for development of floral organs (Yu et al 2000; Clément et al 1996; Rodrigo et al 2000) and fruit setting (Iglesias et al 2003). In grapevine, it has been shown that a nutrient deficiency in the inflorescences could be a cause of flower abscission (Lebon et al 2004) and carbon metabolism is thought to be involved in flower necrosis (Gu et al 1996).

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Little knowledges are available in the literature on the genetic and molecular aspects involved in the abortion of ginoecium; therefore the main objective of this research is the identification of a large sets of differentially expressed genes during the development of the flower and to study their expression in the diverse step of olive flowering in olive.

2. Materials and Methods

2.1. Plant Materials and Growth Condition

Field observations and experiments were carried out in an olive grove of the "Istituto Sperimentale di Olivicoltura" in Spoleto (42°49'N, 12°40'W) at 300 m a.s.l. During inflorescences development of cultivars Leccino and Dolce Agogia, flowers were collected at different stages of development from three weeks before anthesis to full anthesis.

2.2. Nucleic acid extraction, cDNA synthesis and library construction

Total RNA was extracted from flowers with a NucleSpin RNA Plant Kit (Macherey Nagel). Reverse transcriptions (RT) were carried out using Ready-To-Go RT-PCR beads (Amersham Biosciences). cDNA aliquots equivalent to 80 ng of total RNA were used in each RT-PCR reaction. An amount of 500 µg of total RNA from Leccino samples were further purified with the RNAeasy Plus Mini Kit (Qiagen) and prepared for the construction of subtractive (SSH) libraries. Libraries were prepared at Rx Biosciences (Rockville, MD, USA) using a proprietary technology. Two cDNA libraries were constructed to compare Leccino samples collected the 22th and 29th of May: a forward library, where cDNA of 22th of May was subtracted with 29th, and a reverse library. Libraries were analysed by sequencing 1,127 clones at Rx Biosciences using an ABI3700 automatic DNA sequencer and a first bioinformatic evaluation was performed with a CLC Combined Workbench 3 software. Nucleotide sequences retrieved by SSH were screened for vector contamination by using a BioPerl script, and used for contig assembly by using the CAP3 software (http://deepc2.psi.iastate.edu/aat/cap/cap.htlm/).

Computational annotation of the two EST dataset was performed using the Blast2GO software v.2.3.6 (http://www.blast2go.org) using the b2g_jun09 database. Enzyme mapping of annotated sequences was done by direct GO to Enzyme annotation and used to query the Kyoto Encyclopaedia of Genes and Genomes (KEGG – http://www.genome.jp/kegg/) to define the main metabolic pathways involved.

2.3. Novel gene isolation and expression analysis

A PCR strategy was applied to isolate partial nucleotide sequences corresponding to sucrose synthase, beta-glucosidase, beta-mannosidase, mitochondrial ATPase, ribulose-biphosphate carboxylase, long chain fatty acid CoA ligase, ubiquitin protein ligase, mitochondrial elongation factor Ts_{mt}. Actin was used as housekeeping gene to provide a reference for the global transcription level. For quantitative gene expression analysis (qRT-PCR) flower samples were collected 14 days before (16th, 22th, 26th, 29th May) and 4 days after anthesis (4th June), frozen in liquid nitrogen and stored at -80° C until analysis. Total RNA was extracted as previously described. The qRT-PCR tests were carried out on three biological replicates using the LightCycler platform (Roche Molecular Diagnostics). Amplification and detection were performed using the SensiMix Lite kit (Quantace). The 20 µl reaction consisted of 2 µl of cDNA, 80 ng RNA, and 18 µl of the master mix prepared using 4 µl of 5x SensiMix Lite, 3.5 mM final concentration of $MgCl_2$, 0.4 µl of 50x SYBR Green solution, 1.5 µl of Enzyme Mix, 0.5 µM final concentration of the forward and reverse primers (Table 1). The cycling conditions consisted of 10 min denaturation at 95° C followed by 35 cycles of 95° C for 15 s, 62° C for 20 s, 72° C for 20 s. For the quantification of the target gene, we used a DNA standard obtained by PCR of the genes of interest as previously described and determining the concentration by UV spectrophotometry. An external absolute standard curve was constructed with a reference standard in the range 10^7-10^3 copies/reaction. The amount of the target transcripts fell within the range tested, allowing the quantification of the target sample. In addition, parallel assays were run for the housekeeping gene encoding actin.

Table 1: Description of primers described in this study.

Gene	Forward primer	Reverse primer
Actin	5'-TCCTGAGGTTCTTTACCAGCCTTC-3'	5'-CTAGCGCTGTAATTTCCTTGCTCA-3'
Sucrose synthase	5'-CACAGATGAACCGTGTGAGAAATG-3'	5'-GCCTCAACAACAGTCAAACCAAAG-3'
beta-glucosidase	5'-GGTGAATGACTTCCAAAAGGGTTC-3'	5'-AGACATTGTTGTTGCCATGAAGTG-3'
beta-mannosidase	5'-GGGAAACCAATCATGTTGACTGAG-3'	5'-CCGCTTCTTGCACAACTGTAGATT-3'
Fatty-acid-CoA ligase	5'-ATCAGTGCCTGAGATGGGATATGA-3'	5'-CCATCCATCCACAACTACACCTTC-3'
RuBisCO	5'-GCATTCCGAGTAACTCCTCAACCT-3'	5'-TAACGATCAAGGCTGGTAAGTCCA-3'
ATPase	5'-AGAACTTATAATCGGGGACCGACA-3'	5'-ACAGTTGAGCGTTTCTGTCCAATC-3'
Ubiquitin-protin ligase	5'-AAGCTCTTTCCTGTCTCCTCCAGA-3'	5'-CACCCCAAGAAGCATTAGGGATAG-3'
Elongation factor Ts_{mt}	5'-GATGCCTCCTGTGAAGAATGAAGA-3'	5'-AAAGATATTCGGCCAGTCTCCGTA-3'

3. Results and discussion

A total of 1,127 clones were sequenced and, among them, 232 ESTs were selected for further analysis: 148 sequences were singletons while 84 were contigs generated assembling contiguous and overlapping clones. The average length of the sequences analysed was 842 bp with a range of variation from 20 to 2952 bp. BlastX analysis, GO terms mapping and annotation analysis were performed using the Blast2GO software. No BLAST hits for olive could be recorded while cDNA sequences showed similarity with coding sequences present in the genome of the species *Vitis, Populus, Oryza, Arabidopsis* and *Ricinus*, indicating that novel genes have been isolated. Library-specific cDNA repertories were analysed according to the three main vocabularies of gene ontology: cellular component, biological process and molecular function. About 70% of the sequences were annotated: the forward library enclosed 14 non redundant ESTs that account for the 90% of the analysed sequences, indicating that these ESTs could be strongly regulated, while the redundancy in the reverse library was much lower. Concerning the biological processes and molecular function (Table 2), the most represented categories were connected with carbohydrate and starch metabolism, energy metabolism, vescicle-mediated transport and transmembrane transport, genetic information processing and transcription factor.

Table 2: Gene Ontology terms distribution of the differentially expressed ESTs in the vocabularies of biological process and molecular function.

Biological process	*Molecular function*
Fructose metabolic process (1)	Endonuclease activity (1)
Starch metabolic process (6)	Sequence-specific DNA binding (1)
Sucrose metabolic process (6)	Hydrogen ion transporting ATPase activity, rotational
Substituted mannan metabolic process (1)	mechanism (1)
Regulation of transcription DNA-dependent (1)	Aminoacyl-tRNA ligase activity (3)
Nucleoside metabolic process (1)	RNA binding (8)
Protein ubiquitination (5)	Protein dimerization activity (1)
ATP synthesis coupled proton transport (1)	ATP binding (21)
Lateral root development (1)	Glucan 1,3-beta-glucosidase activity (2)
Fatty acid metabolic process (1)	Oxidoreductase activity (12)
Response to cadmium ion (5)	Serine-type carboxypeptidase activity (1)
Ubiquitin-dependent catabolic process (2)	Ubiquitin-protein ligase activity (5)
Mannose metabolic process (1)	Identical protein binding (4)
Response to hormone stimulus (1)	Transcription factor activity (1)
Response to nematode (1)	Zinc ion binding (15)
tRNA aminoacylation for protein translation (1)	Subtilase activity (1)
Protein folding (3)	Mannan endo-1,4-beta-mannosidase activity (1)
Oxidative phosphorylation (4)	Long-chain-fatty-acid-CoA ligase activity (1)
Glucose metabolic process (4)	Cysteine-type endopeptidase activity (2)
Cellular carbohydrate biosynthetic process (4)	Xylan 1,4-beta-xylosidase activity (1)
RNA metabolic process (3)	Hydrolase activity, hydrolizing O-glycosyl compounds (6)
Protein aminoacyl phosphorylation (7)	Cation-transporting ATPase activity (3)
Oxidation reduction (12)	Obsolete_molecular_function (4)
Nucleobase, nucleoside and nucleotide metabolic	Nucleotidyltransferase activity (3)
process (4)	Signal transducer activity (3)
Response to stress (5)	Carboxy-lyase activity (3)
Translation (3)	Protein serine/threonine kinase activity (7)
Signal transduction (5)	Inorganic cation transmembrane transporter activity (3)
Pyruvate metabolic process (3)	Iron ion binding (5)
Cellular aromatic compound metabolic process (3)	Structural molecule activity (4)
Ubiquitin cycle (4)	Electron carrier activity (6)
Vesicle-mediated transport (4)	Calcium ion binding (5)
Intracellular protein transport (3)	DNA binding (6)
Proteolysis (6)	Coenzyme binding (5)
Regulation of catalytic activity (3)	Transcription regulator activity (3)
Response to biotic stimulus (3)	Aspartic-type endopeptidase activity (4)
Aspartate family amino acid metabolic process (3)	UDP-glucosyltransferase activity (3)
Proton transport (5)	Magnesium ion binding (4)
Photosynthesis (3)	Methyltransferase activity (3)
DNA metabolic process (7)	Tetrapyrrole binding (3)
Organelle organization (4)	Heterocycle metabolic process (3)
Regulation of transcription (3)	Electron transport (7)
Serine family amino acid metabolic process (8)	Carbon utilization (3)
Cellular macromolecule catabolic process (4)	

Since it is known that the nutritional theory and assimilates partitioning has a great importance in flower development and ovary abortion, our attention was firstly focussed on the transcriptional profile of sucrose synthase (Fig. 1a). Transcript levels of this gene were high at the beginning of flower development and then decrease at anthesis. Sucrose plays a pivotal role in plant metabolism as signal molecule in assimilate partitioning. In olive, starch content plays a role in the pistil development and it has been observed that starch grains were only present in the ovary, style and stigma of hermaphrodite flowers but not in staminate flowers (Reale et al. 2009). These observation suggest that starch storage tended to be negatively correlated with sucrose synthesis by sucrose synthase. The presence of lower transcript levels for sucrose synthase in Leccino than in Dolce Agogia could be associated with an higher starch content in hermaphrodite flowers. Other genes involved in carbohydrate metabolism which seems to be regulated in flower development were some glucosidases (Fig. 1c, d). Transcript levels for beta-glucosidase declined during development until anthesis, while beta-mannosidase levels increased with an opposite behaviour. These enzymes catalyse the hydrolysis of polysaccharides releasing simple sugars which could be used in the energetic metabolism or in the synthesis of storage materials. The presence of these hydrolases suggests a very quick turnover of cell wall polysaccharides which could be associated with actively growing tissues as developing flowers. The presence of soluble sugars could give a contribution to the flower bud expansion, decreasing of the cellular osmotic potential with the increase of osmotic pressure.

A different transcriptional profile of the long-chain-fatty-acid-CoA ligase in both cultivars was observed (Fig. 1b). In Dolce Agogia the transcript levels increased during time and then decreased quickly at anthesis, while in Leccino they were more stable. This enzyme is involved in a key step of the fatty acid degradation that has, as final product, high amounts of acetyl-CoA. This central intermediate could enter the tricarboxylic acid cycle, generating energy, or could be used in biosynthetic pathways.

Our results showed that while in Dolce Agogia the expression levels of this ligase decreased rapidly, in Leccino, where the proportion of perfect flowers was higher, they were higher and constant during flower development. Moreover it was analysed the transcriptional profile of RuBisCO-large subunit (Fig. 1e) and it has been observed that this gene was down regulated during flower development. The comparison between the cultivars showed that transcript levels were higher in Dolce Agogia in the first phase of development and in Leccino at anthesis. Concerning the energetic metabolism, the mitochondrial ATPase gene (Fig. 1f) was expressed at high levels in both cultivars. Histological observation with *in situ* hybridization had emphasized that this gene is expressed in all the structures of hermaphrodite flowers, mainly in the ovary wall, ovules and embryo sac, and it is absent in staminate flowers (Sgromo et al. 2009). The ubiquitin-protein ligase transcripts gene were detected in both the cultivars analysed but were higher in Dolce Agogia (Fig. 1g). This observation indicated that post-transcriptional regulation could have a role in the control of flower development by activating or repressing numerous genes. Similar observations were reported during the identification of flowering-related genes in *Poncirus trifoliata* (Zhang et al 2009) and it was suggested that the ubiquitin/26S proteasome pathway, which comprise also the activity of the ubiquitin-protein ligase, could play a critical role in various aspect of plant growth and development with a mechanism similar to *Arabidopsis thaliana*. Post-transcriptional regulatory process allow the cells to respond rapidly to intracellular signals and change environmental conditions by adjusting the levels of key proteins.

Finally a gene with high similarity to a mitochondrial translation elongation factor (EF-Ts_{mt}) from tomato was analysed, but its role was not clear (Fig. 1h). Transcript levels for EF-Ts_{mt} decreased during flower buds development in Leccino, and were present at high levels only in the first sample of Dolce Agogia. In tomato, a functional characterization demonstrated that EF-Ts_{m} encodes a functional elongation factor which was induced during fruit ripening.

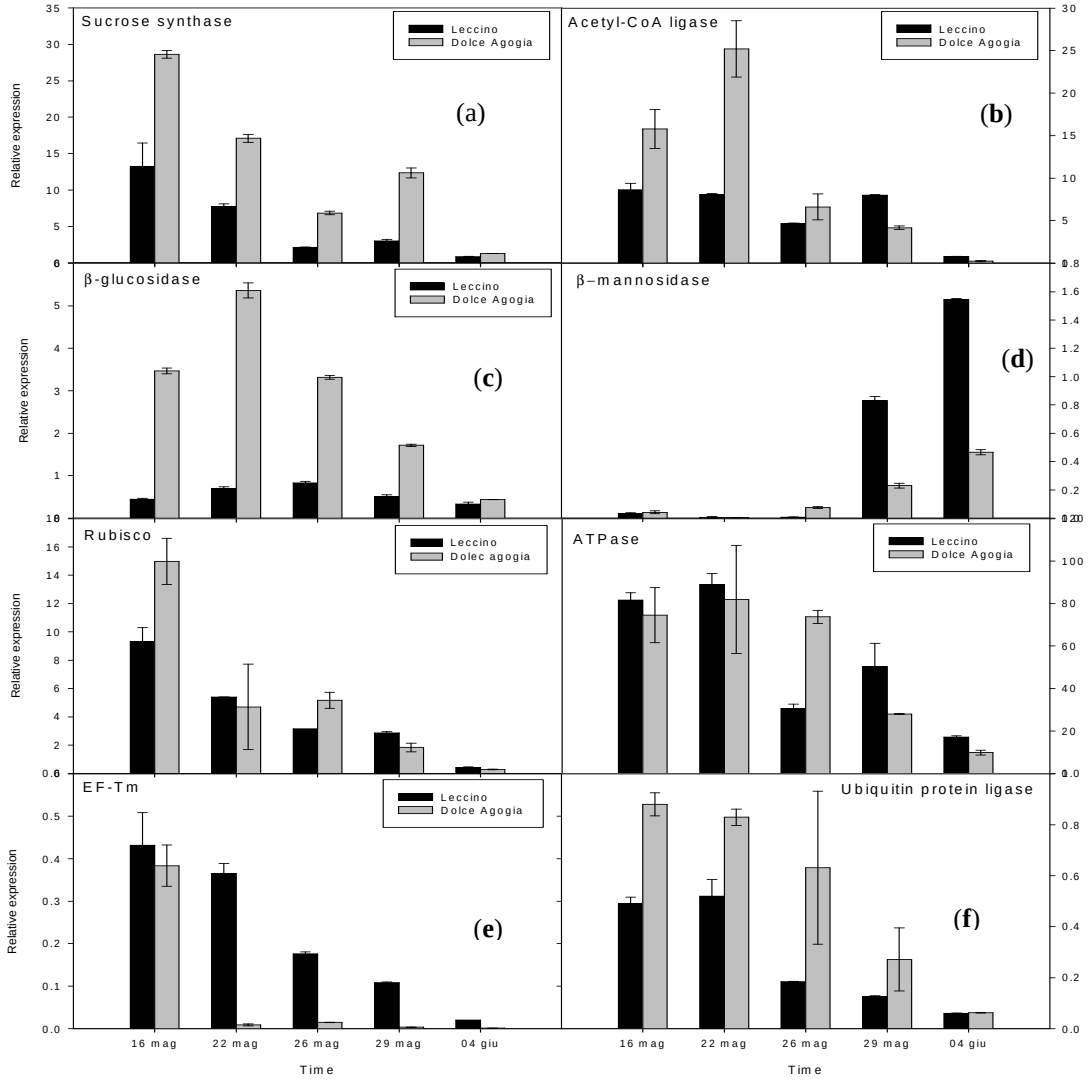

Figure 1: Relative quantities of sucrose synthase, fatty-acid-CoA ligase, beta-glucosidase, beta-mannosidase, mitochondrial ATPase, RuBisCO, mitochondrial elongation factor Ts and ubiquitin-protein ligase in cultivars Leccino (black bars) and Dolce Agogia (grey bars). Data points represent the mean ± SE of at least three replication for the relative expression, which were calibrated by the amount of expression of the actin control.

4. Conclusions

232 ESTs differentially expressed during flower development of Leccino using an SSH method have been isolated. We identified and analysed the time-course transcriptional profile of genes involved in carbohydrate and lipid metabolism, C-fixation in plant, energetic metabolism, genetic information and cellular processing. The transcript levels of most of the analysed genes decrease during flower development. This observation could indicate that before anthesis some metabolic processes slow down, probably waiting for fertilization and the subsequent developmental stage. During flowering transcript levels were differentially modulated in the cv Leccino and Dolce Agogia, providing new insight in understanding gene expression in hermaphrodite and staminate flowers. Taken together, the data we suggest the hypothesis that carbon deprivation and modification of carbohydrate pathways, induces abortion of ginoecium through the programmed cell dead pathway.

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S5-C4

Les huiles d'olive tunisiennes : Caractérisation chimique et sensorielle et valorisation en huiles mono-variétales et labels de qualité

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Résumé

Actuellement, la Tunisie s'est engagée à mettre en place des stratégies de développement axées sur l'amélioration de la productivité et de la qualité et de valoriser les spécificités des zones de production en vue de maintenir les marchés traditionnels et conquérir de nouveaux marchés.
De notre côté, nous avons oeuvré pour valoriser les potentialités oléicoles des ressources génétiques de notre pays par l'élaboration des huiles d'olive tunisiennes monovariétales et la contribution à la création du label « huile d'olive de Tunisie » et des premiers signes de qualité en Huile d'olive.

En effet, dans le cadre d'une convention de recherche-développement, nous avons élaboré dans le respect total des bonnes pratiques de fabrication et d'hygiène, cinq huiles monovariétales appartenant à 4 variétés : *Chétoui Medjez el Bab, Chemlali Sfax- Messouda, Chemlali Maknessy, Chemchali Gafsa et Oueslati El Alaa.* Les résultats montrent que les cinq huiles présentent des caractéristiques physicochimiques, un profil aromatique et des sensations agréables typiques propres à chaque variété et origine. Les tests de préférence réalisés auprès de 300 consommateurs montrent que la majorité des personnes enquêtées ont aimé le fruité de la Chétoui et malgré l'amertume et le piquant inhabituels à ces personnes, l'huile de cette variété a été très appréciée.

Le premier signe de qualité '« IP Huile d'olive Chemlali Monastir » dérive à la fois d'un terroir particulier et d'une variété autochtone bien adaptée depuis des siècles : la Chemlali de Monastir. C'est une huile vierge extra riche en tocophérols (760-1030 ppm) et en stérols (1630 - 2170 ppm). Cette huile est caractérisée par des odeurs de fleur de champs et d'amande verte. Les intensités de l'amertume et du piquant sont légères alors que celle du fruité est moyenne.

Mots clés: Huile d'olive, monovariétale, label, typicité.

Tunisian olive oils: Chemical and sensorial characterization and valorization at monovarietal olive oils and signs of quality

Abstract

Despite the socio-economic importance of the Tunisian olive oil sector and the wide diversity of its genetic resources, Tunisian oils are not highly valued within the national and international markets.

In order to prove the excellent quality and diversity of Tunisian Olive Oil, we have produced, in the frame of the convention between the Technology and quality research unit at the Institut de l'Olivier and the private society's, the monovarietal olive oils: *Chetoui Medjez El Bab, Chemlali Sfax-Messouda, Chemlali Maknessy and Oueslati El Alaa.* These oils were strictly created according to rules of good practices of production and hygiene. Each stage of production was fully and carefully noted in a card to ensure the tractability of the product.

These monovarietal extra-virgin olive oils show physico-chemical characteristics, a flavor profile and pleasant aromas typical to each variety and origin.

The distinctive organoleptic sensations and the variation of taste and aroma of each variety were easily detected and appreciated by the Tunisian and foreign consumers. Each olive oil is distinguished by an origin, a variety and a specific know-how. The investigation consumers in the opinion of 300 persons show that the major part has appreciated the fruity of the Chetoui monovarietal olive oil despite its bitterness and pungency flavor taste.

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In conclusion, these olive oils quality should be valued as a designed origin protected or protected indication and to represent their regions of origin.

Furthermore, in the frame of the convention between the “Regional Commissariat of Resources and Agriculture Developpement” of Monastir and “Instititut de l’olivier, we have cooperated to create the first quality sign of olive oil in Tunisia. Currently, the application is under consideration by the Ministry of Agriculture.

During this research, we have first started by identifying the variety of olive trees within the zones of olive plantation that are suited for establishing the quality sign ‘Olive Oil Chemlali of Monastir’. These are: *Zeramdine, Ouardanine, Moknine, Manzel Kamel* and *Béni Hassen.* As a second step, we have proceeded to establish the pomologic, physiochemical and sensory characteristics of the oil. Finally, we have identified the optimum time of harvest that correspond to the second half of December.

The Olive Oil Chemlali of Monastir is the result of a particular region and specific variety witch is cultivated in those zones for centuries. It is characterized by an aroma of flower fields, and green almond. The intensity of fruity is from medium to light while those of the bitterness and pungent bitter. It is an extra virgin oil olive characterized by: an acidic composition with medium rates of oleic acid (61.5-64%) and a high level of tocopherols ranging between 1030.33 and 757.85ppm. The polyphenols content of this oil varying between 177.14 and 210.4 ppm and a level of total sterols between 1623.82 and 2169 ppm.

Keys words: Monovarietal, olive oil, label, typicity

1. Introduction

L’oléiculture en Tunisie est un secteur stratégique sur le plan socio-économique et environnementale. Actuellement, l’oliveraie compte plus de 65 millions d’arbres, une superficie de 1,7 millions d’Hectares dont 115000hectares de cultures biologique certifiée, et une production annuelle moyenne de 150.000T d’huile d’olive.

Par ailleurs, la Tunisie se positionne en deuxième de point de vue superficie agricole cultivée en oléiculture. Le commerce international de l’huile d’olive assure 50 % du total des exportations agricoles ,5 % des exportations totales et constitue la cinquième source de devises du pays.

Toutefois, l’huile d’olive tunisienne ne dispose d'aucun signe de qualité. La majorité de la quantité exportée soit plus de 95% se fait en vrac laissant cette huile d’olive mal connue et parfois anonyme. Actuellement, notre pays s'emploie à doter l'huile d'olive tunisienne d'une notoriété internationale. Pour ce faire et afin de s'imposer face à la recrudescence de la concurrence, la Tunisie s’est engagée depuis le IX plan de mettre en place des stratégies de développement axées sur l’amélioration de la productivité et de la qualité et de valoriser les spécificités des zones de production en vue de maintenir les marchés traditionnels et conquérir de nouveaux marchés. Pour la réalisation de ces objectifs, nous nous sommes engagé pour contribuer à la valorisation des huiles d’olive tunisiennes à travers la constitution du premier signe de qualité en huile d’olive « huile d’olive Chemlali de Monastir », l’élaboration du cahier des charges du « label de l’huile d’olive de Tunisie » ce qui représente une première mondiale et la production et la valorisation des huiles d’olives tunisiennes monovariétales : Chétoui Medjez El Bab, Chemlali Sfax-, Chemlali Maknessy, et Oueslati El Alaa.

2. Matériel et méthodes

2.1. Matériel végétal étudié dans le cadre de l’élaboration du signe de qualité

Dans le but de contribuer à l’élaboration du signe de Qualité "Huile d’olive Chemlali de Monastir". L’Institut de l’Olivier et le CRDA de Monastir ont œuvré pour la création du premier signe de qualité en Huile d’olive:**«**l’Huile d’olive Chemlali Monastir ». Le dossier est soumis pour avis au Ministère de l’Agriculture, des Ressources Hydrauliques et de la Pêche.

Un cahier des charges du signe de qualité:« Huile d'olive Chemlali Monastir » est élaboré. Il referme le nom du produit, la description physico-chimique et organoleptique de l'huile d'olive et la délimitation de son aire de production à savoir: Zeramdine, Béni Hassen, Moknine, Ouardanine, et Manzel Kamel (Figure 1), les éléments prouvant la provenance de l'huile d'olive de cet aire et la description de la méthode de production des olives de la variété Chemlèli (les pratiques culturales,la période de récolte optimale, la méthode de l'élaboration de l'huile, le procédure de contrôle et les indications spécifiques de l'étiquetage.

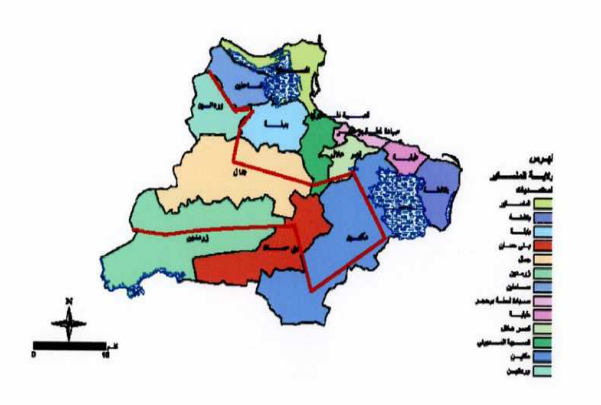


Figure 1: Les zones oléicoles délimitées de la région de Monastir pour l'IP «huile d'olive Chemlali Monastir».

2.2. Matériel végétal étudié dans le cadre de la production des huiles monovariétales

La valorisation des huiles d'olives tunisiennes monovariétales a été réalisée dans le cadre d'une convention entre l'Institut de l'Olivier et les sociétés SOCOHUILE et Huilerie Moderne**.** Les variétés choisies dans cette étude sont la Chemlèli, la Chetoui et la Oueslèti plantées dans quatre localités (Tableau 1).

Tableau 1: Caractéristiques des sites de plantation des variétés étudiées.

Localité & caractéristiques

Variété	Localité	Superficie	Nombre de pieds	% de la Variété recherchée	Date plantation
Chemlali	Messouda – Sfax	152 hect	2600	99%	1911
Chemlali	Sidi Messoud –Maknessy	23 hect	400	99%	1969
Chetoui	Sidi Nasr – Chouache Mjez El bab	8 hect, 47 are	813	98%	1947
Oueslati	El hseynia- El Alaa	9 hect	200	100%	1938

Chaque échantillon est constitué d'environ 2.5Kg d'olives provenant exclusivement de l'arbre et récoltés manuellement à hauteur d'homme sur toute la frondaison pour toutes les variétés étudiées. Aussitôt cueillis, les fruits sont transportés au laboratoire afin de préserver les caractéristiques de qualité. Ils sont effeuillés puis soumis aux différentes opérations pour la détermination de leurs caractéristiques pomologiques et pour l'extraction des huiles qui feront l'objet de différentes analyses physico-chimiques.

2.3. Les analyses effectuées

2.3.1. Analyses pomologiques des olives

L'indice de maturité : L'indice de maturité est un paramètre qui renseigne d'une façon globale sur la maturité des fruits, mais la valeur de cet indice ne reflète pas la réalité du mélange de couleurs d'un échantillon (El Antari et al, 2000).

Le poids moyen du fruit (PMF) : Le poids et les dimensions des fruits et des noyaux sont des caractéristiques variétales. Le patrimoine génétique de la variété a une incidence significative sur ces paramètres (Cimato, 1990 ; Grati Kamoun, 2007).

La teneur en matière grasse: Elle est effectuée par spectrométrie de résonance magnétique nucléaire (RMN). L'appareil est un spectromètre du type "Oxford 4000". Elle est exprimée en matière grasse par rapport au poids sec.

2.3.2. Caractérisation chimique des huiles

- Détermination de l'indice d'acide et de l'acidité dans les huiles, ISO 660 Troisièmes éditions 2009-06-15
- Préparation des esters méthyliques d'acides gras de l'huile d'olive et de l'huile de grignons d'olives COI/T.20/Document : 24 (2001).
- Analyse par CPG des esters méthyliques d'acides gras (ISO 5508)
- Analyses par spectrophotométrie dans l'ultraviolet de l'extinction spécifique aux longueurs d'ondes 232 nm (K232) et 270 nm (K270), COI/T.20/DOC n°1 9/r2V1.
- Détermination du pourcentage d'insaponifiable (ISO 3596)
- Détermination de la teneur en pigments (chlorophylles et carotènes) par spectrophotométrie : selon la méthode de Minguez-Mosquera *et al.* (1991).
- Détermination de la teneur des polyphénols par spectrophotométrie : Le dosage quantitatif des polyphénols est effectué selon la méthode Gutfinger (1981).
- Détermination de la teneur des tocophérols par HPLC (ISO, 9936)
- Détermination de la composition stérolique par CPG: (COI/T20/Doc. n°10)

3. Résultats et Discussions

3.1. Biodiversité des ressources génétiques tunisiennes : Production et Valorisation des huiles d'olives tunisiennes monovariétales

Le Tableau 2 récapitule les résultas obtenus pour les caractéristiques physico-chimiques et sensorielles des huiles monovariétales produites.

Tableau 2: Caractéristiques physico-chimiques et sensorielles des huiles monovariétales produites.

Caractéristiques	*Chemlali Messouda*	*Chemlali Maknessy*	*Chétoui*	*Oueslati*	
					Norme C.O.I. (2009)
Indice de Maturité	2.98	3.01	2.94	2.87	
Acidité	0.20	0.18	0.20	0.20	<0.8
k232	2.05	1.94	1.96	1.94	<2.5
k270	0.08	0.098	0.20	0.098	<0.22
%C16:0	17.1	16.87	9.87	11.27	7.5 - 20.0
%C18:1	60.76	64.04	73.62	74.11	55.0 - 83.0
%C18:2	16.59	14.83	13.49	10.18	3,5 - 21,0
Insaponifiables g/kg	12.88	13.28	14.67	12.52	<15
Stérols totaux (ppm)	1934.08	1638.42	1126.17	1228.57	>1000
Polyphénols (ppm)	128,24	295,3	441,78	103,58	
Tocophérols	959.97	866.96	966.81	372.91	
Chlorophylles (ppm)	3.47	3.43	5.75	4.05	
Carotènes (ppm)	7.54	8.29	20.49	8.29	
Echelle organoleptique de 0 à 5 (fiche CCE)					
Fruité	3	3	3.5	2.5	Vierge Extra
Amer	1.5	0.5	3.5	1	
Piquant	1.5	1	3	2	
Doux	2	2.5	0	2	
Défaut	0	0	0	0	
Notation CCE	7.5	7	7.75	7.25	

Le patrimoine oléicole tunisien jouit d'une haute richesse en cultivars. Chaque variété se distingue par un profil chimique, aromatique et des sensations agréables propres à elle. Cette variation de goût et d'arômes peut entraîner une variation d'utilisation de l'huile et une création de nouvelles habitudes culinaires. Malheureusement, ces particularités et ces sensations agréables ne sont pas perçues par le consommateur tunisien à cause de certaines pratiques incorrectes et courantes dans la chaîne de fabrication de l'huile. En effet, une récolte inappropriée des olives, un long stockage à l'huilerie avant la trituration et système d'extraction mal conduit affectent considérablement la qualité de l'huile produite Montedoro (1989), Grati-Kamoun *et al,* (1999), Koutsaftakis *et al.* (2000), Grati Kamoun, (2007), Grati Kamoun, (2001) et Gomezp-Alonso *et al,* (2007).

3.1.1. Enquête consommateur

Dans le but de mieux valoriser les caractéristiques chimiques et sensorielles des huiles d'olive tunisiennes et afin de faire découvrir au consommateur ces sensations organoleptiques particulières (des notes d'amande verte, d'herbe fraîches,…), nous avons organisé au cours des Salons Méditerranéens d'Agriculture et des Industries Agroalimentaires « SMA 2008, 2009 et 2010 » des séances de dégustation des huiles d'olives monovariétales produites.
Une enquête auprès des visiteurs a été réalisée afin d'évaluer leurs appréciations pour les huiles produites et d'essayer de décrire le comportement du consommateur vis-à-vis des produits de qualité.
L'échantillon est formé de 600 personnes aléatoirement choisies dont 76% sont des hommes, 54% appartiennent à la tranche d'age 31 et 50 et 31% sont âgés entre 21 et 30 ans. La majorité des dégustateurs résident à Sfax environ 82%, 8% sont au Nord, 2% résident au Sahel et 8% sont des étrangers.

3.1.2. Habitudes de consommation chez les tunisiens:

Les analyses montrent que 87% des personnes de l'échantillon ont une tradition de consommation quotidienne de l'huile d'olive. 70% ont des connaissances concernant les différentes variétés d'olivier et ils sont conscients des bienfaits de l'huile d'olive et 74% sont prêts à payer plus cher pour avoir des huiles de qualité à condition que le surplus soit raisonnable.

3.1.3. Connaissances des signes de qualité par les consommateurs

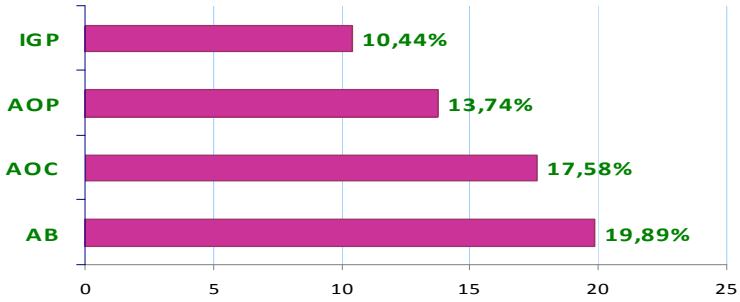


Figure 2: Notoriété des signes de qualité.

Ces résultats montrent que les consommateurs tunisiens ne connaissent pas les signes de qualité qui peuvent être apposés sur les produits alimentaires ainsi que « les promesses » derrière les labels et le taux de notoriété de ces labels sont globalement faibles. Nous constatons que pour l'AOC, signe de qualité qui existe en Tunisie depuis les années cinquante (vins et spiritueux), seuls 17.58% des personnes interrogées l'ont reconnue.

3.1.4. Confiance des consommateurs dans les signes de qualité

La confiance des consommateurs dans les signes de qualité se présente dans le graphique ci-dessous Malgré que les tunisiens connaissent peu les signes de qualité et ignorent la nature des promesses, ils peuvent leur faire confiance (figure 3). Au fait, ils font confiance à la certification en général qui Selon leur avis, elle est synonyme de la bonne qualité du produit.

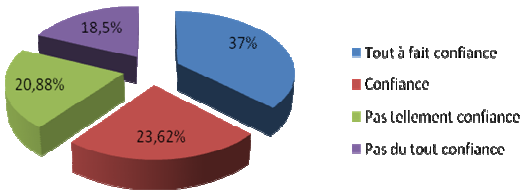


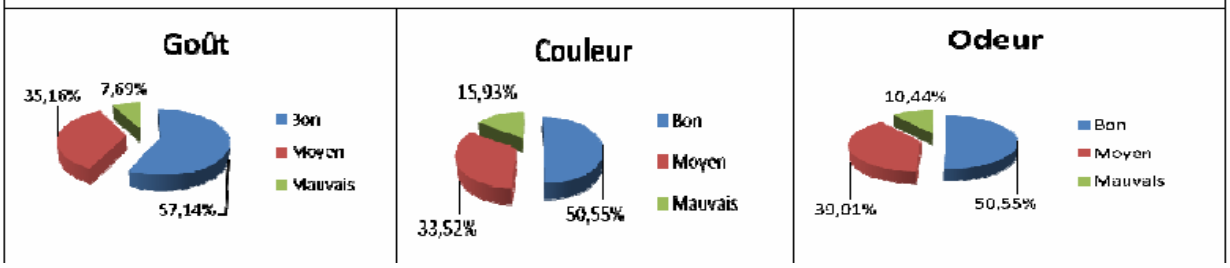
Figure 3: Confiance dans les signes de qualité.

3.1.5. Appréciations des consommateurs concernant les huiles monovariétales

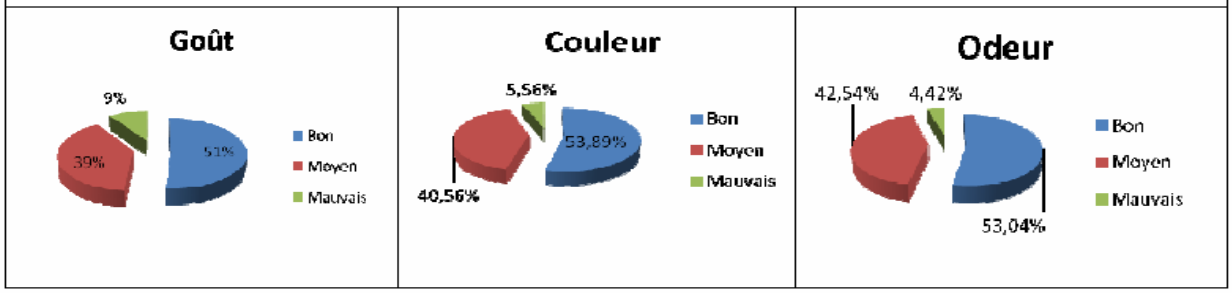
Les études ont montré que le goût, l'odeur et la couleur sont des critères de préférence importants pour les consommateurs tunisiens confirmant ainsi l'étude de Dekhil et D'Hauteville (2006). Ainsi, pour savoir les appréciations des dégustateurs sur les huiles monovariétales produites, on les a interrogé concernant ces trois attributs. L'idée de la production des huiles monovariétales a suscité un grand intérêt chez les visiteurs de différentes nationalités. Ces derniers, lors des séances de dégustation, ont beaucoup apprécié la qualité d'huiles produites. Ils ont rapidement perçu la différence et la spécificité de chaque produit et ils ont même pu distinguer les arômes d'amande verte et de la fraîcheur dans les huiles.

Tableau 3: Appréciation des dégustateurs pour les huiles monovariétales produites

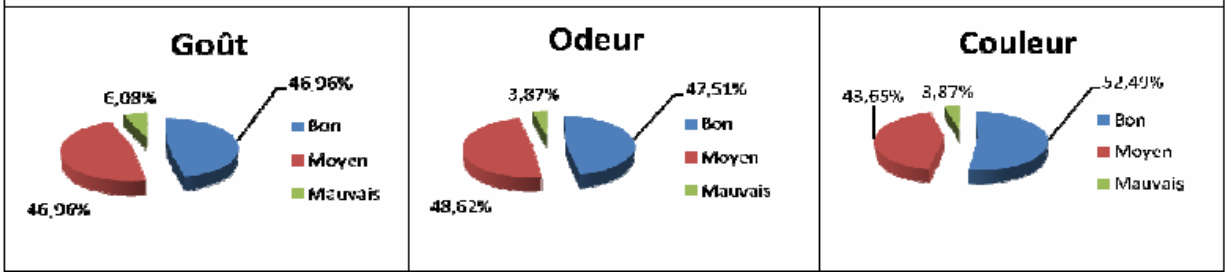
L'huile monovariétale Chemlali Sfax-Messouda



L'huile monovariétale Chétoui Medjez El Bab



L'huile monovariétale Oueslati d'El Alaa



Plus de 50% des dégustateurs ont décrit les huiles monovariétales de la Chemlali Messouda- Sfax, la Chemlali Maknessy et la Chétoui Medjez el Bab comme des huiles ayant un bon goût, une bonne odeur et une belle couleur. Les pourcentages les plus élevés ont été attribués à l'odeur de la Chétoui de Medjez El Bab, la couleur de la Chemlali Maknessy et le goût de la Chemlali Messouda-Sfax. Ils

ont aussi aimé la fraîcheur de la Chétoui et malgré l'amertume et le piquant inhabituels au sfaxiens, l'huile a été très appréciée. Pour finir, on conclu qu'une huile produite dans le respect total des bonnes pratiques de fabrication et d'hygiène ne peut que plaire à son goûteur.

3.2. Contribution à l'élaboration du signe de Qualité « Huile d'olive Chemlali de Monastir

La description du produit est capitale lors de l'élaboration du cahier des charges d'un signe de qualité. De ce fait, cette partie est consacré à l'étude des caractéristiques de l'huile d'olive provenant des aires de production délimitées par le signe de qualité ainsi qu'à la détermination de l'époque de récolte optimale permettant l'obtention d'une huile de qualité supérieure.
Les résultats obtenus à partir des analyses pomologiques, physicochimiques, sensorielles et statistiques effectuées sur les échantillons d'olives et des huiles provenant des zones délimitées au gouvernorat de Monastir à savoir : *Manzel Kamel, Moknine, Béni Hassen*, *Zeramdine* et *Ouardanine,* sont présentés dans le tableau 2.

Tableau 4: Caractéristiques pomologiques, physico-chimiques, et sensorielles de «l'huile d'olive Chemlali Monastir».

Caractéristiques	Zeramdine	Ouardanine	Béni Hassen	Moknine	Manzel Kamel	
PMF	0.96	0.97	1.07	1.02	0.83	
% Pulpe	81.13	79.67	82.33	82.33	79.12	
%Humidité	51.60	53.10	52.80	56.69	53.48	
MG/PF	23.70	22.44	23.43	19.66	20.55	
						Norme C.O.I.
Acidité	0.19	0.18	0.15	0.18	0.17	<0.8
k232	1.62	1.58	1.56	1.61	1.49	<2.5
k270	0.15	0.14	0.13	0.13	0.13	<0.22
%C16:0	16.87	17.37	17.37	17.48	16.34	7.5 - 20.0
%C18:1	62.85	62.89	61.88	61.48	64.12	55.0 - 83.0
%C18:2	15.54	14.51	16.26	15.78	14.34	3.5 – 21.0
Polyphénols (ppm)	177.14	235.26	196.96	267.14	210.4	
Tocophérols totaux (ppm)	893.40	757.07	757.85	790.90	1030.33	
Chlorophylles (ppm)	2.19	4.50	2.18	1.92	4.01	
Carotènes (ppm)	15.87	15.64	17.03	15.75	16.11	
Insaponifiables g/kg	11.0	13.8	13.2	11.6	10	
Stérols totaux (ppm)	2169.78	1623.82	1939.5	2091.76	2039	>1000
			Echelle organoleptique de 0 à 5 (Norme CCE, 2007)			
Fruité	2.28	2.61	2.43	2.46	2.6	
Amer	1.02	2.31	1.07	1.66	1.1	Vierge Extra
Piquant	0.94	1.65	0.87	1.12	1.1	
Doux	1.71	0.3	1.73	1.03	1.75	
Notation CCE	7.41	7.24	7.02	7.13	7.014	

La comparaison des moyennes des différents paramètres pomologiques, physico-chimiques, et sensoriels montre qu'à un risque d'erreur de 5%**,** la différence entre les huiles obtenues à partir des quatre zones étudiées n'est pas significative. Elles peuvent être donc classées dans un même groupe et elles méritent d'abriter un signe de qualité (AOC ou IP).
Sur le plan chimique, les analyses montrent que l'huile d'olive Chemlali Monastir **»** est une huile Vierge Extra, avec une composition conforme aux normes internationales, caractérisées par:

- Une composition acidique avec des taux moyens d'acide oléique (61.5-64%) et élevés d'acides palmitique (16.3-17.5%) et linoléique (14.3-16.3%),
- une richesse en tocophérols avec des teneurs qui varient entre 758 et1030 ppm,
- une teneur en polyphénols qui varie entre 177 et 210 ppm,
- une richesse en carotènes (15.75-17) et en chlorophylles,
- un taux d'insaponifiables inférieure à 1.5% et
- une teneur élevée en stérols totaux qui varie de 1623.82 à 2169 ppm.

L'Huile d'olive "Chemlali Monastir " est un huile d'olive caractérisée par des odeurs de fleurs de champs et d'amande verte. L'intensité du fruité est moyenne à légère alors que celles de l'amertume et le piquant sont légères.

Par ailleurs et afin de garantir la conservation des composés responsables de l'arôme et du goût de l'huile ainsi que les composés mineurs qui protégent l'huile et garantissent sa valeur nutritionnelle et thérapeutique**,** la récolte des olives doit se faire au cours du mois de décembre et plus précisément durant la deuxième quinzaine de ce mois (indice de maturité IM= 3).

4.Conclusion

Malgré l'importance socio-économique du secteur oléicole en Tunisie et la grande diversité génétique, les huiles d'olives tunisiennes ne sont pas très bien valorisées sur le marché national et international. Elles souffrent d'une déficience d'image et de notoriété. Cette étude a mis l'accent sur la haute richesse du patrimoine oléicole tunisien. En outre, elle a confirmé que le facteur génétique, le terroir et le savoir faire ont une grande influence sur les caractéristiques physico-chimiques et le profil aromatique de l'huile d'olive. Les huiles d'olive tunisiennes sont de très haute qualité et possèdent des caractéristiques chimiques et sensorielles spécifiques.

Aujourd'hui, le défi de véhiculer une image valorisante de notre produit à travers son histoire, ses variétés, ses spécificités et ses vertus s'est lancé avec le projet de création du premier signe de qualité "Huile d'olive Chemlali de Monastir" et ne doit pas s'arrêter ainsi que l'élaboration de la Tunisie doit opter rapidement pour une traçabilité déterminant les signes de qualité de l'huile d'olive, en adoptant d'autres appellations d'origine contrôlées AOC, ainsi que des indications de provenance IP.

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Thème 2

Bonnes pratiques de conduite de l’olivier : acquis et innovations

S1-C1

Alternative use of vegetable remains of olive tree orchard in a soil carbon sequestration system

O.M. Nieto[1], **J. Castro**[1], E. Fernández[2]

Abstract

In traditional olive grove a large amount of vegetable remains take place and that have usually burned or rejected. The continuous addition of all the vegetable remains obtained in the pruning, harvests and cleaning of fruits, can be a real alternative for the improvement of the physical-chemical properties of a floor and to be used as mechanism of sequestration of carbon in olive orchards soils. The effect of the continuous addition of olive remains was studied in a olive orchard of Jaén (Spain). The results show like they decrease the annual emissions in 2 t CO_2 ha^{-1} yr^{-1} and with fixation rate of CO_2 of 0,6 $t.ha^{-1}$ yr^{-1}, in relation to a conventional tillage olive orchard and burning the pruning remains.

Key words: vegetable remains, olive tree, soil carbon sequestration

L'utilisation des débris végétaux dans l'oliveraie comme un système alternatif pour la séquestration du carbone des sols

Résumé

Dans l'oliveraie traditionnelle une grande quantité de déchets végétaux sont produits chaque année et sont généralement brûlés ou rejetés. Le retour au champs de tous les déchets végétaux (branches obtenus de la taille des arbres, les feuilles, les grignons d'olive et le nettoyage des fruits), peut être une véritable alternative pour l'amélioration des propriétés physico-chimiques d'un sol et d'être utilisé comme un mécanisme de séquestration du carbone dans les sols des vergers d'oliviers. L'effet de l'addition continue de ces déchets du secteur oléicole a été étudié dans une oliveraie de Jaén (Espagne). Les résultats montrent qu'ils réduisent les émissions annuelles de CO_2 de 2 t ha-1 an-1 et avec les taux de fixation de CO_2 de 0,6 t.ha-1 an-1, en comparaison avec un travail traditionnel du sol de l'oliveraie et le brûlé de branches de la taille.

Mots clés: Déchets végétaux, olivier, sol d'oliveraie, séquestration du carbone.

1. Introduction

In agricultural systems, the relationship between soils, climate change and their impact upon the science of carbon sequestration is well established (Dawson and Smith, 2007). Some authors (Jarecki and Lal, 2003; Hernández et al., 2005; Gómez et al., 2009) have reported that strategies based upon changes in land use and soil management in agricultural soils are potentially important in increasing the soil's organic-carbon (SOC) content and carbon sequestration. Olive trees constitute the most extensive woody crop in Spain, traditional olive management have favoured the constant degradation of these areas (Pastor, 2004). Nevertheless, the introduction of alternative soil-management practices to that of tilling, have decreased erosion and increased fertility (Castro et al., 2008; Gómez et al., 2009). According to the ESYRCE inquiry (MARM, 2008), 57% of olive groves continue to be tilled and only 0.5% mulching with prunings. The routine annual tasks involved in olive cultivation generate a number of residues that are amply suited for recycling as soil covering around the trees, such as the waste left from cleaning the fruit prior to extracting the oil (leaves, green twigs and superficial soil) and the twigs and small branches deriving from regular pruning. On top of this, according to traditional practice, more often than not they are burned, thus creating considerable CO_2 emissions.

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Our aim was to discover the dynamics of organic carbon in the soil using the RothC model to predict the effects of changing the soil-management system from conventional tillage (T) to mulching with shredded olive pruning debris (PD) and the residues deriving from cleaning the fruit (CR).
Some authors have studied the effects of covering the ground with pruning debris. Sofo et al. (2005) underline the importance of this practice to help absorb atmospheric CO_2 and store it as organic matter in the soil. Ordoñez et al. (2001) described an improvement in the physical-chemical properties of the soil and a concomitant increase in fertility. The dynamics of carbon in soils under different land use and management systems have been estimated by models. One of the most widely used is the Rothamsted model for organic carbon turnover in soil (RothC) (Coleman and Jenkinson, 1996) due to its easy application and the success experienced in applying it to sites under diverse agricultural management systems throughout the world (Smith et al., 1997). No data concerning carbon storage in soils after a change in soil management are currently available and neither are there any publications concerning the effects on the soil of continuous mulching with the waste derived from olive-cleaning activities.

2. Materials and Methods

The study area is located in south-eastern Spain, adult olives (cv. *picual*) with a planting density of 82 trees ha^{-1}. The initiafl soil-management system of the grove was T and was applied only to the open gaps between the trees (50% of the total area of the grove). The soil-management system was changed in 1997, which involves spreading shredded olive prunings and residues from the olive-fruit cleaning (leaves, fresh twigs and soil) between the trees. The ground is not tilled and all these residues remain on the surface that covered the soil between the trees (50% of total area of the grove). Soil samples were taken between trees in PD+CR soil after removing the superficial plant-residue layer and, in two neighbouring T olive groves. Three pits measuring 50 x 100 x 50 cm were dug and the samples taken 30 cm deep. Organic carbon in the soil and residues was determined by wet oxidation with dichromate according to the method of Walkley and Black. CO_2 emissions from burning residues were determined accord to 1 g C = 3.67 g CO_2 (IPCC, 2000). The RothC model was used to assess SOC when changed T to PD+CR. In PD+CR carbon inputs were measured in the field as a mean of vegetable-residue inputs. For the T olive grove RothC model was run iteratively in reverse to calculate how much organic carbon needs to enter a soil annually to give the measured amount of SOC. We resorted to data available in the literature concerning tilled olive-grove soils over the last thirty years or more in similar estates in neighbouring areas.

3. Results and Discussion

The SOC contents under T management were slightly lower than available historical data (34.6 ±9.1 t C ha^{-1}), but without being significant, for this reason we assumed that T soil are in equilibrium.

Table 1: Measured and modelled data for the turrover of organic carbon in olive-groves oils under conventional tillage (T) and mulched with residues from pruning debris and olive-fruit cleaning (PD+CR)

Scenario	SOC measured (t C ha^{-1})	IOM (t C ha^{-1})	Input C modelled (t C ha^{-1} yr^{-1})	Input C measured (t C ha^{-1} yr^{-1})	Turnover time (yr)
T equilibrium	26.4	2.0	1.0	-	26
PD+CR 10 years	158.0	2.0	25.3	23.9	6

Table 1: Measured and modelled data for the turnover of organic carbon in olive-grove soils under conventional tillage (T) and mulched with residues from pruning debris and olive-fruit cleaning (PD+CR).

On the basis of the SOC measured and the IOM estimated using the equation of Falloon et al. (1998), the annual carbon input for T was calculated to be 1.0 t C ha^{-1} yr^{-1}. The RothC model estimated the carbon input into the soil of a T olive grove in equilibrium as being 1.0 t C ha^{-1} yr^{-1}, Romanyà et al. (2000) registered similar results for a vineyard in the Mediterranean area, with an annual carbon input of 1.4 t C ha^{-1} yr^{-1}. The only estimates of carbon input for olive groves were reported by Sofo et al. (2005), who registered an annual input as senescent leaves of 0.4 t C ha^{-1} yr^{-1}. The effectiveness of the PD+CR used in our experiment is manifest in the resulting high SOC, which were even higher than those measured in soils T and left to natural vegetation (data no shown). Other authors have also reported increases in these values on changing the soil-management system from T to cover crops (Castro et al., 2008; Gómez et al., 2009) or PD (Ordóñez et al., 2001; Sofo et al., 2005), although they obtained lower levels than the means in our experiment. When mulching with PD+CR the model predicted a continuous increase in SOC, but without reaching a state of equilibrium.

Table 2: CO_2 released into the atmosphere after 10 years tillage (T) and mulching with shredded pruning debris (PD)

	Scenario	CO_2 accumulated to the atmosphere (t CO_2 ha^{-1})
T	Burn pruning debris	24.0
	CO_2 lost from soil	12.0
	Total	36.0
PD	Burn pruning debris	-
	CO_2 lost from soil	15.9
	Total	15.9

Table 2: CO_2 released into the atmosphere after 10 years tillage (T) and mulching with shredded pruning debris (PD).

During the experiment we measured a carbon input at the soil-surface between trees of 23.9 ±14.3 t C ha^{-1} yr^{-1}. After 10 years of change in soil-management system the annual carbon input needed to reach the SOC values between trees at the end of the experiment as estimated by the model were very similar to that measured in soils (Table 1).

CO_2 emissions when the PD were burned in T management and when they were shredded and spread on the ground after 10 years are given in Table 2. As a result of the addition of PD to the soil, the CO_2 that was hitherto released into the atmosphere during the years of tilled management was reduced by more than 55%. With regard to this decrease in CO_2 emission, the RothC model estimated a potential carbon sequestration of 0.5 t C ha^{-1} yr^{-1}. (Table 3). When PD is shredded and spread on the ground, the RothC model predicts a decrease in CO_2 emission of 2 t CO_2 ha^{-1} yr^{-1}. We did not model carbon sequestration after mulching with CRs because this waste matter is normally thrown away. Nevertheless, the total carbon content in the soil showed an increase during the experiment of 13.2 t C ha^{-1} yr^{-1}. The high values of turnover time obtained in T indicate carbon stabilization in the soil, which is to say that carbon migrates slowly from one pool to another. For PD+CR the turnover times were lower, this showed rapid migration of carbon and thus a soil that is not close to equilibrium. Abandoning tillage in favour of using organic waste to cover the ground is considered to be an efficient way of increasing carbon sequestration in agricultural soils. In our experiment we measured a carbon sequestration of 0.5 t C ha^{-1} yr^{-1} with PD cover (Table 3). IPCC (2000) suggested a carbon sequestration potential of 0.3 t C ha^{-1} yr^{-1}. Our results coincide with these values, which supports the idea that recycling PD+CR in olive groves is an effective way of storing carbon in the soil.

Table 3: SOC increase as result of changes in soil management from tillage (T) to mulching with residues (PD+CR). CO_2 reduction and carbon sequestration after the addition of only pruning debris (PD)

	SOC increase (t C ha^{-1} yr^{-1})	CO_2 reduction (t CO_2 $ha^{-1}yr^{-1}$)	C sequestration (t C $ha^{-1}yr^{-1}$)
T to PD+CR	13.2	2.0	0.5

Table 3: SOC increase as result of changes in soil management from tillage (T) to mulching with residues (PD+CR). CO_2 reduction and carbon sequestration after the addition of only pruning debris (PD).

4. Conclusions

When the soil-management was changed from T to PD+CR, carbon storage in the soil improved considerably. Carbon turnover in PD+CR olive-grove soil was quite accurately predicted by the RothC model. A soil-management system that abandons tillage in favour of reusing both pruning debris and the residue from cleaning the olives to cover the ground, constitutes the most effective way of increasing soil quality and diminishing CO_2 emissions in one of the most extensive agricultural enterprises in the entire Mediterranean area.

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S1-C2

Assessing possibilities of quantifying soil fertility of an olive orchard in one integrated index

Gargouri K., Felippe Saiano, Ettore Barone

Abstract

The soil acts physically as a water container, more or less colonized by roots. It also acts chemically as a nutrients provider to the tree. Both of these roles are performed simultaneously by intrinsic characteristic soil properties that as a whole contribute to the definition of the soil fertility. Thus, soil fertility involves a wide range of variable that make very difficult its valuation. The main objective of this work is to try to quantify soil fertility by creating a unique index namely soil fertility index. For this reason the soil quality (fertility) index was estimated by a three-step method modified from Rezai et al. (2005) and Andrews (2001). The first step is choosing of appropriate indicators and selection of the data set, the second is establishing scoring function and weighting factor for the data set indicators in order to standardize them and finally the soil fertility index (SFi) is accomplished by summing the scores for each indicator, dividing by the total number of indicators (Andrews et al., 2004).

Key words: Soil fertility, olive nutrition, soil quality index

Evaluation de la fertilité d'un sol d'oliveraie à l'aide d'indicateurs de l'index de qualité des sols

Résumé

Le sol est le support des cultures et leur réservoir d'eau et de nutriments. Le pouvoir du sol à satisfaire les besoins des cultures est défini comme étant sa fertilité. Celle-ci dépend des caractéristiques intrinsèques du sol et de ce fait d'un grand nombre de variables rendant son évaluation difficile. L'objectif de ce travail est de quantifier la fertilité du sol en créant un indice unique regroupant les principales composantes de ce terme à savoir l'Indice de Fertilité du Sol. Pour ce faire l'indice a été estimé par une méthode en trois étapes telles que proposées par Andrews (2001) et Rezai et al. (2005). La première étape est le choix des variables constituant la base des données. La deuxième est la transformation des variables par une standardisation des valeurs. Ceci est réalisé par l'établissement des fonctions de transformation (score entre 0 et 1) et du calcul du poids de chaque facteur. Enfin, l'indice de fertilité du sol est calculé par l'addition des scores de toutes les variables au point donné et la division de la somme par le nombre total des variables (Andrews et al., 2004).

Mots clés : Fertilité du sol, nutrition des oliviers, indice de qualité du sol.

1. Introduction

The general situation of the olive orchards productivity throughout the World seems to be unsatisfactory. About 70% of the olive orchards are traditional and marginal with a medium to very low productivity due, in a significant degree, to the lack of appropriate orchard management and to low soil fertility (Michelakis, 2002; Touzani, 1998, 1999). All around the Mediterranean basin the olive grove exploits marginal productivity inclining soils (Graaff and Eppink, 1999; Loumou and Giourga, 2003).

Under these conditions, the olive growing system seems to be the only one able to assure the sustainability of the natural resources through their preservation by the maintenance of the soil, the reduction of rainfall's losses, and their exploitation. Olive trees are drought resistant and because of their extensive rooting system are some of the few crops that can survive on only 200–300 mm of annual rainfall (Fresco, 1996). Thus, olive growing is the kind of cultivation that maintained the productive possibility in the barren and dry Mediterranean soils, with very high erosion levels (Loumou and Giourga, 2003). Furthermore, in the southern Mediterranean basin, the olive tree grows mainly

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under arid conditions. The water shortage impact, which represents the most important limiting factor, is conditioned by soil quality, i.e. soil fertility (Braham, 1997; Fernandez-Escobar, 1997). Indeed, the soil quality determines water retention capacity and its availability to the tree. However, the trees have to uptake the nutrients during wet periods, when soil moisture is enough to assure the nutrients transfer. In reality, when these conditions are favourable, the nutrient absorption activity is boosted and the absorbed elements are stocked up in the permanent organs to be used when necessary.

These reserves may be as mineral nutrient or synthesized organic molecules, such as carbohydrates (Fernandez-Escobar, 2001). Therefore, it is necessary to study and to understand the relationship between soil fertility and olive tree nutrition. Several researches had dealt with this topic (Crescimanno, 1975; Zucconi *et al.*, 2001; Peterson and Stevens, 1994; Fernandez-Escobar, 1999; Braham, 1997; Gargouri and Mhiri, 2002; Chaves, 1975). However, none of them dealt with soil fertility as a quantifiable comprehensive parameter. More precisely, they considered this relationship for each fertility component and each nutrient alone, out of the influence of the others. This approach may lead perhaps to a wrong or approximate evaluation of the effects and the importance of each variable underestimating or overestimating its contribution to the olive tree growth and production. Soil quality (SQ) approach may be therefore conveniently used to asses this relationship between soil fertility and olive tree nutritional status. The general definitions for SQ emphasize the capacity of soil to perform services including the production of plants (Doran and Parkin, 1994; Karlen *et al.*, 1998). In addition, descriptions of SQ reflect appreciation for soils' fitness for use (Wander *et al.*, 2002; Larson and Pierce, 1994). Science-based soil quality index (SQi), including inherent and sometimes dynamic properties, provide the necessary integration of information for land managers to make informed decisions about complex issues such as site potential assessment and agroecosystem management (Andrews & Carroll 2001; Rezaei 2003; Rezaei at al. 2005). From an agronomic point of view the best SQi is that one assuring the best plant nutrition status leading to a satisfying level of growth and yield (Andrews *et al.*, 2002). In that case the soil quality indices should be called Soil Fertility index (SFi).

The objective of this study is to elucidate the relationship between soil fertility and olive tree nutritional status through the establishment of a Soil Fertility index (SFi).

2. Material and Methods

2.1. Study area, sampling and laboratory analysis

The olive orchard used for this study is located in central Tunisia (Sfax region - 34.37 N 10.16 E). The orchard covers 134 ha. The trees of the cv Chemlali, 80 yrs old, with very low plantation density (24x24 m apart =17 trees/ha), are conducted under rainfed conditions. The soil is sandy (more than 90% of sand). The fertilization plan consists of 3 – 4 Kg of NH_4NO_3 per tree distributed in spring and autumn (1:2). A 200x200 m grid pattern was established; each intersection point (node) represented a sampling point (figure 1). A total of 27 sampling points (Ii, i = 1 to 27) were identified. Soil samples were

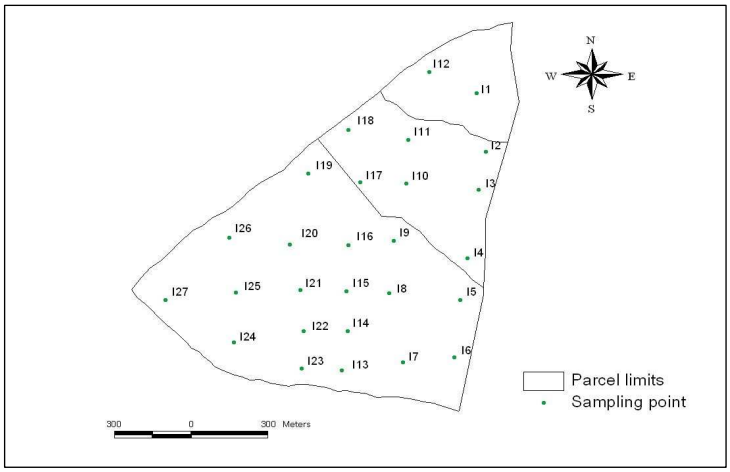

Figure 1: Sampling point repartition on the investigation area.

taken at 1 m depth in mid-July 2005. Each soil sample was collected as follows: four 500g soil cores were taken within 2 m radius of each grid point and one more core right at the intersection point. These 5 samples were mixed thoroughly to provide a bulked sample and to ensure its representativeness. Soil samples were air-dried overnight and passed through a 2 mm sieve. Organic matter content was determined by dichromate oxidation using Walkley and Black method (Pauwel et al., 1992). Total $CaCO_3$ content was determined after application of HCl and measurement of CO_2 produced volume. Olsen method was used to determine available phosphorous concentration. Available K and Na were determined using flam photometer after extraction by ammonium acetate (Pauwels et al., 1992). Soil electrical conductivity was measured on the soil extract saturated soil.

2.2. Determination of wood carbohydrates content

The wood carbohydrates content (WCc) was estimated by the sugars content. For this reason sugars content was determined following sulphuric acid – phenol method (Robyt and White, 1987). A sample of 300mg of fresh matter is mixed with 5ml of methanol 80% and maintained at 70℃ during 30'. Then, 1 ml of the extract is mixed with 1 ml of 5% aqueous phenol and 5 ml of concentrated sulphuric acid. The solutions were allowed to stand for 20' for color development. The absorbance was measured at 640 nm, using a UV-visible spectrophotometer (Jenway 6405 UV/Vis). The total carbohydrates may be estimated using glucose as the standard.

2.3. Estimation of soil fertility index

The soil quality index was estimated by a three-step method modified from Rezai et al. (2005) and Andrews (2001).

Step I: Selection of appropriate indicators and selection of the data set.

The expert opinion method was adopted for the selection of the data set to be used in the generation of the soil fertility index (Andrews et al., 2002). We required a data set that would describe the basic soil functions necessary for obtaining the best olive tree mineral nutrition status. These functions are to provide sufficient nutrients and sufficient amount of water. The selected data set includes soil parameters previously described. Available phosphorus, exchangeable potassium, organic matter content, limestone, gypsum, electrical conductivity, exchangeable Na and chloride contents formed the data set.

Step II. Scoring function and weighting factor for the data set indicators.

The development of soil quality criteria for olive growing in semiarid and arid areas is difficult. An integrated procedure was derived from methods developed by Karlen and Stott, (1994), Andrews et al. (2002a, 2002b) and Rezaei et al. (2005). On the basis of both literature and the observed interactions between data set indicators and plant response variables, scoring of each observed indicator value was performed using non linear scoring curves (Karlen & Stott 1994). Non-linear scoring techniques involve the use of curvilinear scoring functions with a y-axis ranging from 0 to 1 and an x-axis representing a range of site- or function-dependent scores for that variable (Karlen and Stott, 1994; Andrews and Carroll, 2001). CurveExpert v. 1.3 shareware was used to generate the scoring functions. Dependent variable was carbohydrate content in the wood (Drossopoulos and Niavis, 1988; Ulger et al., 2004).

Step III. Calculating the soil fertility index (SFi).

This step is accomplished by summing the scores for each indicator, dividing by the total number of indicators (Andrews et al., 2004). Thus, the SFi is obtained by the following equation:

$$SFi = \frac{\sum_{i=1}^{n} S_i}{n}$$

Where S represents the scored indicator value and n is the number of indicators.

2.4. Statistical analysis

Data were analyzed statistically (SPSS 11.0 software). Classical descriptors were determined, such as mean, maximum, minimum, standard deviation and skewness of data distribution. Also, correlation matrix was calculated for all variables. On the other hand, ANOVA analysis was carried out to identify differences between experimental units for all parameters.

3. Results

Correlation matrix for soil characteristics showed highly significant correlation between electrical conductivity (EC), Cl, and $CaSO_4$ content (correlation coefficients of 0.596 for EC-Cl pair and -0.362

for EC-$CaSO_4$ pair). EC showed also significant correlation with Na and $CaCO_3$ (correlation coefficients 0.272 and 0.282 for the EC-Na and EC-$CaCO_3$ pair, respectively). Na content presented high significant positive correlation with chloride content, (coefficient of correlation 0.514). For this reason only the effect of Na may represent those of EC and Cl. On the other hand, available P_2O_5 and OM showed significant correlation with a coefficient of 0.335. No significant correlation was found for all the other pairs of soil parameters (Table 1). Organic matter showed highly significant differences between sampling points (SP). The minimum values were observed in the southern eastern part of the investigation area: SP I7 and I8 with OM content of 0.21 and 0.23%, respectively. The highest OM content was observed in the southern western part of the orchard at SP I27 level, where OM content reached 0.57%. Two other zones had sampling points with OM value more than 0.5%. In the southern part of the orchard, sampling points I21, I22 and I23, had OM values of 0.53, 0.51, and 0.52%, respectively.

Table 1: Correlation matrix between soil traits pairs.

		P_2O_5	K_2O	Na	OM	$CaCO_3$	$CaSO_4$	EC	Cl
P_2O_5		1							
	Sig.	-							
K_2O		0.196							
	Sig.	0.155	-						
Na	-0.042	0.173	1						
	Sig.	0.764	0.210	-					
OM		0.335*	-0.105	0.235	1				
	Sig.	0.013	0.449	0.087	-				
$CaCO_3$		0.050	0.086	0.344*	0.173	1			
	Sig.	0.0717	0.537	0.011	0.211	-			
$CaSO_4$		0.010	0.088	-0.037	0.021	-0.266	1		
	Sig.	0.941	0.528	0.792	0.878	0.052	-		
EC		-0.014	-0.036	0.272*	-0.131	0.282*	-0.362**	1	
	Sig.	0.921	0.798	0.046	0.343	0.039	0.007	-	
Cl		-0.126	0.182	0.514**	0.008	0.255	-0.341*	0.596**	1
	Sig.	0.362	0.187	0.000	0.956	0.063	0.012	0.000	-

* Significant correlation at the level of 0.05 (bilateral);
** Significant correlation at the level of 0.01 (bilateral).

The eastern part presented organic matter values of 0.56, and 0.52% for I4 and I5. On the other hand, the organic matter contents of the majority of the other SPs were near the mean value of 0.4%.

Limestone distribution in the orchard had also an important variation among the SPs. The minimum value was located in the eastern part of the toposequence, where limestone content was 0.25% at I8. In the same area, low total $CaCO_3$ content was detected: 0.75% and 1.7% for I4 and I5. Conversely, the highest value of 14.5% was observed for I11 which is located in the centre of the northern part of the parcel. The SPs with relatively high $CaCO_3$ content are localised in two zones. The first one is the mid-southern zone: I21, I22, and I23 ($CaCO_3$ content between 12.6 and 13.9%). The second is those of I11 and I10 in the centre of the upper part. Concerning calcium sulphate variation in the soil upper layer of the toposequence, a wide range was observed from 0.01 to 0.8%. The lowest values were located in the eastern part at I4 and I9 levels. In contrary, the highest values were observed in the western part in correspondence to SPs I26 (maximum), I19 (0.65%), I17 (0.65%), and I18 (0.60%).

However, the zone of low $CaSO_4$ content is adjacent to an area with high gypsum content which corresponded to SPs I3 (0.56%), I5 (0.66%), I8 (0.37%), I10 (0.45%), and I16 (0.54%). P_2O_5 contents in the soil showed a moderate variation, with the minimum of 8.73 ppm located at I9, in the mid-eastern part of the orchard. This minimum value is somewhat above the critical level of 8 ppm proposed by Gargouri and Mhiri (2002). The maximum was located at I4, in the eastern boundary of the parcel. Consequently the areas where are located the extreme values of soil available phosphorus content are adjacent. In addition, no poor or, conversely, rich zones were determined. Indeed, the lowest values were neighboring the highest values. For example, I8 (P_2O_5 content = 12.2 ppm) is located near I15 (21.2 ppm). Soil exchangeable potassium contents were highly variable, oscillating between 40 and 181 ppm. Moreover, 11 locations had K_2O content under the critical level of 80 ppm determined for sandy soils in semi-arid regions. These locations were divided in two zones: the northern one and the east-southern one. The northern part is represented by: I2 (70.3ppm), I11 (52.6ppm), I12 (73.8ppm), and I18 (69.9ppm), the upper point of this part is above the threshold, i.e. I1

has an exchangeable potassium content of 84.7ppm. The second area included I6 (55.4ppm), I8 (43.4ppm), I9 (68.7ppm), I14 (78.6), I21 (65.4ppm), I22 (44.3ppm), and I23 (69.5ppm), the soil of the lower part of this area is relatively rich in exchangeable K counting I7 (102.4ppm) and I13 (131ppm). Available Na content variation is less important than K_2O one. Indeed, the variation range is from 50 to 108 ppm. The maximum exchangeable Na concentration is located in the west southern part of the olive orchard (I24). This zone also includes several other sampling points with relatively high exchangeable Na contents. These sampling points are: I25 (93.5ppm), I26 (80.25ppm), and I27 (98ppm). A second relatively rich zone is located in the other side of the southern area of the parcel. Five sampling points formed this area: I4 (83ppm), I5 (92ppm), I7 (81ppm), I13 (85.5ppm) and I14 (97.5ppm). Between these two rich zones is located a poor zone including the significant lowest exchangeable Na values: I8 (57.5ppm), I9 (67ppm), I15 (50ppm), I20 (59ppm), I22 (66.5), and I23 (63.7ppm).

The wood carbohydrates contents showed important differences between the EUs. To understand the relationship between soil fertility and olive tree nutritional status, a Soil Fertility index (SFi) was developed. This index was designed with the aim of quantifying the intrinsic capability of the soil to assure the best nutritional status to the olive tree. For this purpose the leaf carbohydrates content was used as an indicator of olive tree nutritional status (dependent variable), together with several soil parameters such as exchangeable K_2O, available P_2O_5, lime, organic matter, gypsum, exchangeable Na contents and electrical conductivity (EC) of the soil. The scoring method was based on the use of curvilinear scoring functions to transform indicators into dimensionless and, thus, combinable scores (Andrews *et al.* 2002) (Table 2).

Table 2: Scoring function for each component of the data sets*.

Indicator (xi)	Max.observ. value	Bivariate model	R^2	Max.predicted WCc (mg/g)	Scoring functions
K_2O (ppm)	180.72	$Y = 0.5 + 0.002x_i + 1.43x_i^2$	0.12	0.59	$S = 0.85 + 0.003x_i + 2.42x_i$
P_2O_5 (ppm)	27.95	$Y = \frac{0.57}{1 + 128.5\exp^{-12.67x_i}}$	0.83	0.57	$S = \frac{1}{1 + 128.5\exp^{-12.67x_i}}$
OM (%)	0.59	$Y = 0.06 + 2.7x_i - 3.35x_i^2$	0.73	0.6	$S = 0.1 + 4.5x_i - 5.58x_i^2$
Lime (%)	14.5	$Y = \frac{0.57}{1 + 634.04.5\exp^{-3.16x_i}}$	0.82	0.57	$S = \frac{1}{1 + 634.04.5\exp^{-3.16x_i}}$
Gypsum (%)	0.79	$Y = \frac{-7.94 + 102.85x_i}{1 + 153.85x_i + 50.36x_i^2}$	0.85	0.61	$S = \frac{-13.02 + 167.21x_i}{1 + 153.85x_i + 50.36x_i^2}$
Exchang.Na	109.33	$Y = \frac{0.57}{1 + 3.36\exp^{-0.46x_i}}$	0.83	0.57	$S = \frac{1}{1 + 3.36\exp^{-0.46x_i}}$
EC (dS/m)	3.42	$Y = \frac{-16.93 + 3.3x_i^{0.59}}{3.3 + x_i^{0.76}}$	0.82	0.58	$S = \frac{-29.19 + 5.69x_i^{0.59}}{3.3 + x_i^{0.76}}$

*Function Y is wood carbohydrates content and xi is an ascribed indicator or variable value, and S is score from 0–1 based on measured values for xi

The potassium, despite its importance in olive tree nutrition, had very low a coefficient of determination. On the other hand, the phosphorus, sodium and lime scoring functions were based on reaching optimal value. The same trend was observed for organic matter scoring curve but an important decrease takes place after reaching optimal value. Finally, the scoring function for gypsum and electrical conductivity followed the less is better logic (Table 2), i.e. the lower is the amount of gypsum or EC the higher is the leaf carbohydrates content.

Finally, the SFi was calculated by summing the scores for each indicator and dividing the sum by the total number of indicators. The descriptive statistics showed slight high negative skewness which indicate that the SFi distribution shows a long tail on the left. Thus, this parameter is not normally

distributed. Low coefficient of variation (CV = 3.2) was also observed. Indeed, the calculated values vary between 0.8 and 1.

4. Discussion

The soil of the studied area is extremely poor with regards to organic matter content. Indeed, Soyergin *et al.* (2002) stated that the olive trees need at least 1% of OM content to have adequate growth.

Freeman and Carlson (1994) reported an even greater critical limit of 1.5%. The organic matter content is higher in the upper layer of the soil than in depth. Galvez *et al.* (2004), in an olive orchard growing on a toposequence in southern Spain, found that the OM content was relatively higher in the upper horizon than in the underlying ones decreasing from 0.8 to 0.2%, from 0.9 to 0.3% and finally from 1 to 0.5% in the three tested profiles. Nevertheless, Lopez-Granados *et al.* (2002) studied the variation of OM in the horizon 0-10 cm and 10-35 cm in two regions of southern Spain and they did not find significant variation according to depth. In our study the samples were taken from 1 m depth layer, consequently the analytical results represent an average of the contents of surface and deep layers.

Nevertheless, the obtained values are low and, in some SPs, are far under the thresholds. Thus, the studied orchard undoubtedly needs to be enriched in organic matter in order to be able to satisfy plant requirements and to retain, as high as possible, water since it represents the most limiting factor under arid climates. On the other hand, $CaCO_3$ and $CaSO_4.2H_2O$ contents in the studied orchard were adequate for olive tree growth. Indeed, the olive tree tolerates a wide margin of lime content up to very high values (76%). The soil of the orchard is classified as slightly gypsiric (FAO, 2006) since the gypsum content is less than 5%. This condition is favorable to olive tree growth. The lime and gypsum contents showed significant correlation with soil electrical conductivity. The CaCO3-EC correlation is positive and, the lime acts as soluble ion source (FAO, 2006). On the other hand, gypsum-EC correlation is negative which indicate that the presence of gypsum limits the amount of soluble ions.

This result is in contrast with the suggestions of the guidelines for soil description (FAO, 2006). In fact, gypsum (calcium sulphate) is a salt itself, and may increase the salt content. On the other hand, the gypsum may immobilize chloride and sodium in the soil reducing EC, as suggested by Shuman (1993). The significant correlation between EC, exchangeable Na, and chloride is evident since these ions are the main factors increasing the soil electrical conductivity (FAO, 2006). Finally, available P_2O_5 was significantly correlated with OM content. The organic matter is really the principal source of phosphorus in this soil type as suggested by Freeman and Carlson (1994). The development of a model able to integrate all the involved soil parameters seems to be necessary to elucidate this relationship and to estimate the potential growth and yield permitted by soil conditions. The soil fertility index (SFi) developed in this research work tried to integrate all the studied soil parameters. The leaf carbohydrates content was chosen as plant nutrition indicator (dependent variable). However, this parameter, as reported above, did not show high variation in the studied orchard. For this reason the obtained SFi was between 0.83 and 0.98, i.e. a very narrow range. This result clearly indicates the need to widen the sample's size and to find a better indicator of the tree nutritional status plant carrying on with the research work. However, the obtained scoring equations were similar to those obtained by Andrews *et al.*(2002) who worked on annual crops in California. On the other hand, Razaei *et al.* (2005) found a highly consistent index working on annual crops and on a large sample.

In addition, it has to be recognized that the known adaptation mechanisms of trees complicate the establishment of those types of index, which needs long term studies. This establishment of a sound satisfactory soil fertility index may clarify the relationship between soil fertility and plant nutrition highlighting the potentialities of the orchards and, eventually, the need of correction. However, this index clearly needs long-term studies, good indicators and extensive sample. In addition, it seems important, also, to understand the relationship between olive tree nutritional status and olive tree growth, yield and production quality.

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S1-C3

Qualitative and quantitative changes of soil microbial communities as a result of sustainable agricultural practices in an Italian olive grove

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Abstract

The aim of the present work was to evaluate the effects of two soil management systems, so called 'sustainable' (ST) and 'conventional' (CT) on the composition, genetic diversity and carbon substrate utilization of soil microbial communities in a Mediterranean olive orchard. ST system included no-tillage, limited chemical fertilization, and organic matter inputs from drip irrigation with wastewater, spontaneous cover crops and pruning material. CT system was characterized by soil tillage, chemical fertilization, no irrigation and heavy pruning. After seven years of treatments, average olive yield was 8.4 and 3.1 t ha^{-1} yr^{-1} in ST and CT, respectively. CT had a significant higher number of total bacteria and actinomycetes if compared to ST, whereas fungi were significantly lower. In ST, the number of the bacteria involved in the nitrogen cycle isolated from the wetted areas under the drippers (ST-WET) were significantly higher than in inter-row areas (ST-INTER). The patterns of denaturing gradient gel electrophoresis of microbial 16S/18S rDNA showed differences between ST and CT, whereas those of 16S/18S rRNA evidenced that ST-WET clustered separately from CT and ST-INTER. Diversity indexes evaluated by Biolog® assay were significantly different between ST and CT. The results revealed qualitative and quantitative changes of soil microbial communities in response to sustainable agricultural practices that stimulate soil micro-organisms and improve olive yield and quality.

Keywords: sustainable olive growing, cover crops, organic matter, DGGE, Biolog®.

Changements qualitatifs et quantitatifs des communautés microbiennes du sol à la suite de pratiques agricoles soutenables dans une oliveraie italiene

Résumé

L'objectif de ce travail était d'évaluer les effets de deux systèmes de gestion des sols, dits «soutenable» (ST) et «conventionnel» (CT) sur la composition, la diversité génétique et l'utilisation des substrats carbonés par les communautés microbiennes du sol dans une oliveraie Méditerranéenne. ST-système comprenait le non-travail du sol, la fertilisation chimique limitée, et les apports de matière organique à partir de l'irrigation avec des eaux usées, des cultures de couverture et de taille du matériel. CT a été caractérisée par le travail du sol, la fertilisation chimique, ni d'irrigation et de forte taille. Après sept années de traitements, le rendement moyen d'olive a été de 8,4 et 3,1 t ha^{-1} an^{-1} dans le ST et CT, respectivement. CT a eu un nombre plus élevé de l'ensemble des bactéries et les actinomycètes, si on la compare à la ST, alors que les champignons ont été sensiblement plus faible. En ST, le nombre de bactéries du cycle de l'azote isolés du wetted les zones relevant de la goutteurs (ST-WET) étaient significativement plus élevés que dans les zones inter-rangs (ST-INTER). Les dénaturations gradient gel electrophoresis de rDNA 16S/18S ont montré des différences entre les ST et de CT, alors que ceux de 16S/18S rRNA témoigne que ST-WET regroupées séparément de CT et ST-INTER. Les indices de diversité des Biolog® tests étaient significativement différents entre les ST et CT. Les résultats ont révélé des changements qualitatifs et quantitatifs des communautés microbiennes du sol à la suite de pratiques agricoles soutenable qui stimulent les micro-organismes du sol et d'améliorer le rendement et la qualité de l'huile d'olive.

Mots clé: Oliveraie soutenable, couverture végétale, matière organique, DGGE, Biolog®

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1. Introduction

A new approach in olive orchard management is imposed by environmental emergencies, such as soil degradation, water shortage and greenhouse effect (Lal 2004, Hochstrat et al. 2006). Particularly, in semi-arid areas, the use of agronomical techniques able to conserve the natural resources is recommended (Kushwaha and Singh 2005). An integrated approach of culture-dependent and culture-independent methods has provided new tools to study the whole soil microbiota. One of the most useful molecular technique to reveal qualitative genetic (DNA) and functional (RNA) changes in the structure of soil bacterial and fungal communities is based on the characterization of soil-extracted nucleic acids by the amplification of regions of the bacterial and fungal ribosomal RNA gene (16 rRNA

and 18 rRNA, respectively) resolved by denaturing gradient gel electrophoresis (DGGE) (Crecchio et al. 2004). Metabolic microbial community diversity in the structure of soil bacteria communities can be estimated using the Biolog® metabolic assay, based on the ability of microbial isolates to oxidize different carbon and nitrogen sources (Zak et al. 1994). The community-level physiological profiles (CLPPs), obtained by the Biolog® method, are used to differentiate microbial populations from various soil environments or subjected to various treatments (Crecchio et al. 2004).

The present study was performed to explore the effect of sustainable agricultural management systems on genetic, functional and metabolic diversity of soil microbial communities, with a particular emphasis to those involved in nitrogen cycle, by using a combination of culture-dependent and independent methods. The trial was carried out during a 7-year period in an Italian olive orchard under semi-arid conditions. The effects on the productive response of the olive trees and fruit characteristics were also examined.

2. Materials and methods

2.1. Horticultural practices and fruit features

The study was carried out in a mature olive orchard (*Olea europaea* L. - cv Maiatica, a double aptitude variety) located in Southern Italy (Ferrandina - Basilicata Region, 40°29' N, 16°28' E). Olive trees w ere vase trained and planted at a distance of about 8 m x 8 m. The climate in the area is classified as semi-arid. The mean annual temperature ranges from 15 to 17°C. The soil of the experimental grove is a sandy loam (WRB: *Haplic Calcisol*), with a mean bulk density of 1.5 t m^{-3}.

In 2000, the olive orchard was splitted into two plots managed according to sustainable agronomical techniques (Sustainable Treatment - ST) and conventional ones (Conventional Treatment - CT). The ST was irrigated with municipal wastewater treated by a pilot unit and distributed daily from May to October by drip irrigation (6 self-compensating drippers per plant delivering 8 L h^{-1}). Irrigation volume applied over the annual growth season averaged 293 mm (2000-2006). The average annual amounts of N, P, and K distributed by the treated wastewater (293 mm yr^{-1}) were 54, 3 and 50 kg ha^{-1} yr^{-1}, respectively.

The ST soil surface was covered by spontaneous weeds and grasses and mowed at least twice a year. Irrigated trees were lightly pruned each year, in order to improve fruiting potential by controlling the amount of fruiting wood and enhancing flower bud differentiation. Crop residues and pruning material (8.5 t ha^{-1} yr^{-1} dry matter, mean 2000-2006) were left on the ground as mulch. Fertilisers were applied along the growing seasons by a guided fertirrigation, taking into account wastewater and soil chemical composition, and mineral element balance in the orchard system. The CT was grown under rainfed conditions and managed according to the traditional horticultural practices of the area (Xiloyannis et al. 2008), that is by tillage performed 2-3 times per year and mineral fertilization carried out once per year, in early spring, using ternary compounds (NPK 20-10-10 fertilizer at doses ranging from 300 to 500 kg ha^{-1}). In the CT, heavy pruning was performed every two years and pruning residues were burned out of the field.

Fruits were harvested by a trunk shaker and nets. Yield was measured on 12 trees per treatment. Fruit, pulp and stone were dried to a constant weight at 65°C in a forced-draft oven. Pulp percentage and flesh to stone ratio were also determined on fresh weight basis.

2.2. Soil sampling, microbial counts and microbial community metabolic profiles (Biolog®)

In February 2007, three composite samples of bulk soil (20 seven-cm-diameter cores pooled on site per treatment) were randomly collected and immediately stored in sterilized plastic pots at 4℃ after removing visible crop residues. Samples were collected from the top soil layer (0-10 cm) of both treatments, ST and CT. Particularly, in ST soil sampling was performed in the wetted area under the drippers (ST-WET) and in the non irrigated inter-row area (ST-INTER).

Three replicates of 5 g-sub-samples (dry weight equivalent) of each soil sample were suspended in 45 ml sterile 0.1% sodium pyrophosphate-one quarter strength Ringer solution and sonicated for 2 min to disperse microbial cells. Aliquots of ten-fold serial dilutions were spread plated in triplicate on 1/10 strength TSA (Tryptic Soy Agar) medium amended with cycloheximide 0.1 mg ml^{-1} for bacterial counting, and inoculated in MEA (Malt Extract Agar) medium implemented with streptomycin 0.03 mg ml^{-1} and tetracycline 0.02 mg ml^{-1} in triplicate for fungal counting. Counting took place after suitable incubation period (72 h for bacteria and 120 h for fungi) at 28℃. Actinomycetes were isolated by usin g Casein Starch Agar modified supplemented with 0.12 mg ml^{-1} of cycloehximide (Sigma, NY, USA). The isolation of *Azotobacter* was carried out with Brown's substrate modified, whereas the identification of proteolytic bacteria were identified by MPN method in a cultural medium containing gelatine (Oxoid Lim., Hampshire, UK). Ammonifying bacteria were isolated in a liquid cultural medium containing asparagine and incubated at 28℃ for 15 days. *Pseudomonas* were cultured on Pseudomonas Agar Base medium (Oxoid) with the addition of Pseudomonas C-N Supplement (Oxoid).
Sole carbon source utilization patterns of soil microbial communities, also called community-level physiological profiles (CLPPs), were assessed using the Biolog® 96-well Eco-Microplates (AES Laboratoire, France), containing 31 different carbon sources, three times replicated. Data were analysed to determine metabolic diversity indices, including average well colour development (AWCD, the mean of the blanked absorbance values for all the substrates, that provides a measure of total cultural bacterial activity), Shannon's substrate diversity index (H'), substrate evenness (E, equitability of activities across all utilized substrates) and substrate richness (S, the number of utilized substrates), according to Zak et al. (1994). The microplates were incubated at 25℃ in the dark and colour development was measured as optical density (OD) every 24 h over a 144 h period using a Microplate E-Max Reader (Bio-Rad) with a E590-nm wavelength filter, and the data were collected by the Microlog 4.01 software (Biolog, CA, USA).

2.3. Denaturing gradient gel electrophoresis (DGGE)

A direct method was used for DNA and RNA extraction from soil samples by a bead beater system. Samples of 500 mg of soil were processed by FastDNA® Spin Kit for Soil (MP Biomedicals, OH, USA) and RNA Power Soil Isolation Kit (MoBio, CA, USA). Nucleic acids quantity and quality were assayed on 0.7% agarose gel containing 0.5 µg ml^{-1} of ethidium bromide. Extracted RNA was retro-transcribed to c-DNA by RETROscript™ First Strand Synthesis Kit for RT-PCR (Ambion, TX, USA). DNA and c-DNA were amplified in a PCR thermocycler (Bio-Rad Laboratories, CA, USA) with the following primer pairs (MWG-Biotech AG, Germany): i) 968F-1401R for the 16S rDNA gene and ii) FR1-GC and FF390 for the 18S rDNA gene. PCR amplifications were performed according to Crecchio et al. (2004). DGGE was performed by the Bio-Rad DCode™ Universal Mutation detection System (Bio-Rad Laboratories, Hercules, CA, USA). PCR products (10 µl) were loaded into 6% (16S rDNA amplicons) or 8% (18S rDNA amplicons) polyacrylamide gel (37.5:1 acrylamide: bisacrylamide) with an urea-formamide parallel gradient (45-60% for 16S rDNA and 30-60% for 18S rDNA amplicons). Sybr Green I stained gels were photographed with Bio-Rad Gel Doc 2000 documentation system (Bio-Rad Laboratories).

2.4. Fingerprints and statistical analyses

Genetic fingerprints were analysed by the Bionumerics software version 4.5 (Applied Maths, Belgium). The normalization of the profiles in each lane was carried out by loading a standard reference pattern in three different points of the denaturing gel. Profiles comparison and clustering were performed by applying the unweigthed pair-group method using arithmetic average (UPGMA) algorithm, based on the Pearson correlation coefficient (Boon et al., 2002). The values of total and specific microbial groups and Biolog® metabolic indices (AWCD, H', E and S) were treated by analysis of variance (ANOVA).

3. Results

3.1. Tree productive responses, olive characteristics and microbial counts

The olive trees belonging to ST produced almost constantly every year with an average yield of 8.4 t ha^{-1} yr^{-1} (mean 2001-2006) while the CT plants showed a significant ($P < 0.05$) lesser productive level (3.1 t ha^{-1} yr^{-1}) and a strong biennal bearing behaviour with low or no production in 2002, 2004, and 2006 (the so-called "off" years). The starting year of the trial, 2000, was an "off" year for both the examined treatments. Drupes picked from the ST showed a significant amelioration of the their commercial characteristics such as fresh weight, drupe size, pulp percentage, and pulp to stone ratio, which are important parameters for table olives increasing their market value (Table 1).

Table 1: Fruit characteristics and pulp to stone ratio (mean 2001-2006 ± SD) in sustainable (ST) and conventional (CT) treatments. Values with asterisks are significantly different at $P < 0.05$.

Parameter	Unit of measure	ST		CT
Fruit fresh weight	(g)	3.8 ± 0.92	*	2.3 ± 0.78
Longitudinal fruit diameter	(mm)	23 ± 2.17	*	20 ± 2.88
Equatorial fruit diameter	(mm)	17 ± 1.66	*	14 ± 1.79
Pulp	(% on fresh weight basis)	85 ± 3.89	*	78 ± 5.03
Pulp/stone ratio	(on fresh weight basis)	5.8 ± 1.54	*	3.8 ± 1.20

The different soil treatments significantly affected both total cultivable bacteria, significantly lower in ST-WET and ST-INTER ($P < 0.01$), and total fungal counts, significantly lower in CT if compared to the two ST treatments ($P < 0.01$) (Fig. 1a, b). The number of actinomycetes was significantly higher in CT if compared to the two ST treatments ($P < 0.05$), and significantly higher in ST-INTER than in ST-WET ($P < 0.05$) (Fig. 1c).

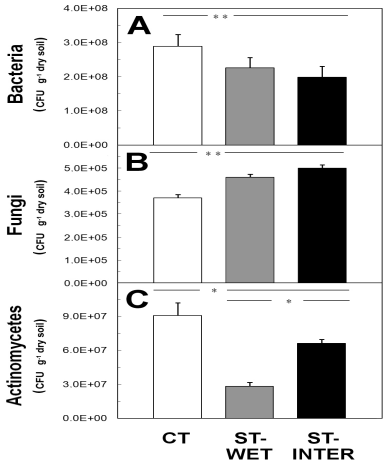

Figure 1: (a) Total bacterial, (b) fungal, and (c) actinomycetes counts in the three treatments: conventional (CT; white bars), sustainable under the drippers (ST-WET; grey bars), sustainable in the inter-row area (ST-INTER; black bars). The values represent the average (± SD) of three independent replicates for each soil treatment. Significance levels:
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

The number of ammonifying bacteria, proteolytic bacteria and *Azotobacter* isolated from ST-WET treatment was significantly higher than in ST-INTER ($P < 0.01$, $P < 0.01$ and $P < 0.001$, respectively) (Fig. 2a, b, c). Moreover, CT significantly differ from the two ST soils both for ammonifying bacteria ($P < 0.05$) and *Azotobacter* ($P < 0.01$) (Fig. 2a, c) being the number of microorganisms in both cases intermediate between ST-WET and ST-INTER. *Pseudomonas* counts were not significantly different between CT and ST neither between ST-WET and ST-INTER (Fig. 2d).

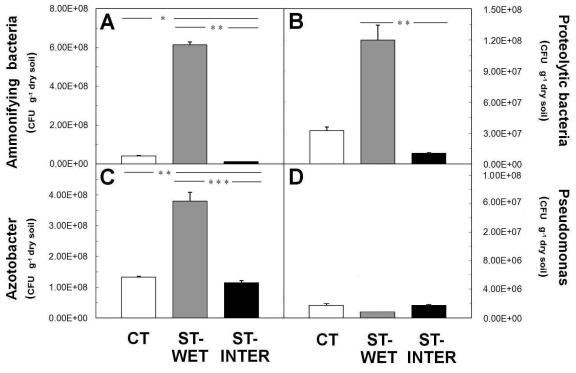

Figure 2: Ammonifying bacteria (a), proteolytic bacteria (b), *Azotobacter* (c) and *Pseudomonas* (d) in the three treatments: conventional (CT; white bars), sustainable under the drippers (ST-WET; grey bars), sustainable in the inter-row area (ST-INTER; black bars). Statistics as in Figure 1.

3.2. Genetic, functional and metabolic fingerprinting

The genetic dendrograms of bacterial 16S rDNA and fungal 18S rDNA showed that molecular patterns of CT were discriminated from patterns of the sustainable treatments (ST-INTER and ST-WET) (Fig. 3a, b). Anyway, Pearson similarity coefficients for 16S rDNA, ranging from 95.1 to 98.5, indicate that electrophoretic profiles relative to bacterial community, were quite similar (Fig. 3a). On the other hand, functional DGGE patterns of rRNA evidenced that irrigated sites under drip emitters (ST-WET) clustered separately from CT and ST-INTER both for bacteria and fungi ribosomal genes (Pearson coefficient = 88.4 and 33,7 respectively) (Fig. 3c, d). The values of Biolog® metabolic indices showed that AWCD and H' were significantly affected ($P < 0.01$ and $P < 0.05$, respectively) by soil treatment (ST vs. CT) (Fig. 4a, b). Moreover, ST-WET significantly differs from ST-INTER soils both for AWCD ($P < 0.01$) and H' ($P < 0.05$) (Fig. 4A, B). The values of E and S showed no significant differences between CT and ST neither between ST-WET and ST-INTER (Fig. 4c, d).

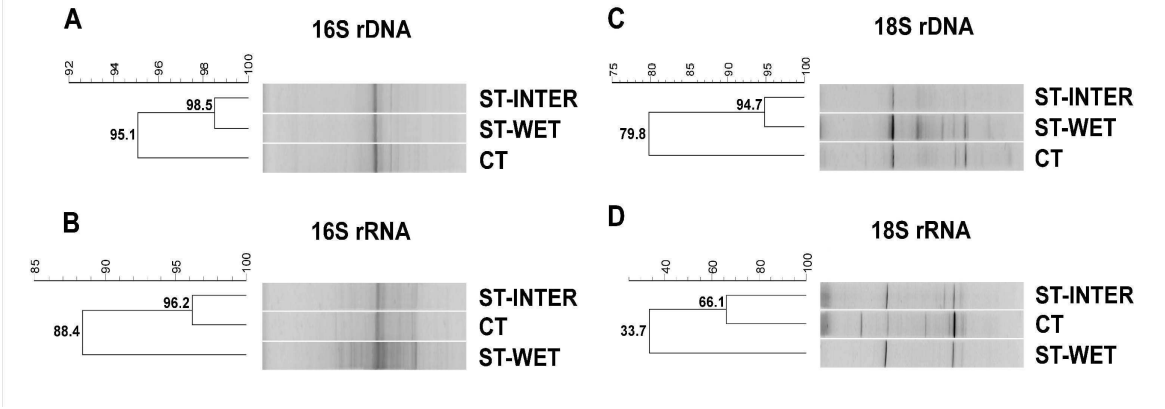

Figure 3: Genetic 16S DGGE fingerprints of soil bacterial communities (a, b) and functional 18S DGGE fingerprints of soil fungal communities (c, d) in the three treatments: conventional (CT), sustainable under the drippers (ST-WET), sustainable in the inter-row area (ST-INTER).

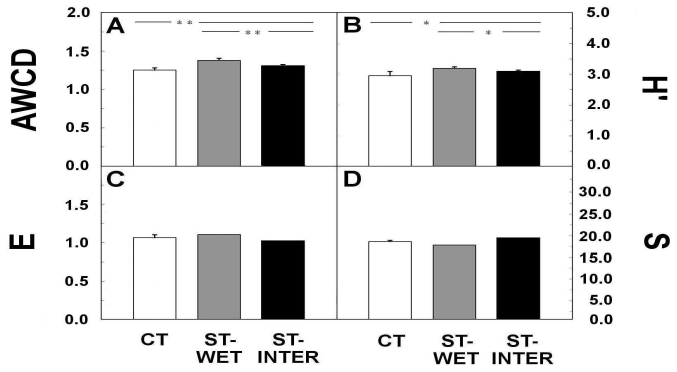

Figure 4: (A) Average well colour development (AWCD), (B) Shannon's substrate diversity index (H'), (C) substrate evenness (E), and (D) substrate richness (S) in the three treatments: conventional (CT; white bars), sustainable under the drippers (ST-WET; grey bars), sustainable in the inter-row area (ST-INTER; black bars). Statistics as in Figure 1.

4. Discussion

The better yield level and fruit quality showed by the olive trees subjected to the ST (Table 1) can be clearly attributed to the adopted orchard management system. First of all irrigation that provided both water and mineral elements supply. In fact, the reclaimed wastewater used in this trial was rich in nutrients (especially N, P, and K) (Table 1), which were distributed by water along the irrigation season and integrated, when it was necessary, by fertirrigations based on orchard requirements.

Total fungal number was significantly higher in ST sites (Fig. 1b), likely because soil fungi rely on external available nutrients (Govaerts et al. 2008) and so respond promptly to changes in organic nutrient matter deriving from cover crops and wastewater irrigation/fertirrigation. The three different orchard management systems also caused significant differences in total bacteria (Fig. 1a).

Particularly interesting are the effects of agricultural management on the number of some of the bacteria involved in nitrogen cycle (Fig. 2). In fact, the results showed that ST-WET site has a higher number of *Azotobacter*, proteolytic and ammonifying bacteria when compared to ST-INTER (Fig. 2a, b, c). The number of *Pseudomonas*, denitrifying bacteria that transform anaerobically nitrates to nitrogen gas or nitrous oxides, was not significantly different between treatments (Fig. 2d). The particular conditions of ST-INTER soils with respect to to ST-WET were reflected by the significantly higher actinomycetes number (Fig. 1c). In fact, actinomycetes (e.g., *Streptomyces*) produce a number of enzymes that help degrade organic plant material, such as lignin and chitin, and so are abundant in soils rich of organic inputs, as ST-INTER soils.

In our study, this effect is clear in both 16S and 18S rDNA genetic DGGE dendrograms, that revealed a discrimination between CT and ST systems (Fig. 3a, c). As for the fungal counts, the effects on bacterial community structures were very likely due to the input of organic matter deriving from cover crops (ST-WET and ST-INTER) and wastewater irrigation/fertirrigation (ST-WET). The observed differences between CT and ST soils were likely due also to the two soil management regimes. In fact, zero tillage with residues recycling and mulching result in a soil with good physical and chemical qualities, and high, stable yields, compared to conventional tillage (Govaerts et al. 2008). In contrary, differences in functional DGGE dendrograms of both 16S and 18S rRNA, that reflect the status of metabolically active microorganisms, indicated that the irrigation regime is the main factor inducing changes in bacterial and fungal communities of sites under the emitters (ST-WET) (Fig. 3b, d).

Soil bacterial metabolic diversity indices estimated by Biolog® CLPP are usually higher in sustainable than in conventional soils (Govaerts et al. 2008). Our results show that the values of AWCD and H' were significantly increased by the ST (Fig. 4a, b). In addition, comparing the two sustainable treatments, AWCD and H' were higher in ST-INTER soils (Fig. 4a, b), where cover crops could be an important discriminating element for microbial substrate utilization. Indices of netabolic diversity do not necessarily reflect the composition of the bacterial communities as two communities can have the same H' value but utilize different substrates.

Our results demonstrated that soil microorganisms respond to the application of a sustainable orchard management with evident benefits for olive yield and quality (Table 1). Sustainable orchards showed a higher microbial complexity and diversity. The study of the response of soil microbiota to different agricultural management systems and the quantitative and qualitative analysis of soil microbial communities could lead to identify agricultural practices that support and stimulate soil micro-organisms in order to improve orchard production.

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S1-C4

Soil management and soil water balance in a mature rainfed olive orchard

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Abstract

An experiment was performed for a 3-year period in a rainfed olive grove located in Southern Italy to evaluate the effect of different soil management techniques on Soil Water Content (SWC). The compared treatments were Sustainable System SS (non-tillage, spontaneous vegetation cover, annual recycling of pruning material) versus Conventional System CS (tillage, no pruning material recycling). Surveys on soil structure and hydrological behaviour were performed. SWC was measured by Bouyoucos blocks placed at different soil depths. Ten years of sustainable soil management increased the storage of rainfall water particularly in the deeper layers. In SS soil macroporosity was higher than CS system and homogeneously distributed along the profile, favouring the vertical water movement down to deeper horizons. In CS the occurrence of soil crusting and compacted layers hindered infiltration and percolation of rainfall water.

Keywords: sustainable olive growing, cover crops, organic matter recycling, soil macroporosity, soil water holding capacity

Gestion comparée de matière organique et de l'eau dans le sol des oliveraies soutenables ou conventionnelles

Résumé

La recherche a été effectuée pendant 3 ans dans une oliveraie non irriguée, située ou Sud de l'Italie, afin d'évaluer l'effet des différentes techniques de gestion sur l'humidité du sol (SWC). On a évalué les effets sur la structure et sur les caractéristiques hydrologiques des sols d'un Système Soutenable SS (organique) et d'un System Conventionnel SC (travail du sol) de gestion du sol. Dix ans de gestion soutenable du sol ont augmenté la capacité de stockage de l'eau des précipitations pour la plupart des couches en profondeur. Dans le SS la valeur de la macroporosité du sol n'est pas élevée, mais elle est répartie de façon homogène en direction du profil, de telles façons à favoriser le mouvement vertical d'eau en profondeur. D'autre part, dans le SC, l'encroûtement et le compactage de la couche superficiel du sol ont empêché l'infiltration et la percolation des eaux de pluie.

Mots clés: Oliveraie soutenable, couverture végétal, recyclage matière organique macroporosité du sol, capacité de rétention d'eau du sol.

1. Introduction

Olive tree (*Olea europaea* L.) is the most widespread crop of the Mediterranean Sea Basin where it grows in semi-arid climates. Since ancient times olive tree is grown under rainfed conditions due to its ability to tolerate drought stress (Xiloyannis et al. 1999; Sofo et al. 2007).

Mechanical tillage is the most common technique used for soil management in olive orchards. Such practice is essentially performed to hinder weed competition for water and nutrients and to reduce soil evaporation by interrupting water capillary rise and augmenting soil surface roughness (Ozpinar and Cay 2006). On the other hand, continuous tillage induces oxidative soil metabolism (high organic matter mineralization) negatively affecting soil physical and hydrological features, such as porosity, soil structure and aggregation. This condition reduces water infiltration rate and triggers runoff and erosion processes (Abid and Lal 2009). Such phenomena are really serious in olive orchards particularly when they are placed on steep lands (Kosmas et al. 1997; Raglione et al. 1999; Francia Martínez et al. 2006; Fleskens and Stroosnijder 2007; Gómez et al. 2009).

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A spontaneous vegetation cover in the olive orchards represents an alternative soil management able to overcome the cited disadvantages linked to the mechanical tillage. Particularly, many researchers reported an improvement of soil water moisture in cover cropped olive groves (Hernandez et al. 2005; Durán-Zuazo et al. 2009). Nevertheless, such environment-friendly practice is not common in semi-arid areas where soil water availability represents the limiting factor for crop production. Instead, under such limiting climatic conditions, more efforts should be addressed to store rainfall water in the soil and to reduce runoff losses.

An experiment, lasted 3 years, was performed in a mature rainfed olive grove located in Southern Italy to evaluate the effect of two different soil management systems, Sustainable (cover cropped with spontaneous species) and Conventional (continuously tilled), on Soil Water Content (SWC). In the Sustainable System, cover cropping was combined with the recycling of olive pruning material, an important source of organic matter internal to the olive grove.

2. Materials and methods

Experimentation was carried out for a 3-year period (2007-2009) in a rainfed olive grove located in Southern Italy (Ferrandina - Basilicata Region, 40°29' N, 16°28' E). Mature trees (*Olea europaea* L. - cv Maiatica) were vase trained and planted at a distance of about 8 m x 8 m. The climate in the area is classified as semi-arid with an annual rainfall of 561 mm (mean 1976-2006). The mean annual temperature ranges from 15 to 17°C.
The soil of the experimental grove is a sandy loam classified as a Haplic Calcisol (FAO WRB 1998). In 2000, the top 60 cm soil layer had a low content of organic matter (12 g kg^{-1}). The key meteorological parameters (air temperature, rainfall, humidity, etc.), were measured daily in 2006, 2007, 2008, and 2009 by a standard weather station close to the trial area. The reference evapotranspiration (ETo), provided by SAL service of the Extension Regional Service (www.alsia.it), was estimated using Blaney-Criddle, radiation, and Hargreaves methods (mean value).

Since 2000, the olive orchard was split into two parts subjected to different soil management system: Sustainable System - SS, and Conventional System – CS. In the SS, the soil surface was covered by spontaneous weeds and grasses which were mowed at least twice a year. Olive trees belonging to the SS were lightly pruned each year. Cover crops and pruning were cut and their residues were left on the ground surface as mulch. The natural vegetation together with pruned material and senescent leaves ensured an annual C distribution of about 5 t ha^{-1}.
The soil of the CS was managed by tillage (milling at 10 cm soil depth) performed 2-3 times per year in order to keep the soil bare. Heavy pruning was carried out every two years but pruned residues were burned out of the olive grove.

With the aim to characterize soil structure, vertically orientated thin sections (5.5 x 8.5 cm) were obtained from undisturbed soil samples collected in November 2007 at different depths (0-10, 10-20, 20-30, 30-40, and 40-50 cm) along the profile in the two different management systems (60 soil thin sections in total). The images were analyzed using the Image-Pro Plus software produced by Media Cybernetics (Silver Spring, MD, USA). Total porosity and pore distribution were measured according to pore shape and size, the instrument being set up to measure pores larger than 50 □m. Pore shape was expressed by a shape factor [$perimeter^2/(4\pi \cdot area)$] so that pores could be divided into regular (more or less rounded) (shape factor 1-2), irregular (shape factor 2-5), and elongated (shape factor >5). These classes correspond approximately to those used by Bouma et al. (1977). Pores of each shape group can be further subdivided into size classes according to either their equivalent pore diameter (regular and irregular pores), or their width (elongated pores) (Pagliai et al. 1984). According to this procedure, the elongated transmission pores (50-500 □m) (Greenland 1977) were quantified. Thin sections were also examined using a Zeiss 'R POL' microscope at 25X magnification to observe soil structure.

Saturated hydraulic conductivity was measured *in situ* using a constant head well permeameter (Model 2800 Guelph Permeameter - Soilmoisture Equipment Corp., Santa Barbara, USA). The measurements were made in May 2007 at 12 cm depth with at least three replicates. In order to evaluate only the vertical water flux, measurements were carried out in confined well by a plastic tube (diameter = 5.5 cm). As the well radius changes respect to the standard condition (radius was 2.25 cm instead of 3.0 cm), the well shape factor C (Reynolds et al., 1986), used in the calculation, was modified according to Zhang et al. (1998).

Volumetric Soil Water Content (SWC - mm mm^{-1}) was measured by means of electrical resistance blocks (Eijkelkamp) installed in November 2006 at different soil depth (25, 50, 75, 100, 150, 200 cm) using a pneumatic driller opening holes of 40 mm. A portable, battery-operated ohmmeter was used to measure resistance. Field measurements were carried out from January 2007 at 10/15-day intervals. Previously, the electrical resistance blocks were calibrated in the laboratory in order to find the best relationship between the measured resistance values and the volumetric soil moisture content of the studied soils.

3. Results and discussion

3.1. The pluviometric pattern of the experimental years

The pluviometric pattern, recorded in the studied area, was quite different among the experimental years. Assuming that the soil water reserve was restored by the precipitation falling from October to March ("recharge period"), the rainfall values recorded in such period were the following: 243.4 mm from October 2006 to March 2007; 288.6 mm from October 2007 till March 2008; 599.6 mm from October 2008 to March 2009. Precipitation felt during the vegetative season (April-September) was 287.8, 248.8, and 376.0 mm in 2007, 2008, and 2009, respectively.

3.2. Soil structure and hydrological characterization

Soil structure characterization by image analysis of thin sections showed similar macroporosity values between the SS and CS treatments at 0-10 cm depth (Figure 1). Conversely, below the 10 cm depth, the macroporosity of the CS drastically decreased to values smaller than 5%, so identifying a very compact soil according to Pagliai (1988). Such condition is a clear effect of the continuous tillage. In addition, the reduction of macroporosity in the CS essentially affected the transmission elongated pores (size class 50-500 µm) which are directly involved in water fluxes within the soil (Figure 1). On the contrary, macroporosity in the SS was homogeneously distributed along the profile, affecting positively water movement in the soil (Figure 1).

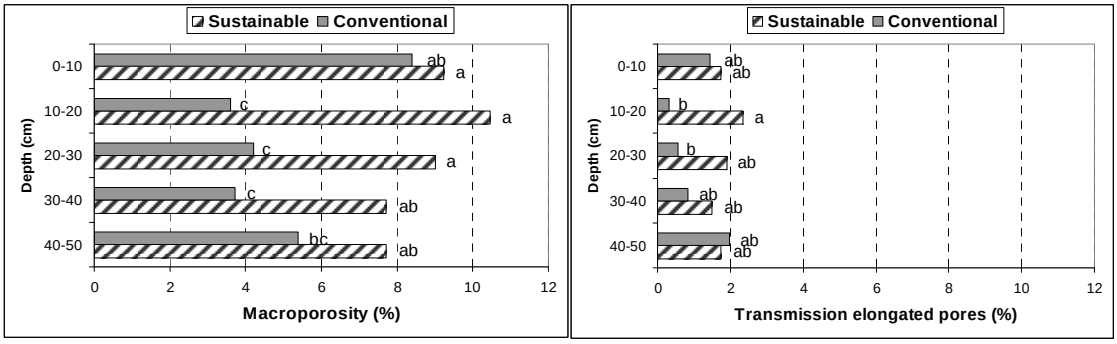

Figure 1: Macroporosity (pore size class: >50 µm) and transmission elongated pores (pore size class: 50-500 □m) at different soil depths in the SS and CS. Bars marked with the same letter are not different (P<0.05) by Duncan's multiple range test.

Differences between the soil management systems were found for K_{sat} measured at 12 cm depth according to the standard procedure for Guelph Permeameter. K_{sat} value was 0.31±0.16 m d^{-1} (mean ± standard deviation) in the SS and 1.08±0.77 m d^{-1} in the CS setting the former in the medium-low K_{sat} class, the latter in the medium class (Rossi Pisa 1997). The highest K_{sat} values measured in the CS were probably due to the recent tillage practice that increased macroporosity of surface horizon. Nevertheless, K_{sat} measurements performed to evaluate the vertical water flux (confined) showed a significant reduction in the CS being equal to 0.013±0.007 m d^{-1} (K_{sat} class: very low) confirming the presence of a ploughpan, as demonstrated by the drastic reduction of the soil macroporosity below the 10 cm layer (Figure 1). In SS, K_{sat} confined was, instead, 0.16±0.07 m d^{-1} lower than the value measured adopting the standard procedure but belonging to the same saturated conductivity class (K_{sat} class: medium-low) (Rossi Pisa 1997).

3.3. Volumetric Soil Water Content (SWC)

SWC was affected by the soil management systems as well as by the different pluviometric pattern recorded in the studied years. As mentioned before, the climatic pattern recorded in 2007 and 2009 was substantially different so providing the occasion to study the response of the two examined soil management systems under diverse pluviometric conditions.

SWC (mm mm^{-1}), measured at different depths in two successive survey dates of the "recharge period" in 2007 and 2009, was reported in Table 1 and 2, respectively.

Generally, SWC was higher in the SS than in CS. Differences between the soil management systems were more evident below the 0-50 cm layer in both the considered years (Table 1 and 2).

At the end of the previous vegetative seasons, November 2006 and October 2008, SWC in both the SS and the CS was the same along the soil profile and at the lowest level (around the wilting point measured at -1.5 MPa). On January 9th 2007, the recharge of rainfall water, fallen from October 2006 (70.0 mm), was evident in the CS soil only in the upper 50 cm (Table 1). Conversely, the soil of the SS not only showed a good moisture level at the 0-50 cm depth but also a gradual water enrichment along its profile. At the end of March 2007, SWC in the CS soil slightly increased especially in the 0-100 layers (31 mm) while in the deepest layers the increase was not important (2.6 mm). In the SS, a moisture increase of 89.6 mm was recorded in the 0-200 cm layer. About the 94% of such increase occurred in the layers below 50 cm (Table 1). The SS was able to retain an amount of water greater of about 45% than that stored by the CS.

Table 1: Soil Water Content (SWC, mean ± standard error) measured 09-01-2007 (t0) and 29-03-2007 (t1) at different soil depths in the SS and CS. Rainfall and ETo of the considered period: 149.0 and 142.5 mm, respectively.

Soil layer (cm)	**SS**			**CS**		
	SWC (t0)	**SWC (t1)**	**SWC (t1 – t0)**	**SWC (t0)**	**SWC (t1)**	**SWC (t1 – t0)**
	mm			Mm		
0 – 50	103.6±7.0	108.6±5.3	5.0	75.1±5.1	85.6±5.4	10.5
50 – 100	84.7±13.5	115.7±1.5	31.0	38.7±1.0	59.2±7.2	20.5
100 – 150	70.0±6.0	104.3±8.6	34.3	37.7±0.0	39.0±1.3	1.3
150 – 200	60.8±5.8	80.1±10.0	19.3	37.7±0.0	39.0±1.3	1.3
Total 0-200	319.1	408.7	89.6	189.2	222.8	33.6

Rainfall recorded from October 2008 till January 4th 2009 was equal to 342.0 mm. Unlike in the previous case, such amount of rainfall was able to wet the soil of the CS at the 0-100 cm depth. At the end of the "recharge period" (March 2009) the soil moisture in the CS was greater than that recorded in 2007 showing a total moisture increase equal to 127.9 mm (Table 2). In the CS a water infiltration was also observed along the soil profile till the deepest layers. On the other hand, the soil of SS reached an optimum water level in all the soil depths already at the beginning of 2009. As a matter of fact, the water increase recorded at the end of March 2009 was of only 7.3 mm (Table 2).

Table 2: Soil Water Content (SWC, mean ± standard error) measured 04-01-2009 (t0) and 31-03-2009 (t1) at different soil depths in the SS and CS. Rainfall and ETo of the considered period: 260.2 and 139.9 mm, respectively.

Soil layer (cm)	SS			CS		
	SWC (t0)	SWC (t1)	SWC (t1 – t0)	SWC (t0)	SWC (t1)	SWC (t1 – t0)
	mm			Mm		
0 – 50	109.7±1.0	110.9±1.4	1.2	94.8±4.2	102.1±2.8	7.3
50 – 100	110.0±1.6	110.0±1.2	-0.1	66.5±7.0	91.2±2.8	24.7
100 – 150	106.0±0.4	111.1±2.2	5.1	37.7±0.0	90.3±1.2	52.6
150 – 200	109.0±4.3	110.1±1.5	1.0	37.7±0.0	80.9±11.8	43.2
Total 0-200	434.7	442.0	7.3	236.6	364.5	127.9

4. Conclusions

Under these experimental conditions, the soil management systems and the annual pluviometric pattern strongly influenced the SWC.

From the obtained data, it is evident that the SS had a higher capacity to store autumn-winter rainfall water than the CS even when the precipitation fallen in this period was scarce. The best structural features found in the SS soil improved the water infiltration so facilitating and speeding the recharge also of the deeper layers which, mostly, supply water to the olive roots under rainfed conditions. In addition, the natural vegetation, while consuming water from the surface layers during the autumn-winter period, increased the water recharge in the soil profile.

Acknowledgments

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S1-C5

Occurrence of *Clostridium perfringens* in olive tree cultivated soils, edible olives and olive oils

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Abstract

Olive tree culture is of important economic significance for Mediterranean countries. The increased knowledge on the soil microflora has raised the question of their role in soils dedicated to the culture of olive tree (*Olea europaea*) and their subsequent presence in olive tree products due to the particular harvesting practice. Among the anaerobes, our interest is focused on *C. perfringens* which is widely distributed in nature and is present in large numbers in the soil, sewage, and in the human and animal intestinal tract and could be served as an indicator of olive products hygienic quality. Thus, 100 samples from olive tree cultivated soils, 50 samples of edible olives and 50 samples of olive oil were collected and examined for the presence *C. perfringens* vegetative and spore forms as well as for other bacterial species. Additionally, all isolated *C.perfringens* strains were tested for their antimicrobial activities against eight antibacterial agents. In soils, C*. perfringens* spore forms were found more frequently (65%), comparing to *C. perfringens* vegetative forms (25%). *C. perfringens* was recovered from 20% of the edible olives samples and from 3% of oil samples, indicating that a systematic monitoring required in order to obtain adequate information for assessing overall quality from public health point of view.

Key words: *C.perfringens,* olive harvesting practice, soil microflora, table olive.

Fréquence de *Clostridium perfringens* au sol de culture d'olivier, aux olives comestibles et à l'huile d'olive

Résumé

La culture de l'arbre d'olivier est d'une importance économique significative pour les pays de la Méditerranéenne. Le rôle des microorganismes au niveau du sol de culture de l'arbre d'olivier (*Olea europeae*) et leur présence aux produits recueillis semble être en connexion avec la technique de récolte. Notre intérêt a été porté sur la bactérie anaérobie *C.perfringens* qui est un germe tellurique, isolé aussi bien du sol et des égouts, ainsi qu'a partir de la flore intestinale humaine et animale. Ce microorganisme pourrait servir comme un indicateur excellent de la qualité hygiénique des produits oliviers. Dans ce but ,100 échantillons du sol de culture d'olivier,50 échantillons d'olives comestibles et 50 échantillons d'huile d'olive ont été recueillis et étudiés pour la présence des formes végétales et sporulées de *C.perfringens*, ainsi que la présence d'autres espèces bactériennes. De plus, nous avons recherché l'activité antimicrobienne des souches *C.perfringens* sur 8 agents antibactériens. La fréquence de formes sporulées de *C.perfringens* aux sols de culture d'olivier semble être supérieure (65%) par rapport aux formes végétales isolées (25%). *C.perfringens* a été isolé dans 20% des olives comestibles et seulement dans 3% des échantillons d'huile d'olive. Il est évident que la technique de récolte ainsi que le suivi systématique de la qualité hygiénique jouent un rôle crucial pour l'arbre d'olivier et ses produits.

Keywords: *Olea europea*, soil, *Clostridium perfringens*, olives, olive oil.

1. Introduction

Olive tree (*Olea europaea*) cultures are of important economic significance for Mediterranean countries, as both agricultural and industrial activities are promoted. In Greece, olive tree plantation keeps a capital role as extended areas of the country are reserved to this culture, which is used both for edible olive and table oil production. Moreover, edible olive as well as table oil consists of the basic exportation product of the country in all over the world.

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Albeit its importance in exportation item, olive and oil production, remains traditional in most areas. Olives are harvested by beating the tree braches with wooden poles to drop them to the ground from where they are collected. Storage of olives before any other processing is the usual practice in countryside. If during storage, water activity and temperature are modified, mould spores could grow in olives were be able to produce toxinogenic substances, as aflatoxins or ochratoxins (Bezirtzoglou E, 2002; Roussos S, 2006). Presence of toxinogenic substances not only decreases the oil olive quality, but in elevated concentrations could threatened the consumers health.

Another important tool for production of quality olive products, is the soil nature where olive trees are cultured. Soil microflora represents a high diversity and seems to be influenced by the use of pesticides, fertilizers and other techniques applied for the culture as the use of treated wastewater (Bezirtzoglou E, 2006). The recent increased knowledge on the anaerobic soil microflora has raised the question of their role in soils dedicated to the culture of olive tree and their subsequent present in olive products.

Among the anaerobes species our interest is focused on *Clostridium perfringens*, which is widely distributed in nature and is naturally present in large numbers in the soil (Matches JR, 1974; Bezirtzoglou E, 2006) sewage and in the human and animal intestinal tract (Bezirtzoglou E, 1996; 1997), where it occurs in both the vegetative and resistant spore forms.

2. Material and Methods.

A 100 samples of soil were collected, coming from the North-West part of Greece, including the geographical areas of Arta (n:25), Preveza (n:25), Ioannina (n:25) and Thesprotia (n:25) (Figure 1). Our samples consist of cultivated soil dedicated entirely to the cultivation of the *Olea europaea.*

All 100 samples from cultivated soil were collected aseptically, in a plastic bag during the harvesting season. Triplicate samples of 500g were collected by the aid of a sterilized spoon instrument at a depth of 10 to 30 cm below the soil surface, in a way that the sample soil include also the cultivated bulb. The soil pH is reported between 4.5-6.5. Subsequently 50 g of thoroughly mixed soil specimen was brought aseptically in a flask. In this same flask, the surface of the cultivated product was scraped from soil and washed out by 450 ml of PBS. Samples were mixed well by vigorous manual shaking before performing any analysis. Samples for physicochemical analysis were placed in 500 ml bottles. At the laboratory, all samples were stored at 5 ± 2^0 C until analyses were completed. All analyses were completed within 24 h of sample collection. All samples were alternatively passed through two sterile membrane filters. The first (20 μm pore size) was used for retention of the soil impurities and the second (0.45 μm) for *C. perfringens*. The growth medium used was lactose-sulfite (L.S. broth) (Bezirtzoglou and Romond, 1990).

Figure 1: Geographical Departments of SW Greece included in our study.

Initially, the membranes were placed into the first tube of a ten-fold dilution series and further diluted to 10^4, and incubated aerobically in a water bath at 46^0 C for 24 h (Figure 2). A portion of each sample was heated for 15 min at 80^0 C and seeded into lactose – sulfite broth to detect spores. Interpretation of results was based on the clouding of the medium from lactose fermentation, presence of a black precipitate (iron sulphate) and gas visible in the inverted Durham tubes within 24 h at 46^0 C.

All isolated *C. perfringens* strains were tested for their antimicrobial activities in Mueller –Hinton agar by applying the following antibiotic discs: amoxicillin (25μg), penicillin G (10 units), kanamycin (30 μg), tetracycline (30μg), streptomycin (10μg),erythromycin (15 μg), chloramphenicol (30 μg) and metronidazole(15μg).

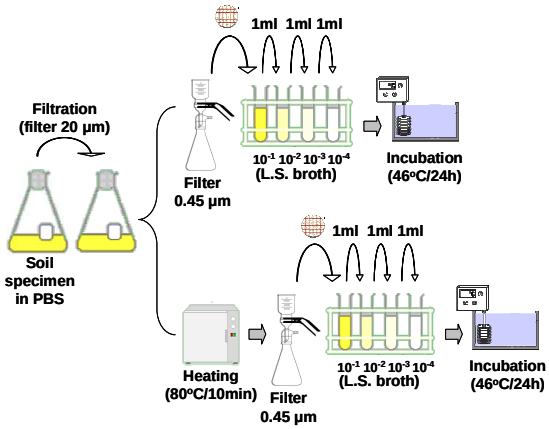

Figure 2: Schematical representation of experimental protocol.

Olive samples (25 g) were collected aseptically in sterilized stomacher plastic bags and transferred to laboratory. After the addition of 225 ml sterile Ringer's solution the samples were homogenized for 5 min by the aid of a stomacher. The rest of the procedure is similar to the one already described for soil.

Olive oil samples were collected from the oil mills in sterile falcon tubes and transferred to the laboratory, diluted (1:5) with warm (35^{o}C) Ringer's solution and homogenized for 5 min before heating and seeding in L-S broth as above.

3. Results

In soil samples, a 65% mean presence of *C. perfringens* spore forms was observed, comparing to the significantly lower presence (25%) of vegetative forms. In Table 1, similar positivity pattern was observed for the area of Arta (72%) and Preveza (68%) in contrast to the those of Thesprotia (60%) and Ioannina (60%) where the positivity percentage of *C. perfringens* were lower. In Table 2 we report the percentage of resistance for *C. perfringens* against the eight antibiotics.

Ten olive samples (20%) and one olive oil sample (2%) were positive for the presence of *C. perfringens* spore forms while no vegetative forms were detected.

Table 1: Occurrence of *C. perfringens* in all sampling areas.

Area	Number of Samples (n)	Vegetative forms	Spores	Total
Arta	25	24%	60%	72%
Preveza	25	16%	56%	68%
Ioannina	25	16%	52%	60%
Thesprotia	25	12%	60%	60%
Mean frequency		17%	75%	65%

Table 2: Percentage of resistant strains of *C. perfringens*.

Agent	Resistant (%)
Amoxicillin	80
Penicillin	70
Kanamycin	53
Tetracyclin	54
Streptomycin	25
Erythromycin	29
Metronidazole	65
Chlorampenicol	54

4. Discussion

The extraction and use of olive oil in the Mediterranean area exists for more than 6000 years and it is linked tightly to the Mediterranean culture, history and habits (Aragon JM and Palancar MC, 2000; Niaounakis M and Halvadakis CP, 2004). Greece is sharing a major position as one of the largest olive producers in Mediterranean area together with Spain, Italy, Turkey, Franch and Tunisia. The Mediterranean area alone provides 98% of the total surface area for olive tree culture and total productive trees, as well as 97% of the total olive production (Niaounakis M and Halvadakis CP, 2004). Constructively, olive products consists of an important economic sector for the European Community and there is a need for extensive knowledge on the olive tree cultivation, with the microbiological aspects included (Boskou D, 1996).

The soil surface consists of an aerobic environment, which comes in disagreement with *C. perfringens* anaerobic nature. However, *C. perfringens* was found in noticeable levels in this study (*Mean frequency:* 65%). Cultivated soils are often associated with animal pasture, manure fertilization and also irrigation with treated wastewater. Thus, these are strong indications of faecal contamination and subsequently occurrence of C*. perfringens* since this organism is a natural intestinal inhabitant (Simon MTP, 1985). Other investigators have shown that this organism can also exist and multiplied in the soil as *nearly* vegetative forms without the necessity of faecal contamination. Moreover, *C. perfringens* possess a specialized enzymatic equipment (Jean, 2004) which may explain how the germ can survive occasionally oxidative stress (Pan et al., 2001; Imlay, 2002). Our results showed that C*. perfringens* vegetative cell recoveries, were lower than spore densities in all cultivated areas, and this was attended to the strict anaerobic nature of the germ. Furthermore, the use of the L.S. broth for *C. perfringens* identification offers considerable advantages as it can detect small numbers of this microbe in samples that also containing a large density of other bacteria, including other clostridia species (Bezirtzoglou et al., 1990). Therefore L-S consists of a rapid and simple technique to differentiate *C. perfringens* bacterial communities of special interest to public health.

All strains of *C. perfringens* were tested for their susceptibility to the following antibiotics; amoxicillin (25μg), penicillin G (10 units), kanamycin (30 μg), tetracycline (30μg), streptomycin (10 μg), erythromycin (15 μg), chloramphenicol (30 μg) and metronidazol (15 μg).

Most of our strains showed resistance (Table 2) to the above antibiotics applied usually for human and veterinary care. Amoxicillin (80%) and penicillin (70%) exhibited the highest percentages of resistance followed by metronidazol (65%), tetracycline (54%), chloramphenicol (54%), kanamycin (53%), erythromycin (29%), and finally streptomycin (25%).

Fermented foods have generally been considered less likely to be vehicles for foodborne infection or intoxication than fresh foods because of the competitive activity and metabolites of the functional microbiota (Panagou EZ, 2006). According to the standard of the IOOC (IOOC, 2004), olive products, when tested by appropriate methods of sampling must be free from pathogenic and spoilage microorganisms as well as toxins that may impose a risk to human health. As our results showed, a minimum percentage of end –product (i.e. edible olives and olive oil) were found positive to spores of *C. perfringens* indicating that under improper handling, storage and distribution there is a risk for consumers.

Organic pollution of the cultivated soil is mainly associated with pesticides, herbicides and fertilizers used in the agriculture. It is then conceivable that the presence of *C. perfringens* as bacterial indicator depend not only on physicochemical conditions, but it is mainly associated with the nature of the organic pollution. Systematic monitoring of the cultivated soil ecosystems must include bacteriological parameters together with chemical indices of organic pollution in order to obtain information adequate for assessing their overall quality. Furthermore, as previous investigators suggested (Panagou EZ, 2006), proper harvesting techniques, standardization and packing in hermetically sealed containers, followed by thermal treatment must be favoured over marketing the end-product in bulk, in order to ensure the food safety and the public health.

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S1-C6

Effet de la solarisation sur la croissance et la longueur de la période juvénile dans des plantes de semis d'olivier

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Résumé

L'effet de la solarisation sur la croissance et la durée de la période juvénile (PJ) de plantes de semis d'olivier a été étudié. Des plantes obtenues par pollinisation libre de 'Manzanilla de Sevilla' et par croisements entre 'Arbequina' x 'Arbosana' et 'Picual' x 'Koroneiki' ont été expérimentées. La solarisation consistait en couvrir le sol par un film plastique noir selon un dispositif Split-plot. La croissance des plantes, la température et l'humidité du sol ont été mesurées. Les résultats indiquent que la solarisation augmenta la croissance. En plus, la solarisation augmenta la température du sol, conserva l'eau d'irrigation et élimina les mauvaises herbes. Les observations préliminaires indiquent un possible raccourcissement de la durée de la PJ par effet de la solarisation.

Mots clés: Amélioration génétique, solarisation, période juvénile, plantes de semis, croissance.

Effect of soil solarization on growth and length of the juvenile period in olive seedlings

Abstract

The effect of soil solarization on growth and on the length of the juvenile period (JP) of olive seedlings has been studied. Seedlings obtained from free pollination of 'Manzanilla de Sevilla' and from crosses between 'Arbequina' x 'Arbosana' and 'Picual' x 'Koroneiki' were tested. Solarization treatment consisted on covering the soil of the trees by a black plastic film in a Split-plot design. Growth, soil temperature and soil humidity were measured. The results indicated that solarization increased clearly the growth of the seedlings. Moreover, solarization resulted in higher soil temperature, conservation of irrigation water; elimination of weeds and smaller number of died seedlings in solarized soils with respect to not solarized. Preliminary data showed that solarization may shorten the JP.

Keywords: breeding, solarization, juvenile period, olive seedlings, growth.

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S1-C7

Photosynthetic performances, osmolytes accumulation and antioxydative activities of three olive-tree varieties subjected to drought

Bechir Ben Rouina, Chedlia Ben Ahmed, Saïd Jilani & Anouar Jribi

Abstract

The recognized tolerance of olive tree to drought and its capacity to grow in shallow, poor quality soils makes the species among the most interesting for cultivation in arid and semi-arid areas. This agronomic interest is enhanced by the fact that, despite the severe rain-fed conditions, olive tree shows a remarkable response to any improvement in the cropping conditions. The effects of water stress on tissue water content, vegetative growth and biomass production, gas exchange, osmolyte accumulation and the antioxidative activities were investigated on fully developed plants of Chemlali Sfax cultivar submitted to severe drought. Low water availability (LW) affected growth and biomass accumulation. Under LW conditions, total leaf area was sharply reduced (26%) due to a combination of leaf growth reduction and shedding of older leaves, minimizing water losses by transpiration. Water stress also caused a marked decline on photosynthetic capacity, and stomatal conductance was the major factor affecting photosynthesis. Drought affected negatively the net photosynthetic rate (5.3 µmol/m^2.s for LW *v*. 10.3 µmol/m^2.s for WW) and stomatal conductance (69.3 mmol/m^2.s *v*. 98.5 mmol/m^2.s). Leaves grown under LW conditions showed decreases in starch, chlorophyll and carotenoid concentrations and plants developed some defense mechanisms against oxidative stress, like the increase in phenolic compounds, proline and soluble sugar concentrations which can improve its antioxidant responses. Furthermore, the activity of catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) increased as leaf water potential decreased.

Keywords: *Olea europaea* L., drought, Pn, gs, proline, antioxidant enzymes.

Performances photosynthétiques, accumulation des osmolytes et activités anti-oxydatives chez trois variétés d'olivier soumis au stress hydrique

Résumé

L'assimilation nette du CO_2, l'accumulation de sucres et de proline, ainsi que l'activité enzymatique anti-oxydative ont été étudiées sur l'olivier, variétés Chemlali Sfax, planté au plein champs dans la région du Chaâl, soumis à un stress hydrique sévère (LW) en comparaison à des oliviers bien irrigués (WW). Chez le traitement stressé, l'accroissement des pousses végétatives et l'activité photosynthétique sont statistiquement réduits par rapport au témoin bien alimenté en eau. Les résultats obtenus montrent que la variété Chemlali Sfax présente une meilleure résistance au stress hydrique, grâce à une régulation stomatique édifiante permettant un meilleur statut hydrique de la plante et une utilisation efficiente de l'eau d'une part et à une activité enzymatique anti-oxydative supérieure (SOD, APX, CAT, PPO). L'accumulation de la proline, de l'amidon et des sucres solubles se trouve majorée ; alors que les concentrations tissulaires en chlorophylles et en caroténoïdes sont réduites. Dans des conditions de précarité des ressources hydriques (LW), la surface totale des feuilles a été réduite de 26% par rapport aux arbres bien alimentés en eau (WW) diminuant au minimum les pertes d'eau par transpiration. La sécheresse a affecté négativement la photosynthèse nette (5.3 µmol/m^2.s pour LW vs. 10.3 µmol/m^2.s pour WW) et la conductance stomatique (69.3 mmol/m^2.s vs. 98.5 mmol/m^2.s). En outre, chez les arbres stressés, les feuilles sont moins riches en amidon, en chlorophylles (a+b) et en caroténoïdes.

Mots clés: *Olea europaea* L., sécheresse, Pn, gs, proline, antioxydants.

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1. Introduction

Olive (*Olea europaea* L.) is an evergreen tree traditionally cultivated in the Mediterranean basin where long periods of soil water deficit are usually present during the dry seasons, for oil and table fruit consumption (Ben Rouina et al., 2007). 95% of world olive orchards area is located in 10 countries of the Mediterranean basin (COI, 1998; Anon, 2001). During the summer months, olive, like other Mediterranean xerophytes, is usually subjected to high solar irradiances, high air temperatures, high vapor pressure deficit and limited water availability in the soil. The reduction of moisture availability anticipated in the climate change scenarios would inevitably add to the problem of water scarcity throughout the Mediterranean region. A survey of the scientific literature revealed that olive tree has important attributes that enable survival and production in drought-prone environments (Ben Rouina et al., 2002a; 2002b; 2007; Ben Ahmed et al, 2009a; 2009b).

In Tunisia, olive tree is the most extended crop not only for its socioeconomic role, nor for the health benefits of olive oil, but also for its great importance in the preservation of green area landscape in semi-arid and arid areas, its limitation of desertification and the prevention of soil erosion and land degradation, and so its involvement in the maintenance of ecosystem durability. Moreover, this species is characterized by its tolerance to contrasting environmental conditions distinguishing the arid climate of Mediterranean type of Tunisia. Olive crop occupies 35% of cultivated lands in Tunisia with a total of 70 millions trees distributed all over the area from the North to the South (1.685 million hectares). Depending mostly on the climatic conditions, the biannual alternate bearing phenomenon characterizing this species leads to the irregularity of production (Prista and Voyiatzis, 2006; Ben Rouina et al., 2007; Ben Ahmed et al, 2009a; 2009b). The intensity of this phenomenon is reinforced under the contrasting environmental conditions of arid climate in Tunisia (Ben Rouina et al., 2007; Ben Ahmed et al, 2009a; 2009b) and that affect the biological cycle of olive tree.

The aim of this study was to identify the drought resistance mechanisms of olive tree and the limitations and damages imposed by water shortage. It was also our goal to investigate how irrigation assists the olive tree to withstand Mediterranean field conditions.

2. Material and methods

The experiment was located in two commercial olive orchards in Chaâl, Tunisia (34°.30'54 N, 10°.20'17 E; altitude 118 ft) between 1999 and 2009. This area is characterized by an arid Mediterranean climate with an annual average rainfall of 184.4 mm (i.e. long term average for the period 1924-2008), mean ET_0 (using the potential evapo transpiration equation of FAO Penman-Monteith) of 1525 mm/year and mean temperatures of 11.1℃ in January and 27.8 ℃ in August. The two adjacent olive orchards 'cv Chemlali Sfax' were managed using the same pruning, fertilizers, weeds control and pest management practices of regional growers. In the first experiment, we investigated the morpho-anatomical, physiological and biochemical adaptations of field-grown olive Chemlali Sfax cultivar with different water availability regimes: drought (LW) and well watered (WW) treatments and we presents data on the leaf-level morphological and structural adaptations to reduce water loss. Leaf measurements included leaf tissue thickness, stomatal density, leaf area, leaf mass per unit area, relative water content, water content at saturation and cuticle transpiration rate. In the second experiment, the effect of watering regime in the vegetative growth, gas exchange and water use efficiency of biomass production were studied. Finally, plant water relations, total soluble sugars, starch, and proline concentrations were investigated.

The water management treatments were:

(i) olive trees in rain-fed orchard under severe drought conditions (LW), were grown on a layer of sand of more than 3 m in depth (sand 92.3%, clay 5.5% and loam 2.2%). The orchard covered an area of 41.3 ha and contained 702 18-year-old olive trees.
The LW orchard received during 2002, 184.4 mm of annual rainfall (Table 1) following two years of abnormal low rainfall (127 mm in 2000 and 87.7 mm in 2001). In order to characterize the effects of water deficit on the plants physiological responses the results recorded in LW orchard were compared to those recorded in a well irrigated one (WW) which received cumulative seasonal water of 415 mm corresponding to 40% of ET_c.

(ii) Olive trees in orchard 2, called WW, were well watered. Olive trees were grown on a layer of sand of more than 3 m in depth (sand 89.1%, clay 6.3% and silt 4.6%). This orchard contained 625, 18-year-old olive trees. The WW orchard was drip irrigated (i.e. two lateral drippers per row of olives) at a continuous rate of 2.4 L/h. plant at a distance of 1 m from the trunk. The average of climatic data recorded in 2002 and the irrigation period was 1 February to 30 October, resulting in a total of 35 irrigations was consigned in Tab. 1.

Table 1: Monthly ET_0 (mm), rainfall (mm), maximum and minimum temperatures (℃) for Chaâl site and irrigation volume (mm) provided in the irrigated plantation.

Month	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	Total
T_x	18.1	18.6	21.8	23.9	28.7	32.6	34.4	35.2	28.2	23.7	19.6	18.2	-
T_n	3.5	6.0	6.8	9.2	14.3	16.8	24.3	20.3	18.3	14.7	8.4	5.4	-
ET_0	43	59	102	135	177	192	229	214	156	109	66	43	**1525**
Rainfall	3.1	6.5	10.2	13.4	27.2	0	0.7	0	11.6	18	45.2	48.2	**184.4**
Irrigation	0	11	16	44	61	72	81	73	34	23	0	0	**415**

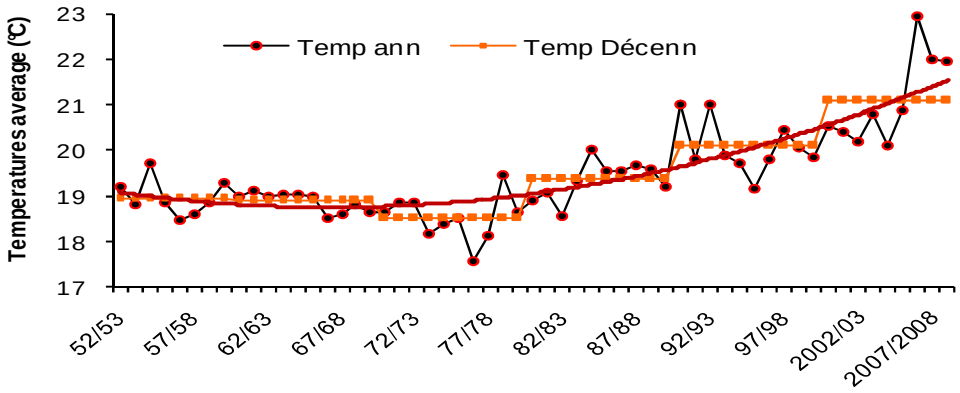
ET_0 = evapo transpiration Penman-Monteith; T_x = maximal temperature and T_n = minimal temperature.

3. Results and discussion

3.1. Environmental effects on olive tree growth

The olive tree development depends on the interaction of major ambient environmental conditions (rainfall pattern, air temperature, solar radiations intensity, atmospheric humidity, air CO_2 concentrations, etc…). Several researches have studied the relationship between olive tree activity and environmental conditions; they have showed that drought and heat are the most important environmental stress factors that disrupt the plant activity (Giorio et al., 1999; Moriana et al., 2002; d'Andria et al., 2004; Tognetti et al., 2004; Ben Ahmed et al, 2009a; 2009b).

Water availability is the principal cause conditioning crops growth. It interacts directly in the plant activity via photosynthesis and transpiration process and affects its growth and fruit set up (Ben Ahmed et al, 2009a; 2009b; Giorio et al., 1999; Moriana et al., 2002) stress factors that disrupt the plant activity (Giorio et al., 1999; Tognetti et al., 2004; Ben Ahmed et al, 2010). In arid area, climate change affects average temperatures and temperature extremes (fig. 1a), timing and spatial patterns of precipitation (Fig. 1b), soil moisture, runoff, and the frequency of disturbances, such as drought. Between 1981 and 2009 global warming is twice more important in arid area of Tunisia than the world average (1.5 - 2 °C against 0.75 °C). This climat e warming is accompanied by a regression between 10 and 20 % of average annual precipitation.



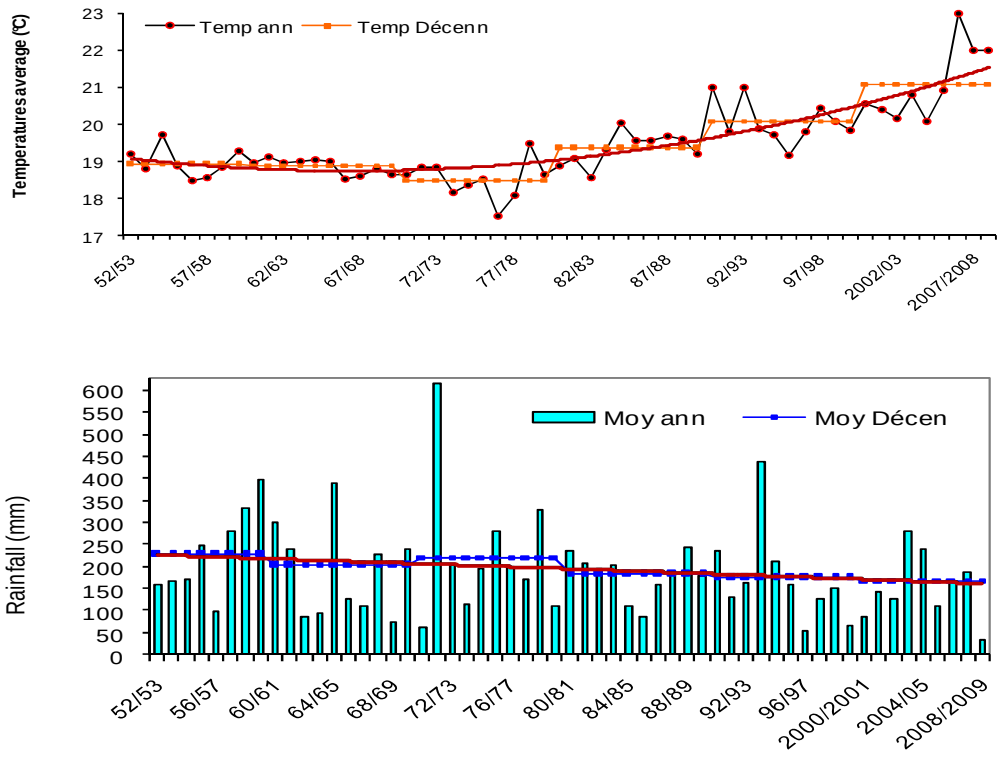


Figure 1: Yearly average of temperature (a) and rainfall (b) at the Chaâl farm between November 1952 and August 2009.

Low water availability (LW) affected growth and biomass accumulation of Chemlali Sfax (Tab. 2). Under drought stress, total leaf area was sharply reduced due to a combination of leaf growth reduction and shedding of older leaves, minimizing water losses by transpiration. Water stress and high temperatures also caused a marked decline on photosynthetic capacity and stomatal control was the major factor affecting photosynthesis (Fig. 2).

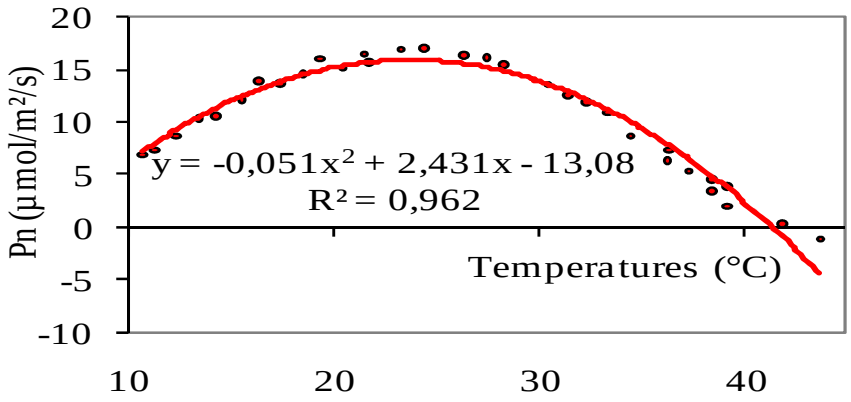


Figure 2: Relationship between net CO_2 assimilation and air temperature on Chemlali Sfax olive tree.

Under drought stress, water use efficiency of biomass production was improved in LW plants (4.5 mg of H_2O cm^{-2} h^{-1}), whereas it decreased in irrigated trees (3.29 mg of H_2O cm^{-2} h^{-1}). Hence, water deficit influenced significantly leaf tissue thickness, stomatal density of mature olive plants, and specific leaf area has often been observed to be reduced under drought conditions and variations in it may be due to variations in leaf thickness and/or variations in leaf density (Tab. 2).

Table 2: Mean values of leaf tissue thickness (µm), stomatal density (stomata mm^{-2}) and leaf characteristics of Chemlali Sfax olive trees subjected to two water regimes.

Treatment	Leaf thickness (µm)			Stomatal density (unit mm^{-2})	Leaf area (mm^2)	Leaf mass area (g m^{-2})
	Total lamina	Spongy mesophyll	Trichome layer			
WW	426.2	239.3	41.5	369	458	248.3
LW	455.5	252.7	43.7	396	401	234.2

3.2. Effects on olive tree water status and CO_2 assimilation

In the study zone, the aridity of the climate and the soil dryness resulted in a noticeable water deficit during a long period of the year. This last deficit was revealed by the recorded levels of RWC (Tab. 3). According to the RWC values, LW plants reached a severe level of water stress (least amount of 46% in August). Therefore, water shortage mechanisms are important for tissue desiccation avoidance, especially when they are coupled with surface reduction and high transpiration resistance of the epidermis (Ben Rouina et al., 2007; Ben Ahmed et al., 2009a,; 2009b). A special form of water conservation is the binding of water to mucilage in cells, ducts and intercellular cavities. Water reserves of this kind can protect the plant from rapid wilting and severe leaf shrinkage.

So, our results revealed that olive cultivars native to dry regions, such as Chemlali Sfax, have more capability to acclimate to drought conditions and climate. This cultivar avoids water loss with high density of foliar tissue and the presence of the thick cuticle and trichome layers. It enhanced their sclerophylly by building parenchymatous tissues and increasing protective structures like both the upper and lower epidermis (tab. 2).

Table 3: Net CO_2 assimilation rate (*A*), stomatal conductance (*gs*), transpiration rate (*E*) and leaf water potential at predawn (Ψ_{PD}) and midday (Ψ_{MD}) of Chemlali Sfax olive tree under contrasting water availability regimes (n = 10).

Watering regime	*A* (µmol m^{-2} s^{-1})	*Gs* (mmol m^{-2} s^{-1})	*E* (mmol m^{-2} s^{-1})	RWC %
WW	14.75a	138.31a	4.05a	92.5a
LW	5.31b	93.10a	3.19b	72.8b
% reduction	36.9	32.7	21.0	21.3

Means within a column flanked by the same letter are significantly not different at P < 0.05 (LSD test).

Stomatal control of water loss was identified as an early response of Chemlali Sfax olive tree to water deficit, leading to limitation of carbon uptake by the leaves (Tab. 3). Non-stomatal factors also play an important role in limiting photosynthesis when olive trees are submitted to prolonged drought under field conditions (Pn = 5.31 µmol m^{-2} s^{-1} for the LW trees *v.*14.75 µmol m^{-2} s^{-1} WW trees). Among such responses, gas exchange is of particular importance in determining the efficiency of water use in response to the limited water resources.

In order to preserve photosynthesis behavior the olive tree, like some plants grown in arid environments, have evolved physiological processes to maintain to some extent tissue turgor and thus stomatal opening [6, 7]. Lowering of leaf potential due to net accumulation of compatible solutes in the cytoplasm such as proline, soluble sugars and phenols, is a well established biochemical mechanism whereby many plants adjust to low soil water availability. The close relationship observed between *Pn* and proline content points to an important role of this osmolyte in the maintenance of photosynthetic activity and therefore in drought tolerance.

3.3. Effects on osmolyte accumulation and the antioxidative activities

Leaves grown under LW conditions revealed signs of oxidative stress. One of the signs was the large reductions in leaf Chl (a+b) and Carotenoids concentration (Table 4). According to [13], the decrease of chlorophyll content is a typical symptom of oxidative stress and may be the result of chlorophyll degradation or be due to chlorophyll synthesis deficiency. Ben Rouina et al., (2007); Ben Ahmed et al., (2009a and 2009b) indicated that expanded leaves exposed to water deficit started to degrade their photosynthetic apparatus, possibly to mobilize resources for the production of new acclimated leaves.

The Chl/Car ratio was significantly lower in olive plants under LW conditions. This result can be used as an early indicator for chlorosis of LW plants and reveals an increased need for photo protection of chlorophylls by carotenoids. It is now well documented that carotenoids are involved in the protection of the photosynthetic apparatus against photo inhibitory damage by singlet oxygen, which is produced by the excited triplet state of chlorophyll.

Table 4: Effects of water regimes on photosynthetic pigments, carotenoids, soluble sugars, starch, proline and total phenols in leaves of Chemlali Sfax olive tree under contrasting water availability regimes (LW = dry weight, FW = fresh weight; n = 3).

Watering regime	Chl a+b mg g^{-1} LW	Carotenoids mg g^{-1} LW	Soluble sugar mg g^{-1} LW	Starch mg g^{-1} LW	Proline µg mg^{-1} FW	Total phenols µg g^{-1} LW
WW	15.87a	3.47a	89.86a	83.12a	73.45a	43.61a
LW	8.65b	2.08b	126.65b	58.76b	225.41b	69.96b

Means within a column flanked by the different letters are significantly different at P < 0.05 (LSD test).

For all tissues tested in this experiment, SOD, APX, and CAT activities increased significantly in LW plants, compared to their respective WW ones. In leaves, this increase was 2.56, 3.36, and 2.07 times, respectively for SOD, APX, and CAT enzymes in comparison to their respective activities in WW-treated plants (Table 5).

Table 5: Anti oxidative enzymes activities of leaves and roots from well watered (WW) and low watered (LW) field-grown Chemlali olive plants.

Treatments	Enzyme activity (units mg^{-1} dw)							
	Leaves				Roots			
	SOD	APX	CAT	PPO	SOD	APX	CAT	PPO
WW	13.4 ± 1.1[b]	2.7 ±0.3[b]	6.5 ± 0.5 [b]	31.0 ± 1.3 [b]	10.7 ±2. [b]	0.7±0.05[a]	3.1±0.04[a]	34.7 ±1.2[b]
LW	32.4 ± 2.1[a]	10.8 ±0.8[a]	12.2 ± 0.6[a]	19.3 ± 1.3[a]	16.3±2.3[a]	0.9±0.06[a]	4.3 ±0.9[a]	14.4 ±1.0[a]

Values represent the means of three samples ± standard deviations. [a] significant differences at the 5% level between enzymes activities levels obtained under well watered and low watered treatments (P≤ 0.05, according to Duncan's multiple range test).

4. Conclusion

The recognized tolerance of olive tree to drought and its capacity to grow in shallow, poor quality soils makes the species among the most interesting for cultivation in arid and semi-arid areas. This agronomic interest is enhanced by the fact that, despite the severe rain-fed conditions, olive tree shows a remarkable response throughout the region where during the summer and drought event, plants were usually subjected to high solar irradiances, high air temperatures, high air vapor pressure deficits and limited water availability.

In water stressed olive trees differences were observed in leaf water status, photosynthetic performance, and pigments content, proline and phenols accumulation. Differences in photosynthesis could be related to differences in soil water apparatus. This tendency suggests an interaction between proline and the antioxidative defense system.

The leaves of Chemlali Sfax olive trees also had a higher succulence index than the other cultivars, which affords protection against sudden wilting and severe shrinkage. It is well adapted to drought conditions as a result of the high density of the foliar tissue and the presence of thick cuticle and trichome layers. However, the development of small leaves may reduce water loss at the whole-plant level. We identified several mechanisms at the morpho-structural level by which Chemlali olive tree cope with drought stress.

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S2-C8

Développement de jeunes plants d'oliviers (*Olea europaea l.)* et apparition de la première floraison

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Résumé

Le développement caulinaire de jeunes plants issus de semis d'oliviers est suivi afin de proposer des critères morphologiques capables de prédire la sortie de juvénilité et l'entrée prochaine en floraison. Le nombre de fleurs apparues à l'échelle de l'arbre est fortement corrélé avec le nombre de fleurs apparues à l'ordre de ramification 4 et 5. Le coefficient de corrélation est respectivement égal à 0,90 et 0,79. Par ailleurs, il est apparu que le nombre de fleurs total est fortement lié avec la hauteur de l'arbre, avec la vigueur observée et avec l'état de ramification atteint (ordre de ramification, nombre de ramifications…). Une analyse en composantes principales portant sur 14 critères morphologiques mesurés sur la totalité de la descendance montrent que les plants ayant développé un nombre de ramifications d'ordre 4 important ainsi qu'un nombre élevé de métamères édifiés par ces derniers et montrant un axe principal important, présentent une forte floraison. Ces paramètres constituent des critères morphologiques intéressants pour prédire l'entrée en floraison chez ces hybrides et plus généralement chez l'olivier.

Mots clés: Olivier, juvénilité, passage à la floraison, critères morphologiques, analyse en composantes principales.

Development of young olive trees (*Olea europaea L.)* and occurrence of the first flowering

Abstract

The patterns of vegetative growth were followed on seedling olive trees. The objective of this research was to propose a set of morphological variables as predictors of tree capability to enter into reproductive phase. The number of flowers was strongly correlated with the number of flowers appeared in fourth and fifth botanical order. The correlation coefficient was respectively equal to 0.90 and 0.79. It appeared that the number of flowers was strongly dependent with the height of the tree, with tree vigour and finally the ramification order reached and the number of branches. A set of 14 variables was proposed and relations between variables were studied by component analysis. The plant which developed an important number of branches on the fourth order, high metameres number on the same order and a long axis, presented an intense flowering. These morphological variables constituted interesting morphological criteria to predict the flowering occurrence at these seedlings and more generally on the olive tree.

Keys words: Olive tree, juvenility, flowering occurrence, morphological criteria, component analysis.

1. Introduction

La phase juvénile est un stade propre de la plupart des systèmes biologiques et notamment de toutes les formes de vie supérieure. Elle pourrait être définie généralement comme une phase au cours de laquelle l'organisme est dépourvu de la capacité de se reproduire (Wareing, 1959; Zimmerman, 1972; Borchert, 1976a). La durée de la période juvénile est un des problèmes rencontrés lors de l'amélioration génétique de la plupart des espèces connues comme c'est le cas du pommier et du poirier (Visser, 1970; Zimmerman, 1977; Costes et al., 2004) et de l'olivier (Lavée et *al.,* 1996; Garcia et *al.,* 2000).

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La juvénilité est définie comme un ensemble de caractéristiques physiologiques et morphologiques manifestées par les semis durant la phase étalée depuis la germination jusqu'à la production des premières fleurs (Hackett, 1985; Monteuuis, 1988). Lors de la transition de la phase juvénile à la phase adulte, l'apparition de la floraison est davantage liée à la formation d'un nombre minimum de nœuds, à l'acquisition d'une taille minimale (Wareing, 1959; Kozlowski, 1971; Borchert, 1976 a et b; Wesley, 1976) ou à l'acquisition par la plante d'un degré de différenciation minimum qu'en nombre de jours, de mois ou d'années (Barthélémy, 1988, 1991). La vigueur semble avoir une relation sur l'entrée en floraison des jeunes plants (Visser, 1970; Santos- Antunes et al., 2005).Le passage à la floraison semble s'inscrire aussi dans une organisation spatio-temporelle de la plante (Barthélémy, 1991, 1997; Crabbé, 1991).

Le présent travail a pour objectif (i) d'étudier les relations simples (corrélations) pouvant exister entre les différents paramètres de végétation et de la floraison (ii) de proposer une série de paramètres morphologiques capables d'évaluer la sortie de la juvénilité et l'entrée prochaine en floraison de jeunes plants issus d'oliviers à l'aide d'une analyse en composante principales portant sur des paramètres phéno-morphologiques et 81 individus (plants).

2. Matériel et méthodes

2.1. Matériel végétal

Nous avons disposé au début de ce travail d'une descendance d'hybrides de 1086 plants. Les plants sont issus de croisements réalisés sur plusieurs années de semis réalisés entre 1994, 1995 et 1996. Ces croisements sont regroupés selon l'origine des croisements : soit *Chemlali* en porte graines (parent femelle) avec différents génotypiques (pollinisateurs), ou encore des croisements réciproques de la *Chemlali* en pollinisateur (parent mâle) avec d'autres génotypes porte graines (parent femelle), des croisements de différents génotypes ou autres cultivars autres que la Chemlali, des autofécondations et des croisements libres issus de la *Chemlali*.

Après élevage en serre dans des conditions normales (ne permettant pas de hâter l'allongement des plants faute de moyen), les 1086 plants sont plantés en Décembre 1997 et en Avril 1998 dans un verger de comportement dans la région de Sfax située à 240 km au sud de Tunis (étage bioclimatique aride supérieur) Les arbres sont menés en forme libre sans aucune intervention de taille.

2.2. Paramètres mesurés et analyse statistique

Nous avons réalisé une description complète de 81plants et nous avons repéré les 41 arbres qui sont sortis de la floraison. Les sites de floraison sont localisés au niveau de l'arbre entier à travers les différents ordres de ramification. Nous avons établi une quarantaine de variables relatives au développement aérien de l'arbre, à l'architecture élémentaire développée et la quantification de la floraison à l'échelle de l'arbre.

Les variables retenus sont les suivantes: $\mathbf{V_a}$**:** âge de l'arbre; $\mathbf{V_p}$**:** port de l'arbre; $\mathbf{V_0}$: Hauteur première charpente; $\mathbf{V_1}$: Hauteur de l'arbre; $\mathbf{V_4}$: périmètre du tronc; $\mathbf{V_6}$**:** Nombre de ramifications total; $\mathbf{V_8}$**:**Nombre de métamères total; $\mathbf{V_{15}}$: nombre de ramifications longues d'ordre 3; $\mathbf{V_{17}}$: Longueur moyenne de ramifications longues d'ordre 3; $\mathbf{V_{18}}$: : angle que font les ramifications d'ordre 3; $\mathbf{V_{19}}$: somme des métamères édifiés par l'ordre 3; $\mathbf{V_{20}}$: nombre de ramifications longues d'ordre 4; $\mathbf{V_{22}}$: longueur moyenne des ramifications d'ordre 4; $\mathbf{V_{23}}$: angle que font les ramifications d'ordre 4; $\mathbf{V_{24}}$: somme des métamères édifiés par l'ordre 4;$\mathbf{V_{25}}$**:** nombre de ramifications longues d'ordre 5;$\mathbf{V_{27}}$**:** longueur des ramifications d'ordre 5;$\mathbf{V_{28}}$**:** angle que font les ramifications d'ordre 5;$\mathbf{V_{29}}$: somme des métamères édifiés par l'ordre5; $\mathbf{V_{30}}$: Nombre de fleurs total; $\mathbf{V_{39}}$**:** Nombre de métamères édifiés par le chemin le plus court jusqu'à la première fleur;$\mathbf{V_{40}}$: Ordre de ramification maximal.

Des corrélations entre les variables ont été établies par le test de Pearson à l'aide du logiciel *SPSS* (version 10.0). Compte tenu des fortes corrélations obtenues entre les différents paramètres, nous avons réalisé une analyse en composantes principales sur les mêmes individus mais avec seulement 22 variables à l'aide du logiciel *Xlstat* (version 2007.6).

3. Résultats

3.1. Localisation de la floraison selon les différents ordres de ramification

A l'apparition de la floraison sur les différents arbres, les sites sont localisés au niveau de l'arbre entier à travers les différents ordres de ramification. Le nombre de fleurs total développées à l'échelle de l'arbre, est en étroite relation avec le nombre de fleurs développées à l'ordre botanique 4 (R=0,90**), le nombre de fleurs développées à l'ordre botanique 5 (R=0,79**) et en second lieu avec le nombre de fleurs développées à l'ordre botanique 3 (R=0,55**), à l'ordre 2 (soit R=0,54**) et enfin à l'ordre 6 soit avec un coefficient de corrélation égal à 0,44.

3.2. Relations entre la floraison et les critères morphologiques

3.2.1. Relations linéaires simples

Selon le Tableau 1, il apparaît que le nombre de fleurs est en relation avec la hauteur de l'arbre (R=0,25*) et avec la vigueur de l'arbre c'est à dire le périmètre du tronc (R=0,30**). Le nombre de fleurs montre une relation hautement significative avec le nombre de ramifications longues développées (R=0,34**) et avec le nombre total d'entre- nœuds édifiés par ces derniers (R=0,47**). Le nombre de ramifications longues développées à l'ordre botanique 3, 4 et 5 ainsi que le nombre total d'entre-nœuds édifiés par ces derniers et leur direction, notent une relation très étroite avec le nombre de fleurs développées.

Tableau 1 : Corrélation entre le nombre de fleurs total moyen par port et les différentes variables.

Variables	Coefficient de corrélation (R)
Hauteur arbre	0,25*
Périmètre du tronc	0,30 **
Nombre de ramifications longues	0,34 **
Somme des entre-nœuds édifiés	0,47 **
Nombre d'entre-nœuds formés jusqu'à l'apparition de la première fleur	0,44 **
Ordre de ramification où est apparu la première fleur	0,44**
Nombre de ramifications longues d'ordre botanique 3	0,35**
Somme des entre-nœuds édifiés par les ramifications d'ordre3	0,26*
Nombre de ramifications longues d'ordre botanique 4	0,60 **
Angle que fait les rameaux d'ordre 4 avec le porteur	0,32**
Somme des entre-nœuds édifiés par les ramifications d'ordre4	0,55 **
Nombre de ramifications longues d'ordre botanique 5	0,31**
Longueur des ramifications d'ordres 5	0,48 **
Somme des entre-nœuds édifiés par les ramifications d'ordre 5	0,28 *
Angle que fait les rameaux d'ordre 5 avec le porteur	0,56 **

* : Corrélation significative au seuil de 5 %. ** : Corrélation hautement significative au seuil de 1%.

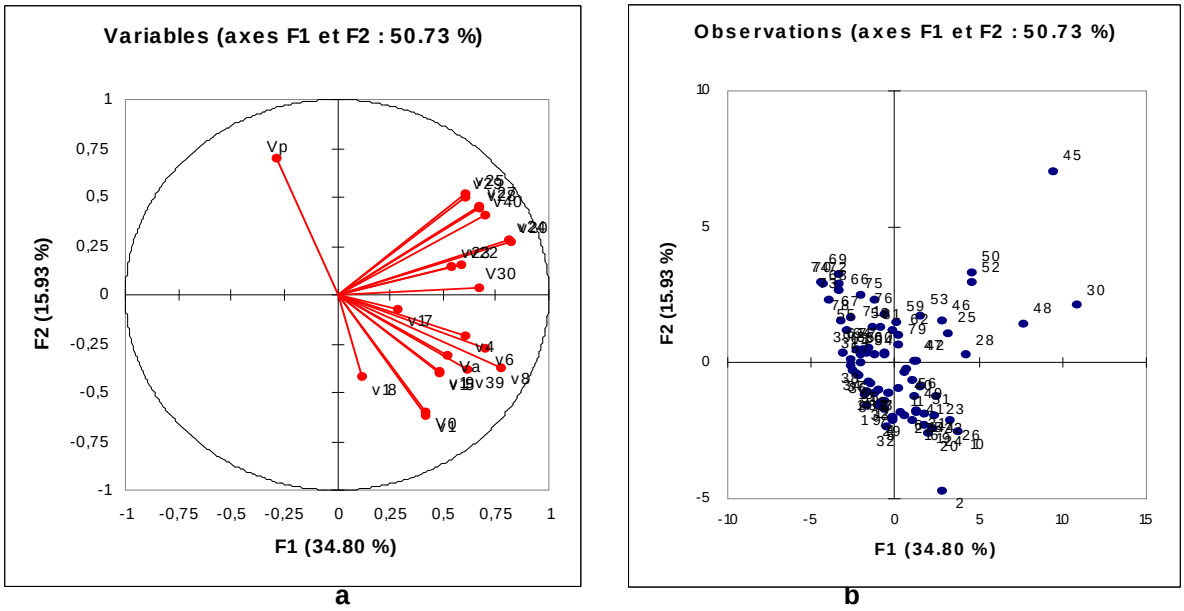
3.2.2. Analyse des paramètres morphologiques à l'aide d'une analyse en composantes principales

L'analyse en composantes principales conduit à diagonaliser la matrice de corrélations donnant les valeurs propres avec le pourcentage d'inertie expliquée pour chacune. Les quatre premières valeurs propres expliquent près de 69,5% de l'inertie totale. La première valeur explique près de 35% de l'inertie totale, la seconde 16% de l'inertie, la troisième de 11,5% et enfin la quatrième 7%. Les variables utilisées pour comparer les différents individus sont exprimées dans la nouvelle base de vecteurs propres en quatre facteurs, ce qui permet d'établir les coefficients de corrélation entre les variables et les facteurs principaux des axes (Tableau 2).

Tableau 2: Corrélations des variables (R) avec les quatre premiers axes.

Variables	Axe 1	Axe2	Axe3	Axe4
V_a	**0,51**	-0,30	-0,43	0,31
V_p	-0,28	**0,69**	**0,50**	0,17
V_0	0,41	**-0,59**	-0,39	-0,08
V_1	0,42	**-0,61**	-0,46	0,03
V_4	**0,60**	-0,21	-0,12	0,41
V_6	**0,70**	-0,27	0,28	-0,30
V_8	**0,77**	0,36	0,36	-0,24
V_{15}	0,48	-0,40	**0,65**	-0,25
V_{17}	0,29	-0,07	0,05	**0,58**
V_{18}	0,12	-0,41	0,17	0,02
V_{19}	0,48	-0,38	**0,70**	-0,12
V_{20}	**0,81**	0,27	0,00	0,01
V_{22}	**0,58**	0,15	0,25	**0,50**
V_{23}	**0,53**	0,14	0,37	0,32
V_{24}	**0,81**	0,27	0,01	0,04
V_{25}	**0,60**	0,62	-0,24	-0,25
V_{27}	**0,67**	0,45	-0,27	-0,21
V_{28}	**0,67**	0,44	-0,21	-0,16
V_{29}	**0,60**	0,60	-0,23	-0,26
V_{30}	**0,66**	0,03	-0,03	-0,15
V_{39}	**0,61**	-0,38	-0,29	0,08
V_{40}	**0,70**	0,40	0,12	0,23

La Figure 1a représente la projection des points variables selon le plan P_1 factoriel formé par le premier et le second facteur expliquant 51 % de la variabilité.



a b

Figure 1: Projection des variables selon le plan factoriel P_1 formé par l'axe 1 et 2 (a). et représentation des individus ayant une forte contribution au plan factoriel P_1 (b).

Le premier facteur expliquant à lui seul 35 % de la variabilité, montre que toutes les variables évoluent dans le sens positif de ce dernier à l'exception de la variable V_p évoluant dans le sens contraire. Les variables montrant une forte contribution à l'axe1 sont: le périmètre du tronc (V_4), le nombre de ramifications total (V_6) et la somme des entre-nœuds édifiés par les différentes ramifications (V_8), le nombre de ramifications à l'ordre 4 (V_{20}), la somme des métamères édifiés par ces derniers (V_{24}) ainsi que le nombre total de fleurs apparues à l'échelle de l'arbre (V_{30}). Le nombre de ramifications à l'ordre 5 ainsi que les caractéristiques de croissance des ces ramifications (V_{25}, V_{26}, V_{27}, V_{28} et V_{29}), l'ordre de ramification maximum atteint (V_{40}) et le nombre de métamères édifiés jusqu'à l'apparition de la première fleur (V_{39}), augmentent dans le sens positif de l'axe. Cet axe caractérise la relation pouvant exister entre la floraison et l'état général de l'arbre aussi bien au niveau de la vigueur de l'arbre que la différenciation morphologique atteinte par les structures raméales de l'arbre. Il apparaît aussi que le port général ne montre pas une influence directe sur le passage à floraison des arbres.
Le second facteur (expliquant 16 % de la variabilité) oppose d'une part le port de l'arbre (V_p) et d'autre part les variables de la hauteur de l'arbre (V_1) et de sa première charpente (V_0). Cet axe semble caractériser le port de l'arbre et de sa hauteur.

La représentation des individus sur le plan factoriel P_1 fait ressortir des groupes distincts (figure 1b). Ainsi, les arbres se trouvant sur la droite de l'axe 1, se caractérisent par un nombre important de fleurs (V_{30}), une ramification importante (V_6), un nombre de ramification d'ordre 4 et 5 ($V_{20,}$ V_{25}) et un nombre important de métamères édifiés par ces derniers ($V_{24,}$ V_{29}) ainsi qu'une vigueur importante (V_4). D'autre part, les individus se trouvant à gauche de l'axe 1, indiquent les arbres peu fleuris ou encore juvéniles (V_{30} faible) et dont la ramification est peu développée et l'ordre de ramification ne dépasse pas 3.
L'axe 2 sépare d'une part les plants dont la hauteur (V_1) et la hauteur de la première charpente (V_0) sont importants vers le bas de l'axe et d'autre part les individus dont la hauteur est peu importante vers le haut de l'axe. La variable port de l'arbre (V_p) augmente dans le sens positif de l'axe indiquant les individus à port buissonnant ou mixte vers le haut et les arbres à port arborescent vers le bas.

3. Discussions & Conclusions

Le but de cette recherche est d'analyser le phénomène de l'apparition de la floraison qui se caractérise par l'omniprésence d'une double question: Quand et où une structure florale va t-elle s'initier dans le système d'axe ramifié que constitue le végétal? Des critères phéno-morphologiques sont recherchés comme indicateurs de l'entrée prochaine en floraison de jeunes oliviers.

Ainsi, le nombre de fleurs apparues à l'échelle de l'arbre, est en étroite relation avec la hauteur de l'arbre ou encore l'importance de l'axe principal de l'arbre. Ces résultats confirment les recherches antérieures réalisées par différentes équipes de recherches ayant travaillé sur les aspects morphologiques accompagnant la sortie de la juvénilité étudiée chez différentes espèces (Borchert, 1976 a et b; Koslowski, 1971; Wesley, 1976; Zimmerman, 1972, 1977). Il est aussi apparu que la floraison est aussi en relation avec le périmètre du tronc c'est à dire la vigueur de l'arbre. Ces résultats corroborent ceux de Visser (1970) sur pommier et ceux de Msallem (2002) et Santos-Autunes et al. (2005) sur olivier. Une recherche de Nesme et al. (2005), a porté sur la mise au point de paramètres morphologiques permettant d'estimer la vigueur chez le pommier qui est à son tour un excellent indicateur de la mise à fruit.
Le nombre de fleurs apparues est fortement corrélé avec celui des fleurs apparues à l'ordre 4 et 5. Au niveau de l'architecture de l'arbre et lors de la transition de la phase juvénile à la phase adulte, la formation des structures reproductrices s'inscrit dans une séquence précise et ordonnée de différenciation végétative dans une organisation spatio-temporelle donnée de la plante. La floraison est une étape obligatoire du programme morphogénétique de l'espèce. Ces résultats corroborent les observations de Barthélémy (1988, 1991) sur les espèces tropicales et de Crabbé (1991) sur les espèces ligneuses plus généralement.
Il apparaît ainsi que les arbres entrent plus rapidement dans la phase reproductive quand le maximum d'axes est maintenu sur l'arbre d'où la nécessite d'un nombre minimum d'interventions d'opérations de taille pendant le jeune âge. Costes et al. (2004) étudiant le développement de jeunes plants de poirier jusqu'au passage à la floraison, constatent que les arbres ayant développé une augmentation des ramifications sylleptiques durant les deux premières années de leur développement, c'est à dire une augmentation de la ramure, montrent une entrée en floraison plus précoce. Ce paramètre peut être utilisé dans les programmes d'amélioration pour sélectionner les génotypes les plus précoces au niveau de l'entrée en floraison (Sansavini et Musacchi, 1994).

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S2-C9

Phenology and olive (*Olea europaea* L. cv Frantoio) fruit maturation monitoring in Tuscany

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Abstract

Olive (*Olea europaea* L.) is an evergreen tree and one of the most important tree crops in Mediterranean agro-ecosystems. Maximization of olive productivity at the end of the growing season requires detailed knowledge on fruit growth and oil accumulation processes. To achieve this objective, in the last decade, regional agricultural services have adopted crop management strategies based on field monitoring networks, physical and chemical analyses, meteorological data sets, and internet-based services. In this work we describe the Decision Support System (DSS) implemented by our research group on the web site of ARSIA – Extension Service for Agriculture in Tuscany, Italy – for phenology and olive fruit maturation monitoring.

Keywords: DSS, internet-based-services, meteorological data, maturity index.

Phénologie et pilotage de la maturation du fruit d'olive (*Olea europaea* L. cv Frantoio) en Toscane

Resumé

D'olive (*Olea europaea* L.) est un arbre à feuilles persistantes et l'une des cultures d'arbres les plus importants dans les agro-écosystèmes méditerranéens. La maximisation de la productivité d'olive à la fin de la saison de croissance requiert des connaissances approfondies sur la croissance des fruits et des processus d'accumulation d'huile. Pour atteindre cet objectif, dans la dernière décennie, des services régionaux de l'agriculture ont adopté des stratégies de gestion des cultures basées sur les réseaux de surveillance sur le terrain, des analyses physiques et chimiques, météorologiques ensembles de données et services basés sur Internet. Dans ce travail, nous décrivons la Decision Support System (DSS) mis en œuvre par notre groupe de recherche sur le site web de ARSIA - Prolongation du service de l'agriculture en Toscane, Italie - pour leur phénologie et de suivi de maturation des fruits d'olive.

Mots-clés: DSS, internet-based-services, des données météorologiques, l'indice de maturité, Toscane.

1. Introduction

Tuscany is among the major regions in the world devoted to the cultivation and production of high quality olive oil. However, the olive culture sector in Tuscany, as in many others Italian regions, very often suffers for the small farms dimension that makes difficult to introduce innovations aimed to improve olive oil quality. In order to cope with these needs, the public administrations are involved in the developing of services that enable farmers and technicians to plan more accurately their orchard management strategies. It is in this scenario that since 2005 ARSIA – Extension Service for Agriculture in Tuscany started the implementation of a Decision Support System (DSS) for assessing olive phenology and the degree of ripeness of the olive fruit. The system has been created using the knowledge gained over the years through the web site agroambiente.info (Ricciolini and Guidotti 2004) and general software architecture of the DSS for grape ripening (Sebastiani et al., 2004). Olive DSS is based on a monitoring network of olive farms and an agro-climatic network of meteorological stations. The final objective is to provide real-time and on-line information on the olive phenology and fruit ripening to the various actors of the olive oil sector. Another objective is to create a large database that can be used for the development of agro-climatic models useful in determining the dynamics of the phenology and fruit ripening in olive.

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2. Material and Methods

The DSS on the ripening of the olives was developed using a single variety, Frantoio that is the most common olive oil cultivar in Tuscany. The monitoring network was activated in 2005 selecting a group of olive farms spread across a homogenous climatic area of Tuscany, which was also characterized by the cultivation and production of high quality olive oil. The DSS is freely available by technicians, farmers and researchers and is integrated with the other services provided by ARSIA (http://agroambiente.info.arsia.toscana.it/arsia/arsia) (Figure 1).

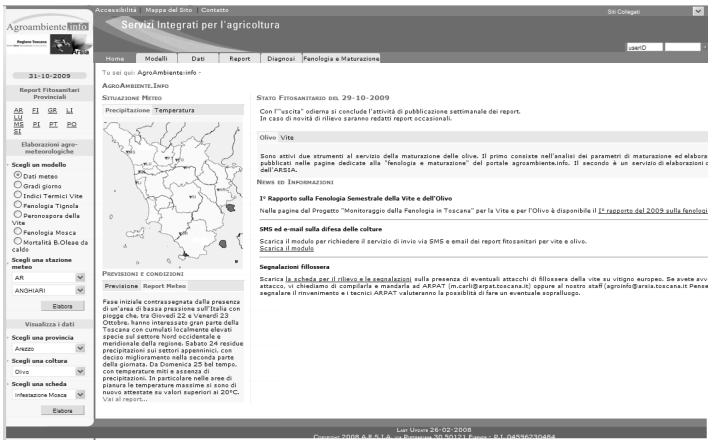

Figure 1: the website of ARSIA http://agroambiente.info.arsia.toscana.it/arsia/arsia.

In each farm a group of olive trees (10-20 plants) is selected on the basis of their representativeness and proximity to the meteorological stations. On these plants, phenological measurements are performed using a BCCH simplified phenological scale during the year at variable time intervals (monthly, biweekly and weekly according to the predicted speeds of the phenological changes). In the period of olive fruit ripening (mid September – early December) a sample of 500-600 grams of olives is collected weekly for specific physical-chemical analysis in laboratory. The parameters evaluated are: pulp-stone ratio, average weight of fruits (g), relative humidity (%), oil content (% on dry weight) content in polyphenols (as gallic acid, g kg^{-1} dry matter), maturity index according to Uceda and Frias (1975). All the phenological and physical-chemical analysis are loaded in the DSS database that is also updated daily with data from the network of weather stations.

3. Results

The DSS is accessible through internet and from the main page you can activate several links having the following functionality: a) display pages containing the analytical (phenological and physical-chemical) data of the farm monitored b) processing meteorological data c) obtain weekly reports on olive fruit ripening process (Figure 2, 3 and 4).

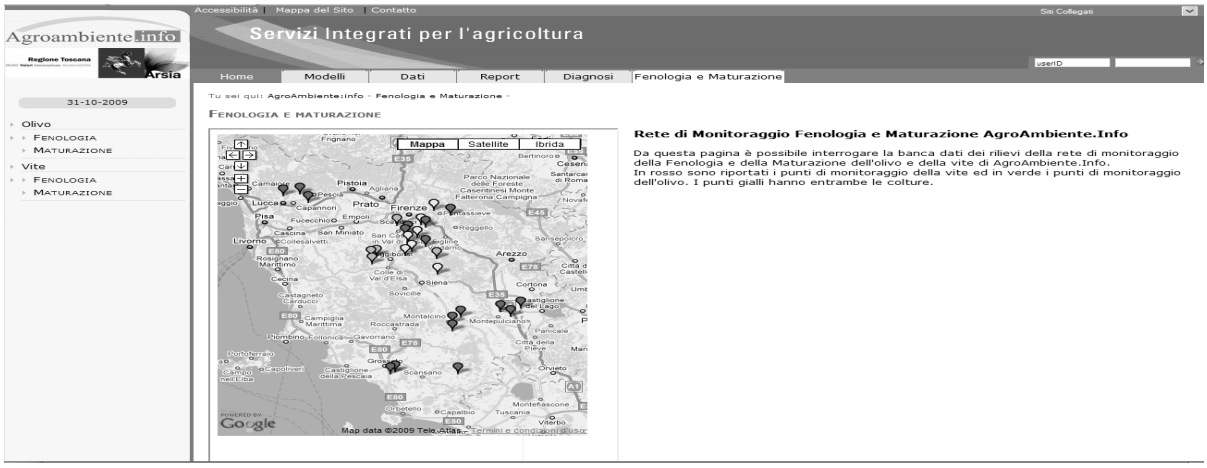

Figure 2: the main page of the olive and grape DSS with the links to the phenology and fruit ripening web pages.

For every new connection the olive DSS executes a query to the database by creating Web pages: those reporting the phenological and physical-chemical data on site (farms) (Figura 5) or those elaborating the metereological patterns. Using some computing functions integrated into the DSS the users can calculate for each weather station the actual value of the Degree Day. The calculations are based on data collected by the agro-meteorological network ARSIA at individual weather stations (Figura 6).

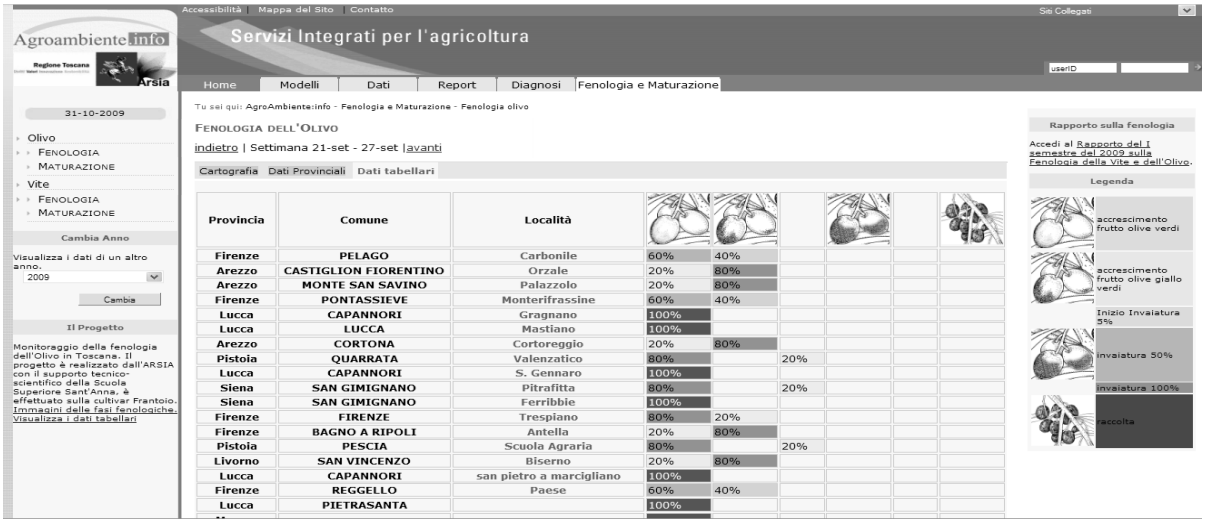

Figure 3: the main page of the olive DSS for phenology monitoring. In this page is possible to display the phenological stage present in that specific week (21-27 September) for the different farms monitored.

Figure 4: the main page of the olive DSS for olive fruit ripening monitoring. From this page is possible to access to the analytical data (left bottom) and to the reports (right).

The results of these years of DSS testing on phenology and olive fruit ripening have demonstrated the possibilities of the system for delivering and updating this information in real time, plus the ability to make this service accessible and easily usable by other entities engaged in territorial analysis.

Research institutions, regional government, consortiums and associations of producers may thus have a common tool for sharing and analyzing data, and for the development of agro-climatic models useful in determining the dynamics of phenolgy and olive ripening in Tuscany.

Figure 5: the page reporting the physical-chemical data for olive fruit ripening on each site monitored. In this example the data for the week 12-18 October are shown.

Figure 6: the page reporting the meteorological elaborations: Degree Day (DD) at different thresholds (10 and 7.5 degrees) or period for 2009. In the other columns the differences in day (plus or minus) to attain the same degree days are also reported.

Finally, in table 1, we report the numbers visited DSS pages in the year 2008 and 2009 (still in progress). It is clear the improvement in the number of visited pages in 2009 in respect to 2008 and the distribution along the different months with a clear peak in October for ripening web pages.

In conclusion the field monitoring networks, providing the physical and chemical data on olive ripening and phenology, together with meteorological data sets, and internet-based services proven to be a useful and appreciated system for real time monitoring and support decisions at olive orchard, research and administration level.

Table 1: Number of visited pages in 2008 and 2009 dived per months.

Months	2009		2008	
	Phenology	**Ripening**	**Phenology**	**Ripening**
January	3805	395		
February	7316	98		
March	8683	145		
April	5711	133	39	56
May	4699	166	189	397
June	5782	290	223	134
July	18321	167	5537	306
August	21287	217	6934	391
September	19311	499	6088	646
October	12085	1965	4423	1822
November			2706	611
December			2862	232

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S2-C10

Growth, chemical composition of vegetative tissues, total per plant uptake and utilization efficiency of Mn, Fe, Zn, Ca, Mg, K and P by the olive cultivar 'Chondrolia Chalkidikis', when cultivated in three soils with different physicochemical properties

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Abstract

Three-month-old rooted olive cuttings (*Olea europaea* L., cv. 'Chondrolia Chalkidikis', about 20-25 cm in height) were grown outdoors for 140 days (from the 30th of May until the 17th of October), in three soils (parent material marl, gneiss schist. and peridotite), to find out possible differences between them concerning growth, total per plant uptake and utilization efficiency of Mn, Fe, Zn, Ca, Mg, K and P. Plant growth was not affected by the soil type. Total per plant Mn, Fe and Mg content was significantly greater in the peridotite soil, compared to the gneiss schist and marl soils. Finally, the utilization efficiency of Mn, Fe and Mg was significantly lower in the peridotite soil, compared to the other two soils.

Key words: Nutrient uptake; nutrient use efficiency; olive cultivar; 'Chondrolia Chalkidikis'; parent material.

Croissance, composition chimique de tissus végétaux, absorption et efficacité d' utilisation de Mn, Fe, Zn, Ca, Mg, K et P par la variété d'oliviers 'Chondrolia Chalkidikis', cultivés sur trois sols différents

Résumé

Des plants d' oliviers (*Olea europaea* L., variété 'Chondrolia Chalkidikis', de haut 20-25 cm), âgés de trois mois, ont été cultivés pendant 140 jours (du 30 mai jusque au 17 octobre), à la campagne, sur trois sols (matériau d'origine marl, gneiss schist, et peridotite), pour investiguer si des différences statistiquement significatives existaient entre eux, en ce qui concerne la croissance, l' absorption et l' efficacité d' utilisation des éléments Mn, Fe, Zn, Ca, Mg, K et P. La croissance des plantes n'a pas été influée par le type de sol. La quantité totale des plants aux Mn, Fe et Mg a été significativement plus grande au sol de peridotite, qu'aux autres deux sols. Finalement, l'efficacité d'utilisation des Mn, Fe et Mg a été significativement plus basse au sol de peridotite, qu'aux autres deux sols.

Mots clés: Absorption ; efficacité d'utilisation des éléments ; variété d'oliviers ; 'Chondrolia Chalkidikis' ; maternel génétique parentale.

1. Introduction

Differential uptake and utilization efficiency of several macro- and micronutrients by different plant species, or cultivars have been observed by many researchers (Damon and Rengel, 2007; Jiang, 2008; Rengel and Damon, 2008). Furthermore, in the cases of micronutrient deficiency the chosen genotypes should have high uptake and transport capacity to the leaves of the particular element (Rengel, 2001). In addition to that, high internal nutrient utilization efficiency is also another property that one genotype should have under those conditions (Jiang and Ireland, 2005; Jiang, 2006). However, no attention has been paid until now concerning the influence of soil type when the same genotype/cultivar is cultivated, on its' nutrient uptake and utilization efficiency. On the other hand, although olive tree is considered as a species having great capacity to survive and produce in low-fertility soils, almost nothing has been reported so far concerning nutrients' (especially micronutrients') absorption, distribution and utilization efficiency.

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The aim of the present investigation was to study the influence of the soil type when olive trees are cultivated, on their growth, absorption and utilization efficiency of seven (micro- and macro-) nutrients. For that purpose, the olive cultivar 'Chondrolia Chalkidikis' was chosen for investigation, since it is very resistant to cold and widely cultivated for table olives production in the region of central Macedonia, of northern Greece. Three soils, from different parent material (Marl, Gneiss schist and Peridotite) and with different physicochemical properties, were chosen as a medium for planting, as they are the three basic soil types on which 'Chondrolia Chalkidikis' is cultivated in the area of Thessaloniki, central Macedonia, Greece.

2. Materials and Methods

2.1. Plant material and soil sampling

Three-month-old rooted olive cuttings (*Olea europaea* L., cv. 'Chondrolia Chalkidikis'), about 20-25 cm in height were grown outdoors for 140 days (from the 30th of May to the 17th of October) under ambient conditions, in black plastic bags, containing three kg of soil. The plants were randomized and separated, based on their height and initial total fresh weight, in three similar groups (corresponding to the three soils used) and six replicates per soil were included. The total number of plants was 18 (3 soils X 6 replicates). Three soils, derived from different parent material (Marl, Gneiss schist. and Peridotite) and having different properties, were included in the study. The soils were collected from the area around the city of Thessaloniki, i.e. Epanomi (Marl), Souroti (Gneiss schist.) and Galatista (Peridotite) and represent the three basic soil types on which olive tree is growing in the region of Thessaloniki, Macedonia, northern Greece. The soil samples were collected from the upper 60 cm of each soil, where most of the olive root system is usually growing. During the period of experimentation, the plants were irrigated, three times a week until the end of August and two times a week from the beginning of September to the 17th of October, with 200 ml of distilled water.

2.2. Soil sample analysis

After collection, soil samples were dried at room temperature, separated from stones and sieved to pass a 10 mesh screen before analyses. General chemical analyses, as well as extraction of micronutrients, were conducted in each one of the three soils. General chemical analyses of the soils included the pH measurement, the % content of organic matter, the exchangeable cations Ca, Mg and K, the particle size analysis and the % $CaCO_3$. The pH was measured in soil with distilled water (solution 1:1) (Bates, 1964), the organic matter with the $K_2Cr_2O_7$ method (Allison, 1965), the exchangeable cations according to the ammonium acetate method of pH 9 (pH 9 was chosen in order to reduce the solubilization of $CaCO_3$ as the pH values of the three soils were above 7) (Bower et al., 1952; Alifragis and Papamichos, 1995), the particle size analysis according to the 'Bouyoucos' method. Finally, the % $CaCO_3$ content was determined with the calcium meter method, while the extraction of Mn, Fe, Zn in the three soils was conducted with the DTPA method (Alifragis and Papamichos, 1995).

2.3. Plant growth parameters

Once the plants were harvested (at the 140th day), shoot length, as well as fresh and dry weight of leaves, stems, roots and total plant fresh and dry weights were measured. Samples were initially weighed (fresh weight), then washed with tap and afterwards with distilled water, dried at 75^{o}C for 24 hours, and weighed again (dry weight).

2.4. Mineral concentrations of plant tissues and total plant uptake of Mn, Fe, Zn, Ca, Mg, K and P

After the plants were divided into stems, roots and leaves, washed once with tap and twice with distilled water, dried at 75^{o}C for 24 hours and milled to a fine powder to pass a 30 mesh screen. A portion of 0.5 g of the fine powder of each sample was dry-ashed in a muffle furnace at 515 ^{o}C for 5h. Then, the ash was dissolved in 3 mL of 6 N hydrochloric acid (HCL) and diluted with double distilled water up to 50 mL and the concentrations of the elements Ca, Mg, K, Mn, Fe and Zn were determined by atomic absorption spectroscopy (Perkin-Elmer 2340, Waltham, MA, USA). The concentrations of the microelements were expressed in μg/g. D.W., while those of macronutrients in % D.W. Multiplying the concentration of each nutrient (μg or mg/g. D.W.) found in each plant part by its dry weight, the content (absolute quantity) of each nutrient per plant part at the end (at the 140th day) was calculated.

By addition of the nutrient contents of different plant parts, total nutrient content (μg or mg) per plant, and thus total nutrient uptake per plant was computed. Finally, the nutrient utilization efficiency of each nutrient (NUE), which is defined as the amount of biomass produced per unit of nutrient, was further calculated (Chapin and Van Cleve, 1991).

2.5. Statistics

All data were statistically analyzed by using the SPSS software package (SPSS 16.0.1. for Windows, Chicago, IL) and, particularly, for comparison of means of all the variables measured in each one of the three soils, Duncan's test was performed, for $P \leq 0.05$.

3. Results

3.1. Physicochemical properties of the three soils

The physicochemical properties of the three soils are shown in Table 1.

Table 1: Physicochemical properties of the three soils.

Soil	Sand	Clay	Loam	Texture	Organic matter (%)	pH	$CaCO_3$ (%)	P/Olsen (p.p.m.)	Ca	Mg	K	Mn	Fe	Zn
	%								meq /100g. soil			mg/ kg soil		
Marl	62.4	14.8	22.8	SCL	2.88	7.63	3.5	28.0	36.90	2.25	1.40	7.0	3.0	1.5
Gneiss schist	68.4	20.8	10.8	SL	1.68	7.15	1.3	15.0	10.68	2.17	1.20	6.0	5.0	1.5
Peridotite	52.4	26.8	20.8	SCL	4.44	7.97	15.4	17.5	36.21	2.46	6.70	12.0	4.0	1.0

As it is clear from the above table, the soils from parent material Marl and Peridotite were sandy-clay-loam (SCL), while that from Gneiss schist was sandy-loam (SL). The organic matter content (%) of the Gneiss schist. soil was relatively low, while that of the other two soils was sufficient and ranges between 2.88 and 4.44%. The $CaCO_3$ (%) content was medium (15.4%) in the Peridotite soil, while in the other two soils was very low. Among the exchangeable cations, Ca dominates in the Marl and Peridotite soils (which were saturated in Ca), while in Gneiss schist was approximately 27% of that in the other two soils. The greatest concentration of the DTPA extractable Mn was recorded in the Peridotite soil, while in the other two soils it was only about 50% of that in the previous one.

3.2. Plant growth

All the growth parameters studied (shoot length, fresh and dry weights of all tissues, total plant fresh and dry weight) did not differ significantly between the three soils, in which plants of 'Chondrolia Chalkidikis' were grown (data non-shown).

3.3. Mineral concentrations of plant tissues and total plant uptake of Mn, Fe, Zn, Ca, Mg, K and P

The concentrations of seven nutrients and the total per plant uptake of them are shown in Table 2. It is clear from that table that the total plant content of Mn, Fe and Mg was significantly greater in the Peridotite soil, than in the other two soils. In contrast to that, the concentrations of Mn, Fe and Mg in leaves did not differ between the three soils (Table 2).

Table 2: Mineral concentrations of roots and leaves and total per plant content of seven nutrient elements in the olive plants, grown in the three soils studied.

Vegetative tissue	Soil	Mn	Fe	Zn	Ca	Mg	K	P
		mg/kg d.w.			% d.w.			
Roots								
	Marl	41b	1790b	20b	0.93a	0.22b	0.76a	0.11a
	Gneiss schist	42b	1810b	26a	0.64b	0.27ab	0.74a	0.14a
	Peridotite	60a	3930a	18b	0.81ab	0.33a	0.71a	0.07b
Leaves								
	Marl	22a	54a	11a	0.95a	0.08a	0.81a	0.15a
	Gneiss schist	17a	48a	11a	0.94a	0.08a	0.89a	0.21a
	Peridotite	21a	62a	14a	0.99a	0.10a	0.97a	0.16a
Total plant content		mg			mg			
	Marl	0.70ab	17b	0.43a	248a	34b	231a	38a
	Gneiss schist	0.60b	17b	0.43a	190a	35b	195a	41a
	Peridotite	0.90a	42a	0.42a	232a	49a	231a	34a

The different letters in each column symbolize significant differences between the three soils, for $P \leq 0.05$ (SPSS; Duncan's test). The same also applies in Table 3.

3.4. Utilization efficiency of Mn, Fe, Zn, Ca, Mg, K and P

Table 3 shows the utilization efficiency of Mn, Fe, Zn, Ca, Mg, K and P in the three soils. As it is clear from that table, the utilization efficiency of Mn, Fe and Mg by the olive cultivar 'Chondrolia Chalkidikis' was significantly lower in the Peridotite soil, than in the other two soils.

Table 3: Nutrient utilization efficiency of seven nutrient elements in the three soils when 'Chondrolia Chalkidikis' was grown.

Soil	MnUE	FeUE	ZnUE	CaUE	MgUE	KUE	PUE
	mg d.w. of the plant/ total micronutrient content in μg			mg d.w. of the plant/ total macronutrient content in mg			
Marl	44.61a	1.87a	75.75a	134.58a	984.20a	144.02a	885.25a
Gneiss schist	45.22a	1.59a	63.72a	147.36a	790.37b	143.80a	683.33a
Peridotite	33.65b	0.76b	75.13a	139.20a	658.25c	139.58a	945.28a

4. Discussion

According to Therios (2005), the most appropriate soils for growth and fruiting of olive trees are the sandy-loam (SL), after sufficient fertilization with N, K and P and with adequate water content. However, the low C.E.C. capacity of the Gneiss schist soil (only 17%, compared to those of 43% and 48% of the Marl and Peridotite soils, respectively, data non-shown), together with the low organic matter content (Table 1) were possibly the basic reasons why plant growth (as expressed by shoot length and total plant weight) was not greater in the Gneiss schist soil, compared to the other two sandy-clay-loam soils (data non-shown).

The DTPA extractable Mn concentration in the Peridotite soil was two times greater, than in the Gneiss schist soil (Table 1). This is possibly the reason why total per plant Mn content was significantly greater in the Peridotite soil, compared to the Gneiss schist soil. Nevertheless, the concentrations of Mn in leaves did not differ between the three soils. This happened because significantly greater concentrations of Mn were recorded in the root system of the olive plants when they were grown in the Peridotite, than in the Marl and Gneiss schist soils (Table 2). Therefore, in the case when 'Chondrolia Chalkidikis' was grown in the Peridotite soil, although absorbed significantly greater quantity of Mn, limited transport of Mn from the root system to the leaves took place, than in the case when it was grown in the Marl soil (29.6% of the total plant Mn was distributed in leaves and 57% was 'retained' in the root system, compared to 19.9% in leaves and 68.6% in the root system and 19.5% and 69.7% when it was grown in the Gneiss schist and Peridotite soils, respectively, data non-shown). Based on the above remark, it could be concluded that 'Chondrolia Chalkidikis' was more Mn-efficient (greater percentage of the total plant Mn distributed in leaves) when was grown on the Marl soil, than on the Peridotite one. According to Jiang and Ireland (2005) and Jiang (2006), Mn efficient wheat cultivars owned this ability to a better internal utilization of Mn, rather than to a higher plant Mn accumulation. In our case, significantly lower utilization efficiency of Mn (expressed as mg total plant d.w. /μg of the total Mn content) took place in the Peridotite soil, compared to the other two soils (Table 3). However, between Gneiss schist and Marl soils, the olive plants grown on the Marl soil had better internal utilization efficiency (greater percentage of the total Mn content distributed in leaves).

The concentrations of Fe in the three soils were approximately at the same level (Table 1); however, the olive plants accumulated significantly greater amount of Fe when they were grown in the Peridotite soil. According to Maruyama et al. (2005), who made a comparison of iron availability in leaves of barley and rice, the difference in the Fe acquisition ability between these two species was affected by the differential mugineic acid secretion. Maybe a similar mechanism (i.e. greater or different kind of organic secretions from the root system of the olive plants in the Peridotite soil and formation of Fe-organic complexes), is true for 'Chondrolia Chalkidikis' in the three soils. Although in the Peridotite soil olive plants accumulated significantly greater amount of Fe, compared to the other two soils, the greatest part of it was accumulated in the root system (Table 2), and limited transport from root system to leaves took place (96.7% of the total plant Fe quantity was retained in the root system and only 1.3% of it was distributed in leaves, while for example in the Marl and Gneiss schist soils the corresponding percentages were 92.5% and 3.0% and 95.7% and 2.1%, respectively, data non-

shown). Furthermore, the concentrations of Fe in leaves did not differ between the three soils (Table 2). From all the above data, it could be concluded that 'Chondrolia Chalkidikis' was more Fe-efficient (as it was also more Mn-efficient) when was cultivated in the Marl soil (due to better internal utilization), than in the Peridotite and Gneiss schist soils. From Table 3 it is clear that Fe utilization efficiency (expressed as mg total plant d.w. /μg of the total Fe content) was significantly greater in the Marl and Gneiss schist soils, than in the Peridotite one. However, between Marl and Gneiss schist soils, the olive plants grown in the Marl soil had better internal utilization efficiency (greater percentage of the total plant Fe content distributed in leaves, as also happened with Mn).

The concentrations of all the macronutrients in leaves did not significantly differ between the three soils, as well as the total per plant quantities of them, with the exception of Mg (significantly more Mg accumulated by the olive plants in the Peridotite soil); however, the greatest part of it accumulated in the root system. It is of great importance that although the concentrations of Mg in the three soils were approximately at the same level, olive plants accumulated significantly greater quantity of it when they were grown in the Peridotite soil, than in the other two soils. This finding could be ascribed to a possible differential colonization of the root system by mycorrhiza between the three soils (colonization by different fungus genus/ species, or/and different percentage of root system colonization by fungus). In other experiments we did with 'Chondrolia Chalkidikis' and another two olive cultivars, we found two different arbuscular mycorrhiza fungus genus (*Glomus* and *Gigaspora*) and different colonization of the root system by fungus between these three soils (Chatzistathis, unpublished data). Better mineral nutrition with P and Mg of different plant species, inoculated with different mycorrhiza fungus genus, than when they were inoculated, has been found by Arines and Vilarino (1989), Liu et al. (2000) and Nogueira and Cordoso (2002). Furthermore, according to Satter et al. (2006), the uptake and utilization efficiency of N, P, K, Ca and Mg by *Acacia mangium* seedlings was significantly influenced by arbuscular mycorrhiza inoculation. It is possible in our case that the significantly lower utilization efficiency of Fe and Mg in the Peridotite, compared to the other two soils, could be also owned to a different colonization of the root system by mycorrhiza fungus between the three soils, as that described previously. In contrast to that, the significantly lower utilization efficiency of Mn in the Peridotite soil was probably due to almost twice greater concentration of it in that soil, compared to the other two (Table 1), as no significant difference in total plant biomass existed between the three soils (data non-shown).

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S2-C11

Thrips Infestation as a New Record in Egyptian Olive Groves

Essam Agamy [a], Monir El-Husseini [b] and Mohamed El-Kholy [c]

Abstract

New damage symptoms on leaves of olive (*Olea europaea*) were noticed for the first time in Egypt by M. El-Kholy in March 2008 identified as sand blasting effect as the discovery was accidentally preceded by a sandstorm. In December 2008 and April 2009 the persisting infestation on sampled leaves were identified by an investigating institution as *Alternaria alternata*. Treatment with fungicides was not effective. Following the flowering and fruit set season of 2009 other damage symptoms (which were never seen before) have been observed on fruitlets and developing fruits. Therefore M. El-Kholy inspected different olive growing regions, ascertaining that the infestation is widespread in two regions; El-Arish on the northeastern coast (31° 07 □ 32□ N – 33° 48 □ 44□ E) and inland Ismailia (30° 28 □ 28□ N – 32° 07 □ 30□ E). Some growers perceived this new damage as caused by Peacock Spot *(Spilocaea oleaginea)* which is confined in certain areas in the country and others contributed it to malnutrition. Close-up images of the damage symptoms on both leaves and fruits were emailed to olive agronomists and entomologists in all producing countries around the Mediterranean, Australia and the USA. Neither the symptoms nor the probability of the causative agent were identified except by Dr. Mohamed Ali Triki, Institut de l'Olivier of Tunisia and Dr. Leandro Ravetti, Modern Olives, Australia that it might be caused by a piercing sucking insect, mentioning that the symptoms were not seen before. The unknown insect was isolated and with the assistance of Dr. Essam Agamy, and Dr. Monir El-Husseini the insect was identified as thrips. For identification, images of microscopic slides were emailed to specialists in almost all olive producing countries. In September 2009 the thrips was classified as *Dendrothrips ornatus* by Dr. Enrique Quesada Moraga, Associate Professor, Agricultural and Forestry Engineering School, University of Cordoba. Later it was classified by Dr. Monir El-Husseini as *Dendrothrips eremicola* Priesner which belongs to the desert fauna of Egypt and was never mentioned previously as pest on any crop in the country.

The slashing and feeding activities by the mouthparts cause silvery bronze streaks on the attacked leaves with closely visible yellow lesions, mostly round-shaped with bleached centers at the spots where sap has been sucked. The vein skeleton of olive leaves resists the thrips attack due to denser parenchyma tissue; therefore the lesions exist around the central vein in an elliptical shape. The more damage to the leaves the more they give a leathery texture feeling. Feeding on the olive leaf tips has been rarely observed. Severely affected leaves have shown premature defoliation. Highly infested trees appear very unhealthy due to defective photosynthesis and transpiration, exhibiting a significant decline in yields. It has been also noted that the thrips attack flower ovaries when in full bloom and also the small fruitlets immediately after setting causing either abortion or malformed fruits. The damaged surviving fruitlets from this initial attack will develop further but will have scabby silvery scars, deformation and/or splitting. As a result, damaged table fruits must be discarded and the oil produced will be of inferior quality.

Since the insect is not registered in Egypt, M. El-Kholy carried out 14 different trials on trial blocks in his grove with different commercially approved insecticides. Some were not effective at all and the most effective biological ones in suppressing population were based on Abamectin and Spinosad. Other controlling measures are being applied which involve monitoring with sticky blue traps and sanitation of grove surface from leaves, litters, and pruning leftovers. Scratching the soil under the trees with a garden fork helps in exposing the prepupal and pupal stages thus interrupting the life cycle.

The emergence of this pest is attributed to different possibilities which are being under investigation, among which is disturbing the natural fauna and flora balance in the desert ecosystem by reclamation activities and growing monoculture on large areas. Current activities to resolve the problem involve:

1- Collecting information to construct a trusted data base on the status of this pest in Egyptian olive plantations concerning distribution, host plants, susceptibility of olive varieties, biology and generations.

2- Selecting a proper and safe biopesticide for rapid suppression of thrips population on olive trees.

3- Searching for effective natural enemies of *D. eremicola* in the virgin desert ecosystem on its original host plants for developing as biological control agents on commercial level.

4- Fostering close cooperation between scientists and olive growers to transfer knowledge and technology through both field days and farmer-to-farmer extension to understand the relationship between the different ecological aspects of the desert ecosystem and the new pest problem to benefit from and avoid emergence of future new infestations by pests.

The case indicates clearly the importance of regional and interregional cooperation on common crops.

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S2-C12

La mycorhization : une méthode biotique pour améliorer la croissance et contourner le stress abiotique chez l'olivier (*Olea europaea L.*)

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Résumé

L'inoculation en pépinière par une mycorhyze à vésicules et arbuscule (MVA) *Glomus intraradices* de jeunes plantes d'oliviers a montré une meilleure installation de la symbiose mycorhzienne chez Chemlali et Meski soldée par une amélioration de la croissance, de la ramification du système racinaire et de la biomasse végétative et racinaire. A l'exception de Zarrazi, les racines des plantes mycorhizées contiennent plus de sodium que les témoins. Alors que dans les feuilles des plantes mycorhizées, la teneur en Na reste inférieure à celui des plantes non mycorhizées. Ceci nous a permis de conclure que la souche mycorhizienne a amélioré le mécanisme d'exclusion du sodium au niveau des racines chez certains cultivars.
En terme de ce travail, les résultats obtenus ont montré que l'inoculation des plantules en pépinière a permis la réduction du stress salin, chez les cultivars étudiés, quand les doses Na Cl de l'eau d'irrigation sont inférieures à 75 mM.

Mots clés : Olivier, mycorhize, *Glomus intraradice,* Croissance, , salinité, Exclusion sodium

Mycorrhization: a biotic method to improve olive tree (*Olea europaea L.*) growth and to mitigate abiotic stress

Abstract

The inoculation in nursery by arbuscular myccorhizal fungus (AMF) (*Glomus intraradices)* of young plants of olive trees showed a better installation of the of symbiosis at Chemlali and Meski varieties balanced by an improvement of the growth, root system ramification and the vegetative and root biomass.
With the exception of Zarrazi cv., the roots of inoculated plants contain more sodium than the witnesses. While in the leaves of the mycorrhized plants, Na rate remains less than non-inoculated plants. This allowed us to conclude that the mycorrhizal stump improved the mechanism sodium root exclusion of the tested cultivars. In terms of this work, the obtained results showed that the AMF *Glomus intraradices* inoculation of the plants in nursery allowed the attenuation of the salt stress in studied cultivar when the Na Cl water rate's less than 75 mM.

Key words: Olive tree, mycorrhize, *Glomus intraradices ,* growth, salinity , Na exclusion,

1. Introduction

L'utilisation des mycorhizes revêt une importance particulière sous des climats arides et semi arides où la salinité des sols réduit l'apport par la plante des fertilisants phosphaté et azoté. Ainsi, certains travaux ont pu montrer que la tolérance des plantes à la salinité peut être améliorée par les symbiotes (Dixon et al., 1993). L'efficience de ces symbioses entre le champignon et la hôte reste toutefois liée, en plus des conditions pédoclimatiques, aux espèces symbiotiques mises en jeu.
En dépit de leur rareté, les études ont montré que l'olivier est parmi les espèces qui peuvent être infectées par les endomycorhizes (MVA). Inoculée par *Glomus mosseae* les racines des jeunes plantes d'oliviers des cultivars Moraïolo, Leccino et Frantoï sont devenues plus ramifiées permettant aux plantules une meilleure croissance (Citernesi et al., 1998). L'inoculation des plantules de différents cultivars avec des champignons mycorhiziens a montré une efficacité importante à surmonter les contraintes de culture (Hayman et al., 1976; Roldan – Fajero et Barea, 1986).Des études plus récentes (Porcel et al., 2006) ontv montré que les endophytes engendrent chez les plantes des transformations morphologiques et physiologiques pour tolérer les contraintes du milieu. La colonisation mycorhizienne diminue les effets osmotiques chez les plantes occasionnés par la toxicité de Na+ (Ruı´z-Lozano and Azcon, 2000 ; Rabie and Almadini, 2005)

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2. Matériels et condition d'inoculation et de stress salin

Les boutures semi ligneuses enracinées et ayant le même âge et relativement le même nombre de racines adventives, des variétés Chemlali, Zalmati Meski et Zarrazi, sont repiquées dans des pots de 1.5 l remplis de substrat préalablement stérilisé (pour éliminer autre organisme vivant**)**. Le substrat est mélangé à 2:1 constitué de sable grossier et de tourbe.

Pour chaque cultivar deux lots de 10 plantules sont choisis, l'un mycorhizé (M) au niveau de chaque plantule par 3 g de substrat infecté par une souche améliorée de *Glomus intraradices* (Premier Tec , Quebec, Canada*).* L'autre lot non mycorhizé (NM) est pris comme témoin. Les pots sont placés en serre d'acclimatation durant deux mois. Puis ils ont été transférés sous ombrière à l'abri des pluies .Les plantules reçoivent périodiquement et équitablement de l'eau courante stérilisée. Chaque mois la longueur totale des pousses végétatives est mesurée.

Après 9 mois, des plantules des deux lots sont séparées de leurs substrats par un lavage soigneux. La biométrie des différents types de racine (racines adventives, ordre 1 R1), ordre 2(R2) et ordre 3 (R3)) ainsi que les poids frais et secs de la partie végétative et racinaire de chaque plantule sont procédés. Le rendement en matières sèches (MS%) dans les feuilles, le bois et les racines a été obtenue après dessiccation des organes dans une étuve à 74℃ pendant 48 heures.

Le pourcentage de colonisation des racines par *Glomus intraradices* est déterminé par la méthode de colorisation et d'observation de Phillips and Hayman (1970).

Pour étudier l'effet du stress salin sur les plantules mycorhisées comparées à un témoin (plantules non mycorhizées), des plantules âgées de 6 mois des 4 cultivars sont repiquées dans les mêmes conditions citées précédemment. Durant un mois toutes les plantules (inoculées et témoins) sont irriguées avec l'eau ordinaire de robinet stérilisée. Un mois après l'inoculation, un traitement salin est réalisé en irriguant les plantules avec de l'eau contenant les concentrations suivantes (25, 50, 75 et 100 mM de NaCl) durant 11 mois. Pour éviter le choc de l'accumulation excessive de NaCl, Les doses NaCl sont fractionnées en les répartissant entre les irrigations.

Les deux essais sont conduits en dispositif expérimental aléatoire avec 2 traitements (mycorhizé:M et non mycorhizé: NM) et 10 répétions pour chaque traitement. La signification entre les résultats est réalisée avec une analyse de la variance (ANOVA) et la comparaison entre les moyennes est faite moyennant le test Duncun.

3. Résultats et interprétations

3.1. Effet de *Glomus intraradices* sur la ramification racinaire

Les résultats obtenus (Fig.1) montrent que le champignon a provoqué chez leurs hôtes une amélioration de la ramification du système racinaire. En effet, si le nombre des racines adventives n'a pas beaucoup changé, celui des racines latérales du 1er ,2ème et 3ème ordre a augmenté, de même pour les longueurs totales. Ayant des diamètres variant entre 1 et 2 mm ces racines sont les sites préférentiels des endomycorhizes. Concernant le taux de répartition des racines, la figure (1) montre qu'une dominance des racines d'ordre 3 (85% du système racinaire) chez les plantes M de Chemlali et Meski (70%). Cette répartition est au profit des racines d'ordre 1 et 2 de Zalmati M (30% et 60%) et de Zarrazi M (35% et 50%).

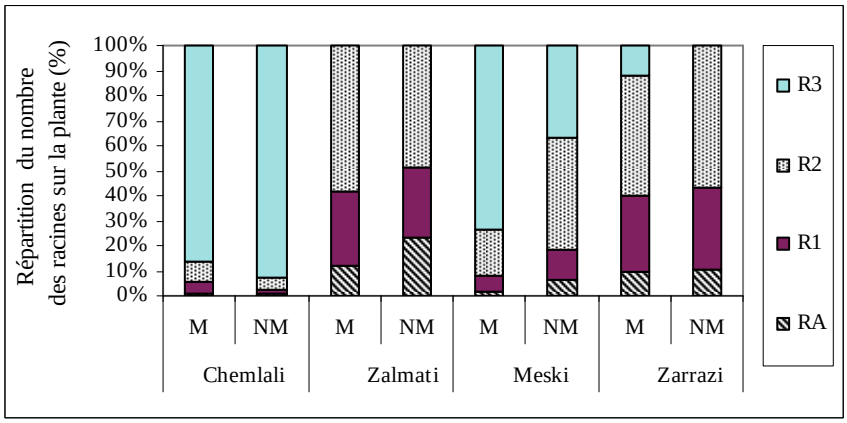

Figure 1: Répartition du nombre des racines RA, R1, R2 et R3 des plantes M et NM avec RA : racines adventives ; R1, R2 et R3 : racines d'ordre 1, 2 et 3.

Les chevelures racinaires les plus intenses sont observées chez les plantules mycorhizées de Chemlali, Meski (fig.1) L'analyse statistique montre des différences hautement significatives au seuil de 1% pour le même cultivars entre les plantes mycorhizées et non mycorhizées. Des résultats identiques sont obtenus sur le cultivar Frantoïo et Leccino inoculée avec *Glomus mosseae* (Gm). Chez ces cultivars la symbiose a augmenté la longueur et le nombre du 1er, 2 ème et 3 ème ordre. Les racines adventives ne sont pas affectées par Gm (Citernesi et al. 1998). D'autres études ont montré aussi que les mycorhizes, par le développement de leur mycélium, modifie l'architecture des racines (Smith et Read, 1997; Berta et al. 1995). L'analyse morphométrique du système racinaire chez *Vitis vinifera* (Schellenbaum et al. 1991), *populus* sp. (Hooker et al. 1992), *Platanus acerifolia* (Tisserant et al., 1992)et *Prunus ceracifera* (Fortuna et al., 1998) a montré une amélioration de la ramification du système racinaire des plantes mycorhizées.

3.2. Effet de *Glomus intraradices* sur la biomasse végétative et racinaire

Dans le but de quantifier l'effet de *Glomus intraradices (*Gi***)*** sur la croissance des plantes nous avons déterminé le rendement en matières sèches (MS%) = poids sec/poids frais x 100) au niveau de tous les organes de la plante**.** L'examen du Tableau (1) montre que les biomasses racinaires et végétatives sont plus importantes chez les plantes mycorhizées. Positivement Corrélés avec le poids frais et sec du matériel végétal, les rendements en matière sèche (MS) sont nettement améliorés avec la mycorhization. Chez le cultivar Chemlali la MS des feuilles, du bois et des racines passe de 26,78; 43,77 et 44,45% dans le témoin à 37,83; 50,5 et 51,24% chez les hôtes. L'analyse statistique révèle une différence significative au seuil 5% entre les taux des plantes mycorhizées et non mycorhizées. De même pour Meski ou le taux en MS est plus important chez les plantes mycorhizées. La diminution dans les biomasses végétatives et racinaires chez les plantes mycorhizées de Zalmati et Zarrazi est probablement liée à l'absence de racines tertiaires chez celles-ci.

Tableau 1: Effet de *Glomus intraradices* sur pendement en matières sèches (%).

Cultivars	Mycorhization	Matière sèche (%)		
		Racine	Bois	Feuille
Chemlali	M	37,83	50,5	51,24
	NM	26,78	43,77	44,45
Zalmati	M	28,91	44,36	41,21
	NM	37,66	44,59	41,72
Meski	M	47,24	55,71	45,231
	NM	34,35	45,94	42,88
Zarrazi	M	33,33	42,87	40,6
	NM	36,99	43,64	41,64

M : plante mycorhizée ; NM : plante non mycorhizée

3.3. Colonisation mycélienne des racines

Les observations microscopiques périodiques des racines chez les plantes mycorhisées ont montré que la colonisation mycélienne a concerné exclusivement les racines d'ordre 2 et 3 ayant des diamètres variant entre 0,5 et 1 mm.

En se basant sur le nombre de racines colonisées par rapport aux racines observées un taux de colonisation mycorhizienne du système racinaire a été calculé pour chaque cultivar. Les résultats consignés dans le tableau 2 montre que les système racinaires des cultivars Chemlali et Meski détiennent les taux les plus importants de colonisation mycorhizienne avec respectivement 62% et 49%. Les résultats obtenus révèlent que les taux de colonisation sont en étroite relation avec la morphologie racinaire des cultivars (Tab.2). En effet, les cultivars Chemlali et Meski ont le système racinaire le plus envahi par le mycélium du champignon et le plus développé par la présence de racines fines d'ordre 3 dont le diamètre est inférieur ou égale à 0.5 mm.

Tableau 2: Taux de colonisation mycorhizienne du système racinaire des cultivars avant le traitement salin.

Cultivar	Nombre de racines observées	Nombre de racines colonisées	Taux de colonisation (%)
Chemlali	157	97	62
Zalmati	120	35	29
Meski	135	66	49
Zarrazi	125	28	22,4

Des résultats similaires ont été notés par d'autres auteurs sur d'autres espèces. En effet, sur blé, Sanders et Sheikh (1983) ont constaté une corrélation positive entre le développement du système racinaire de quelques lignées et celui du taux de mycorhization. Sur 4 espèces agro forestières, Guissou et al. (2001) ont signalé une amélioration de l'absorption minérale chez les plantes mycorhizées ayant un bon développement racinaire.

3.4. Réponse des plantes mycorhizées à la salinité

3.4.1. La croissance végétative

Généralement, chez les plantes mycorhizées (M) et non mycorhizées (NM), la croissance végétative évaluée par la longueur cumulée des pousses végétatives tend vers la baisse progressivement sous l'effet de la salinité (Fig.2). Cette diminution est plus prononcée chez les témoins (NM). La salinité a diminué, en fonction du temps, l'infection mycorhizienne de *Glomus intraradices* (Gi). Toutefois, l'effet de la salinité a été légèrement atténué avec *Glomus intraradices* chez tous les cultivars (Fig.2). En effet, la croissance des plantules mycorhizées des 4 cultivars, s'est maintenue stable après 10 mois avec la dose 75mM NaCl en comparaison avec le témoin (NM) dont une forte chute de la croissance y est observée avec la même dose. Gi a relativement retardé l'effet du stress salin chez les plantes mycorhizées. Les résultats ont montré aussi que, l'effet de Gi diminue quand NaCl augmente dans le substrat d'élevage et que la dose 100 mM exerce un effet inhibiteur voire létale sur Gi dés son installation (Fig. 2).

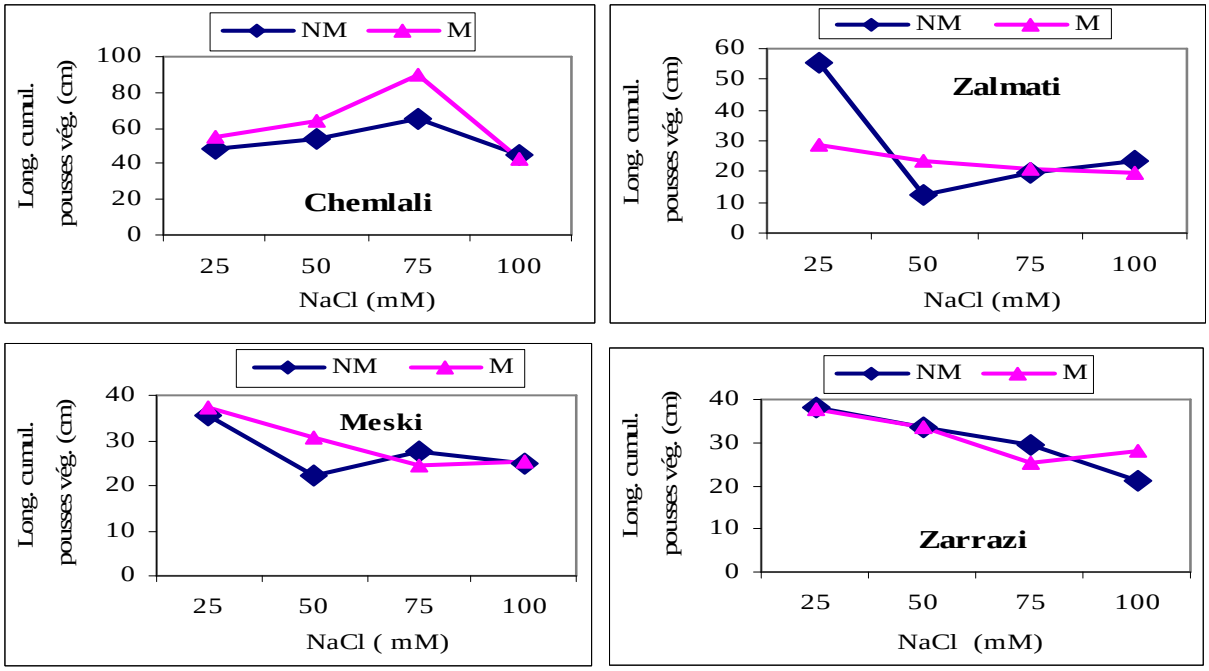

Figure 2: Effet de la mycorhization sur la croissance végétative des plantules irriguées avec différentes concentrations en Na Cl (mM).

En terme de cet essai de mycorhization des plantes d'olivier soumises à un stress salin, les résultats obtenus ont montré que la symbiose endomycorhizienne excercée par **Glomus intraradices (Gi)** a permis de réduire l'effet de NaCl chez la majorité des cultivars avec des doses inférieures à 75 mM. Dans le cas des doses faibles à modérées de NaCl (25 et 50 mM) Gi améliore la croissance des plantules sans toutefois être affecté par la salinité. Des résultats similaires ont été observés sur deux cultivars italiens Carolea et Nocellara del Bellice (Briccoli Bati et al.,1994), qui ont noté une diminution dans l'infection mycorhizienne avec l'augmentation de la salinité. Les mêmes auteurs ont rapporté que les fortes doses de sel ont réduit le statut minéral des plantes mycorhizées des deux cultivars. En effet, Requena et al., (1996; 2001) ont rapporté que la salinité affecte la viabilité des hyphes mycorhiziens qui constituent les principales sources de la propagation des champignons et des arbuscules qui représentent le site privilégié des échanges entre les deux partenaires de la symbiose Gianinazzi-Pearson et al.(1988).Cette amélioration de la croissance sous stress salin s'explique par le fait que Gi développe un système mycélien qui aboutit à l'amélioration du potentiel hydrique des plantes stressées. Par conséquent une meilleure absorption d'eau et de sels minéraux. Cette amélioration de la tolérance à la salinité des plantes mycorhizées a été déjà signalée sur d'autres espèces (Ruı´z-Lozano and Azcon, 2000 ; Rabie and Almadini, 2005)

4. Conclusion

Les tests d'inoculation de *Glomus intraradices* ont amélioré significativement aussi bien la croissance végétative que racinaire des cultivars Chemlali et Meski et un peu moindre pour le cultivar Zarrazi. Pour Zalmati il n'existe pas de différence significative entre les plantules inoculées et témoins. Les racines des plantes inoculées sont plus ramifiées.Généralement, chez les plantes mycorhizées et témoins, la croissance végétative, évaluée par la longueur cumulée des pousses végétatives, tend vers la baisse progressivement sous l'effet de la salinité. Cette diminution est plus prononcée chez les témoins. La salinité a diminué, en fonction du temps, l'infection mycorhizienne de Gi. Toutefois, l'effet de la salinité a été légèrement atténué avec *Glomus intraradices* chez tous les cultivars comparé avec le témoin, tout en signalant que l'effet de Gi diminue quand NaCl augmente dans le substrat d'élevage et que la dose 100 mM exerce un effet inhibiteur voire létale sur son installation.

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S2-C13

Recrudescence du dépérissement de l'olivier causé par les champignons telluriques en Tunisie

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Résumé

Cette présente étude a pour but d'élucider l'étiologie de la maladie du dépérissement de l'olivier sévissant dans plusieurs oliveraies tunisiennes et d'étudier sa répartition géographique. La maladie provoque le flétrissement, le dessèchement et la mort total des oliviers de différents âges et cause des dégâts considérables en pépinière et en plein champ. Les investigations au champ et au laboratoire ont montré que plusieurs champignons telluriques sont responsables des pourritures au niveau du collet et des racines des oliviers dépéris. Il s'agit de *Fusarium solani, Fusarium oxysporum, Fusarium lateritium,Rhizoctonia bataticola, Cylindrocarpon destructrices, Rhizoctonia solani*, *Verticillium dahliae*, *Corticium rolfsii, Armillaria mellea, Phytophthora* sp., et *Pythium* sp. Le pouvoir pathogène de ces champignons a été confirmé par inoculation des jeunes plants d'oliviers de la variété Chemlali. Récemment, une importante évolution spatio-temporelle de la verticilliose de l'olivier causée par *Verticillium dahliae* Kleb., a été enregistrée dans plusieurs régions du pays (Sfax, Sousse, Monastir, Sidi-Bouzid, Kairouan, Mahdia, Tataouine, Zaghouane,…). Tous les nouveaux foyers sont découverts dans des périmètres irrigués où l'olivier est cultivé en intercalaire avec quelques espèces légumières sensibles à ce champignon.

Most Clés : Olivier; dépérissement; flétrissement, pourriture des racines, champignons telluriques.

Increesing of olive trees dieback caused by Soilborne fungi in Tunisia

Abstract

The objective of this study was to elucidate the etiology of the dieback and death of olive tree in several olive growing areas in Tunisia and to determine its geographical distribution. Olive trees are being severely affected by wilt, die-back and death. This disease cause serious problems of mortality in nurseries and olive fields. Our Surveys and laboratory isolation, revealed the presence of complex of soilborne fungi causing collar and root rot of olive trees. These fungal species are *Fusarium solani, Fusarium oxysporum, Fusarium lateritium, Rhizoctonia bataticola, Cylindrocarpon destructans, Rhizoctonia solani*, *Verticillium dahliae*, *Corticium rolfsii, Armillaria mellea Phytophthora* sp., and *Pythium* sp. The pathogenicity of these soil borne fungi proved that they are the causal agents of olive trees decline in Tunisia. Recently, an important spread of *Verticillium dahliae* kleb. was observed in several olive growing regions of Tunisia (Sfax, Sousse, Monastir, Sidi-Bouzid, Kairouan, Mahdia, Tataouine, Zaghouane,…). The disease occurred mainly in some orchards where olive trees were intercalated with some sensitive vegetables to this pathogen.

Keywords: *Olea europea*, die-back, wilt, decline, root rot, soil borne fungi.

1. Introduction

L'olivier (*Olea europaea*) occupe une importante place parmi les espèces fruitières cultivées en Tunisie. Elle occupe le quatrième rang mondial en nombre de pieds d'olivier avec environ 66 millions d'arbres, et le deuxième rang en termes de superficie, de l'ordre de 1,7 millions d'hectares, soit près du tiers des terres arables du pays (Jardak, 2008). Ces dernières années, plusieurs cas de dépérissement sont signalés sur des jeunes plants d'oliviers issus de boutures herbacées en pépinière et en plein champ et aussi sur des oliviers âgés. Les mauvaises reprises après plantations, les dépérissements et les mortalités les plus couramment observés sont en général causés par des agents pathogènes responsables de graves pourritures au niveau du collet et des racines. Plusieurs

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champignons telluriques tel que *Cylindrocarpon destructans, Phytophthora megasperma, P. palmivora, Pythium irregulare, Sclerotium rolfsii, Armillaria mellea*, *Rosellinia necatrix*, *Fusarium* spp., *Rhizoctonia* spp. et *Verticillium dahliae,* se sont montré à l'origine des symptômes de flétrissement et de dépérissement partiel ou total de l'olivier dans plusieurs pays du bassin méditerranéen. (Al Ahmed, 1984; Boulila, 1993, 1994 a et b, 2001; Sanchez Hermandez et al., 1995, 1997, 1998; Porras *et al*., 2003; Lucero, 2006; Jardak, 2008). La présente étude a pour objectif de déceler l'importance des dégâts causés par les principaux agents de dépérissement en Tunisie.

2. Matériel et Méthodes

2.1. Importance de la maladie et description des symptômes

Des prospections de terrains et des enquêtes ont été menées dans plusieurs régions oléicoles du pays dans le but d'étudier l'importance des dégâts sur les oliviers dépéris en plein champ et en pépinière, de préciser les caractères pathologiques et de repérer les principaux foyers en Tunisie. Cette étude consiste également à décrire les symptômes caractéristiques de la maladie et d'étudier l'évolution du dessèchement sur les arbres dépéris. Elle comprend d'abord une description de la morphologie externe (organes aériens,…) puis une étude de l'anatomie interne basée sur l'examen des tissus internes (système vasculaire) au niveau des organes attaqués.

2.2. Isolement et identification

Des échantillons de bois ont été prélevés au niveau du collet, des racines de la partie vivante au-dessous de la zone nécrosée des rameaux attaqués, sur des oliviers malades au champ et en pépinière afin d'isoler d'éventuels agents pathogènes. Ils sont soigneusement désinfectés à l'alcool 95°, lavés à l'eau distillée stérile, puis découpés en petits fragments ensuite déposés dans des boites de Pétri contenant du milieu PDA et incubés à 25°C . Après cinq jours, chaque colonie fongique isolée (excepté les saprophytes) est transférée sur plusieurs milieux spécifiques (SNA, Prune medium, Water Agar) supplémenté de Streptomycine et/ou de Cefotam à raison de 200 mg l^{-1}. L'identification des champignons isolés est basée sur les critères morphologiques des organes fructifères produits dans les différents milieux, comme décrit dans plusieurs clés d'identifications (Booth, 1966; Dhingra and Sinclair, 1978; Maas, 1998; Agrios, 2004; Nasraoui, 2000 and 2006; John F. Leslie, 2006). Cependant pour les Pythiacées, les colonies fongiques isolées sont transférées sur les milieux spécifiques des oomycètes: Oat Meal Agar (Triki *et al*., 2001) contenant 300 mg l^{-1} de Streptomycine ou bien sur milieu Bonnet (Bonnet *et al*., 1980) incubé à 25°C. Leur identification est eff ectuée en se basant sur les clés de Ribeiro (1978); Waterhouse (1968); Van der Plaats-Niterink (1981) et Jeffers et Martin (1986).

2.3. Test de Pathogénie

La pathogénie de tous les champignons isolés est confirmée par inoculation des jeunes plants d'oliviers de la variété Chemlali, âgés de deux ans. L'inoculation est effectuée par trempage des racines bien lavées à l'eau courante et légèrement blessées (au niveau de la racine pivotante) pendant une heure dans la suspension de spores de chaque champignon ajustées à 10^6 spores ml^{-1}. Cependant, pour le cas de *R. bataticola* et *R. solani*, l'inoculation des jeunes oliviers est également effectuée par immersion du système racinaire dans des solutions de sclérotes et de mycélium respectivement. Les jeunes plants du lot témoin sont blessés puis trempés pendant une heure dans l'eau distillée stérile. Enfin, tous les plants sont transplantés dans des pots en polyéthylènes contenant un substrat stérile (tourbe : sable, 1 :1 v/v), puis incubés dans une chambre de culture dans des conditions de forte humidité. Les plants sont irrigués deux fois par semaine.

2.4. Etude de quelques facteurs abiotiques contribuant à la manifestation de la maladie

Dans le but de vérifier l'impact de certains paramètres abiotiques tels que la salinité, la texture du sol, et le mode de conduite (entretien,…) sur la manifestation de la maladie, une enquête de terrain a été effectuée dans plusieurs parcelles infestées et dans quelques pépinières situées dans des aires géographiques différentes.

3. Résultats et Discussions

3.1. Importance de la maladie et description des symptômes

Le dépérissement de l'olivier est une grave maladie qui attaque souvent les jeunes plants en pépinière en plein champ dans plusieurs régions oléicoles de la Tunisie notamment dans le centre et le nord du pays (Fig. 1). Les investigations au champ et en pépinière montrent l'importance des dégâts sur les jeunes oliviers à cause de l'évolution rapide de la maladie. Les symptômes apparaissent généralement dès le début du printemps et sont presque identiques avec la majorité des champignons telluriques. Ils se manifestent au début par un changement de couleur au niveau de la frondaison (du vert sombre au vert clair puis au jaune). A un stade plus avancé, l'olivier souffre d'un jaunissement localisé à évolution ascendante à partir de la base de quelques rameaux suivi par un desséchement localisé qui débute à partir des jeunes pousses (Figure 2). Enfin la maladie fini par l'envahissement total de l'arbre (évolution descendante) lorsque la dégradation atteint la totalité du collet et/ou des racines.

3.2. Isolement et identification

Les isolements fongiques réalisés, à partir des échantillons de bois, de collet et de racines prélevés des oliviers malades, ont permis d'avoir plusieurs isolats fongiques qui se sont révélées appartenir à différents genres. L'identification morphologique basée sur l'utilisation des milieux sélectifs et/ou semi sélectifs suivant plusieurs clés d'identifications a montré que les champignons fréquemment isolés en pleins champs et en pépinières sont *Fusarium solani, Fusarium oxysporum, Fusarium lateritium, Rhizoctonia bataticola, Cylindrocarpon destructrices, Rhizoctonia solani*, *Corticium rolfsii, Phytophthora* sp., et *Pythium* sp. (Booth, 1966; Waterhouse, 1968; Dhingra and Sinclair, 1978; Ribeiro, 1978; Bonnet *et al*., 1980; Van der Plaats-Niterink, 1981 ; Jeffers et Martin, 1986; Maas, 1998; Agrios, 2004; Nasraoui, 2000 et 2006; John F. Leslie, 2006). L'espèce *Armillaria mellea* est souvent décelée sur des oliviers adultes souffrant d'asphyxie suite à une importante stagnation d'eau dans des sols lourds.

Des dégâts plus accentués se traduisant par des enroulements multiples au niveau du système racinaire sont également observés en plein champ sur des jeunes oliviers âgés de deux ou trois ans. Ces déformations racinaires sont notamment causées dès le stade pépinière, où le jeune olivier issu de bouture herbacée élevé émet au début une puissante racine pivotante, et se trouve par la suite rapidement limitée par les parois de conteneurs trop petits. Les étranglements et les lésions observées au niveau du système racinaire favorisent souvent l'invasion par les champignons telluriques. Ainsi, plusieurs cas de dépérissement sont observés sur des jeunes oliviers suite à un synergisme marqué entre *Fusarium solani* et *Rhizoctonia bataticola*.

3.3. Émergence de la verticilliose de l'olivier en Tunisie

La Verticilliose de l'olivier causée par *Verticillium dahliae* Kleb. a été décrite pour la pr emière fois en Tunisie dans une oliveraie de la délégation de Mahres à Sfax (Triki *et al*., 2006). Les symptômes de cette maladie se manifestent de façon sectorielle, soit sur branche, soit sur charpentière ou simplement quelques rameaux. La verticilliose induit sur les parties attaquées un flétrissement unilatéral puis les symptômes se généralisent. Les rameaux attaqués portent des feuilles qui s'enroulent en forme de gouttière vers leur face inférieure et perdent leur coloration verdâtre pour virer au brun clair, ce qui induit leur dessèchement complet. Sur les branches attaquées, les jeunes pousses peuvent apparaître partiellement ou totalement desséchées. Des coupes transversales ou longitudinales au niveau des tissus malades montrent souvent un brunissement du bois. Le système radiculaire de quelques arbres malades présente parfois des altérations et un brunissement au niveau du cylindre central.

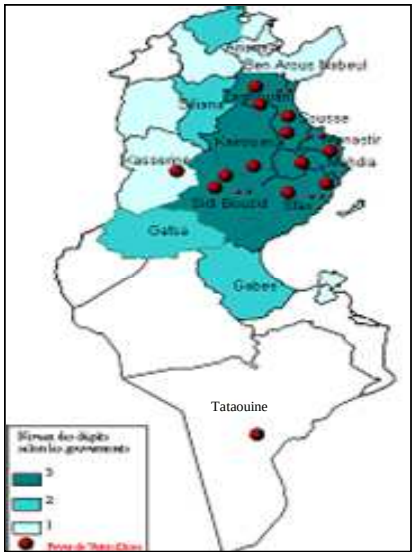

Figure 1: Cartographie des Foyers de dépérissement de l'olivier en Tunisie (● = foyers de Verticilliose de l'olivier).

Figure 2: Evolution des symptômes de dépérissement sur un jeune olivier.

Les études épidémiologiques réalisées ces dernières années dans les principales oliveraies tunisiennes montrent une importante évolution spatio-temporelle de cette maladie dans plusieurs oliveraies situées dans différentes régions de divers étages bioclimatiques (Sfax, Sousse, Monastir, Sidi-Bouzid, Kairouan et Zaghouane,…) (Fig. 1). Les investigations au champ et au laboratoire ont permis de mettre en évidence la présence de *Verticillium dahliae* Kleb. Tous ces nouveaux foyers sont découverts dans des périmètres irrigués où l'olivier est cultivé en intercalaire avec quelques espèces légumières (tomate, piment, pomme de terre, aubergine,…) sensibles à ce champignon.

Les travaux de caractérisation moléculaire des isolats de *Verticillium dahliae* sont basés sur l'amplification de séquences répétées (rep-PCR) et par l'amplification aléatoire de région de génome (RAPD). Parmi 20 amorces testées, uniquement dix ont été sélectionnées pour la qualité des profils et la productibilité des produits amplifiés (Fig. 3). En effet, les amorces sélectionnées ont révélé une variabilité de la structure génétique entre les isolats de *Verticillium dahliae* (Fig. 3). Ainsi, cette étude est fondamentale pour former des groupes d'isolats de structures génétiques homogènes liés aux sites géographiques de prélèvement des échantillons porteurs de la maladie.

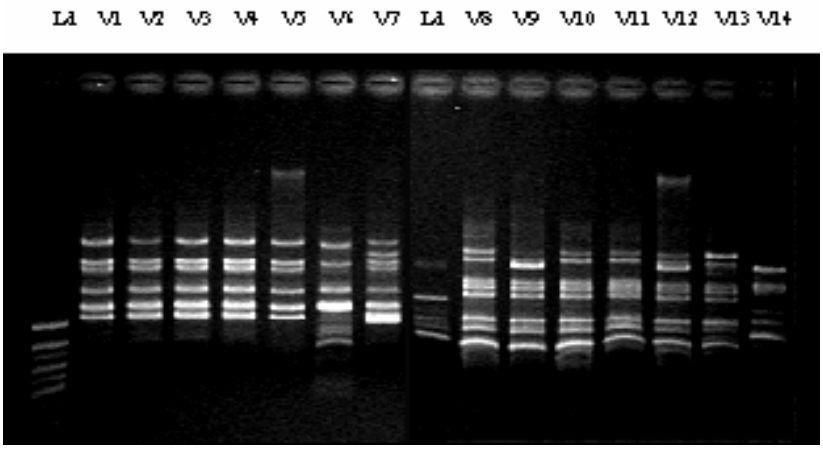

Figure 3: étude de la variabilité génétique de quelques isolats de *V. dahliae* par RAPD (Amorces OPD1 et OPK2 ; puits 2 à 8 et puits 10 à 16 = isolats de *V. dahliae*).

3.4. Tests de pathogénie

L'inoculation des jeunes plants d'oliviers de la variété Chemlali par les champignons telluriques isolés, confirme leur pathogénie par la production des symptômes caractéristiques de flétrissement, de jaunissement et de dessèchement à partir de la quatrième semaine de croissance. Toutefois, les cinq espèces fongiques *F. solani*, *R. solani*, *C. destructans, V. dahliae, Sclerotium rolfsii* et *R. bataticola* se sont avérées les plus agressives. D'ailleurs, les plantes inoculées par ces champignons sont totalement dépéries et présentent des fortes nécroses au niveau des collets et des racines. Il est également à signaler que les isolements fongiques effectués à partir des organes attaqués confirment la pathogénie de toutes les espèces fongiques inoculées.

3.5. Effet de quelques facteurs abiotique sur la manifestation de la maladie

Le phénomène de dépérissement des jeunes plants d'oliviers résulte d'une détérioration générale et graduelle des arbres pouvant se produire à cause de plusieurs facteurs biotiques et abiotiques ou de leur interaction. Les investigations au champ ont montré que les maximums d'attaques (80% environ) sont obtenus dans des sols lourds à forte rétention d'eau. Ces sols non filtrant et trop argileux favorisent la stagnation d'eau qui provoque la pourriture du collet et/ou des racines des jeunes oliviers et l'invasion par les champignons telluriques qui provoquent le dépérissement partiel ou total de l'arbre. D'autres facteurs comme la salinité de l'eau d'irrigation, la carence ou la toxicité d'un élément minéral et l'entretien de la parcelle (désherbage, façon culturales,…) peuvent contribuer fortement à l'installation de la maladie. En effet, le dépérissement peut être complexe et liée à l'invasion par un parasite de faiblesse qui précède un état de stress provoqué par un autre facteur non parasitaire (salinité, blessures,…). Il est à noter que le dépérissement des jeunes oliviers est souvent rencontré dans des parcelles ayant des espèces maraîchères sensibles à ces champignons telluriques en intercalaire avec l'olivier ou qui étaient cultivées précédemment.

4. Conclusion

Cette étude a montré que les nouvelles plantations d'oliviers sont menacées par des attaques de dépérissement en pépinière et en plein champ, dans plusieurs régions de la Tunisie. La plupart des symptômes observés sur olivier correspondaient à ceux décrits ailleurs dans plusieurs pays du bassin méditerranéen. Les investigations au champ et au laboratoire ont montré que la maladie est causée par un complexe de champignons telluriques qui sont capables d'induire des pourritures au niveau du collet et des racines. L'évolution rapide de la maladie dans l'espace et dans le temps justifie l'importance des dégâts en pépinière et en plein champ. En Tunisie, les hautes températures du sol (25-30℃) en printemps et en été et le recours à l 'irrigation sont des conditions favorables à l'infection durant cette période. La présente étude a montré également que le dépérissement de l'olivier est sous l'influence de plusieurs facteurs abiotiques tel que la température, l'humidité, le type de sol, la salinité et la fertilisation. Ainsi, il est évident que les oliviers placés en situation difficile à cause d'un stress abiotique seront plus sensibles à une attaque par les champignons pathogènes que d'autres placés dans des conditions optimales. D'autre part, l'importante évolution spatio-temporelle de la verticilliose de l'olivier dans plusieurs dans différentes régions de divers étages bioclimatiques de la Tunisie nécessite l'étude de la diversité génétique des isolats de *Verticillium dahliae* et de leur pathogénie sur plusieurs variétés d'oliviers et hybrides issus de croisements dirigés (sensibilité variétale) dans le but de définir une stratégie de lutte efficace contre cette grave maladie.

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S3-C14

Approche de la virulence de l'hylesine *Hylesinus oleiperda* (*Coleoptera Scolytidae*) vis-à-vis de quelques variétés d'olivier dans la région de Sfax

Mohieddine Ksantini [(1)] et Zina Aouem[(2)],

Résumé

Dans la perspective de rationaliser la lutte contre l'hylésine *Hylesinus oleiperda* (Coleoptera, Scolytidae), il convient de disposer, pour les nouvelles plantations oléicoles, de variétés d'olivier résistantes à l'attaque du scolyte. Disposant de deux collections variétales de l'OTD Bouzouita et Boughrara, cette étude a pour objectif de déterminer la virulence de l'hylésine vis-à-vis de quelques variétés autochtones d'olivier à huile et de table : Chétoui Tunis, Chemcheli Gafsa, Chemleli Sfax, Chemleli Ontha, Zarrazi Zarzis, Zalmati Zarzis, Ouesleti, Barouni du nord, Meski, Marsaline, Besbesi, Sayali et une variété étrangère la Picholine. Cette étude a reposé sur le dénombrement minutieux des plaques de l'hylésine sur 4 arbres par variété étudiée. Les résultats préliminaires obtenus dans les deux collections montrent que les variétés Chétoui Tunis et Chemcheli Gafsa sont les variétés tunisiennes les plus infestées par le scolyte. L'analyse statistique a permis de distinguer deux groupes bien distincts :
- Le premier groupe est composé de : Chemleli Sfax, Besbesi, Zarrazi Zarzis, Chemleli Ontha, Zalmati Zarzis, Marsaline, Sayali, Ouesleti et Picholine.
- Le deuxième groupe, le plus attaqué, est composé de Barouni, Meski, Chétoui Tunis et Chemcheli Gafsa.
Au champ, les résultats se confirment par les craquelures présentes sur le tronc des variétés les plus sensibles (Meski, Chétoui Tunis et Chemcheli Gafsa) alors qu'elles sont absentes sur les variétés de moindre sensibilité (Chemleli Sfax, Besbesi, Chemleli Ontha, Zarrazi Zarzis, Zalmati Zarzis, Marsaline et Sayali).

Mots clés : Hylésine, olivier, sensibilité variétale, lutte raisonnée.

Abstract

In the perspective to rationalize the fight against Hylesinus *oleiperda* (*Coleoptera*, *Scolytidae*), it invites to arrange, for the new olive orchards, olive tree varieties resistant to the scolyte attacks.
Arranging two variety's collections of OTD Bouzouita and Boughrara, the aim of this study to determine the virulence of *H. oleiperda* face to some autochthonous varieties of olive tree: Chetoui Tunis, Chemcheli Gafsa, Chemleli Sfax, Chemleli Ontha, Zarrazi Zarzis, Zalmati Zarzis, Ouesleti, Barouni, Meski, Marsaline, Besbesi, Sayali and a foreign variety the Picholine.
This study consists in the precious numbering of plates of *H. oleiperda* on 3 to 4 trees by studied variety.

The exploratory results gotten in the two collections show that Chetoui Tunis and Chemcheli Gafsa are the most infested are the Tunisian varieties. The statistical analysis permitted to distinguish two distinct groups:

- The first group is composed of: Chemleli Sfax, Besbesi, Zarrazi Zarzis, Chemleli Ontha, Zalmati Zarzis, Marsaline, Sayali, Ouesleti and Picholine.
- The second group, the less attacked, is composed of Barouni, Meski, Chetoui Tunis and Chemcheli Gafsa.

In the field this results are confirmed by the existence of flakings on trunks of the most sensitive varieties (Meski, Chetoui Tunis and Chemcheli Gafsa). Whereas these flakings are absent on less sensitives ones (Chemleli Sfax, Besbesi, Chemleli Ontha, Zarrazi Zarzis, Zalmati Zarzis, Marsaline and Sayali).

Key words: *Hylesinus oleiperda*, olive tree, variety's sensitivity, reasoned struggle.

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1. Introduction

L'hylésine *Hylesinus oleiperda* est un coléoptère de la famille des *Scolytidae* qui s'attaque préférentiellement aux jeunes oliviers âgés de 4 à 25 ans et peut entraîner le dépérissement voir la mort totale de l'arbre.

Les attaques de l'hylésine sont caractérisées par l'apparition, sur l'écorce du tronc et des branches charpentières, des oliviers attaqués, de plaques de couleur marron portant des trous de pénétration et de sortie des adultes, qui en évoluant ce craquelle et cause l'affaiblissement puis le dépérissement de l'arbre.

Partout dans le monde, où il est signalé, les recherches sur ce scolyte sont presque inexistantes. Les seuls travaux sont conduits en Tunisie vers la fin des années cinquante par Touzeau (1957) puis furent repris par Jardak et al, (1997, 2002, 2004) à l'Institut de l'Olivier vers la fin des années quatre vingt dix.

Les moyens de lutte préconisés se limitent à quelques traitements chimiques à base de Deltamétrine (le Décis) ou d'un mélange Décis- Diméthoate.

L'objectif de ce travail est de disposer, à partir des variétés locales, de plants d'oliviers plus ou moins résistants pouvant être préconisés pour l'installation des nouvelles plantations.

2. Matériel et méthodes

2.1. Biotope d'étude

Tous les travaux ont été conduits dans les parcelles de l'agrocombinat de Bouzouita sises à Boughrara. On a disposé, de deux collections variétales :

La première, de l'ordre de 6,5 ha, est composée de 600 plants issus de boutures herbacées âgés de 10 à 14 ans selon les années de plantation, à la densité de 8 sur 12. Elle compte une centaine de variétés locales et étrangères.

La seconde, de 12 ha est composée de 284 plants greffés, en 1990, sur des portes greffes Chemleli plantés selon l'écartement normal de la région de Sfax, soit 24 sur 24. On y compte une soixantaine de variétés locales et étrangères.

2.2. Les variétés étudiées

Les variétés retenues pour mener cette étude sont :

- Parcelle I, 11 variétés: Chétoui Tunis, Chemcheli Gafsa, Chemleli Sfax, Chemleli Ontha, Zarrazi Zarzis, Zalmati Zarzis, Ouesleti, Barouni du nord, Meski, Marsaline, Besbesi, Sayali et une variété étrangère la Picholine.

- Parcelle II, 8 variétés : Chemcheli Gafsa, Oueslati, Chétoui Tunis, Meski, Barouni du nord, Zalmati Zarzis, Zarrazi Zarzis et la Picholine.

Le nombre d'arbres analysés par variété est variable selon leur disponibilité dans chaque parcelle, tel que présenté dans le Tableau 1 :

Tableau 1 : Nombre d'arbres analysés par variété et par parcelle.

Variétés	Parcelle I	Parcelle II
Chemleli Sfax (Chl sf)	12	*
Meski (Mk)	6	4
Chemcheli Gafsa (Chm)	5	3
Zarrazi Zarzis (Zr)	5	4
Barouni du nord (Br)	5	3
Chétoui Tunis (Cht)	4	4
Zalmati Zarzis (zl)	4	4
Chemleli Ontha (Chl Ontha)	4	*
Marsaline (Mr)	3	*
Besbesi (Bs)	2	*
Sayali (Syl)	2	*
Ouesleti	*	4
Picholine	*	4

2.3. Technique employée

L'étude de la virulence de l'hylésine vis-à-vis de différentes variétés d'oliviers, disponibles en même temps dans le même verger et dans les mêmes conditions de culture, repose sur le dénombrement des anciennes et nouvelles plaques sur le tronc et toutes les branches de chaque arbre analysé. Pour éviter tout risque d'erreur et de confusion, les plaquettes anciennes sont marquées par une croix alors que les nouvelles sont encerclées.

Parallèlement au dénombrement des plaques, on a pris soin de gratter quelques unes pour relever la structure des populations du phytophage au moment de l'étude (été 2006).

3. Résultats et discussion

3.1. Structure de la population de l'hylésine

Les travaux conduits par Jardak et al, (1997) signalent que l'hylésine effectue 2 générations par an. La première est au printemps et la seconde en automne.

En Juillet (période de déroulement de cette étude) l'hylésine se trouve sous sa phase de nuisible endophyte à l'intérieur de l'écorce.

L'observation à la loupe binoculaire des plaques, ramenées au labo après grattage de l'écorce, a montré la présence de tous les stades préimaginaux : des œufs, des larves, des nymphes en plus des femelles présentes encore dans les plaquettes.

Bien que tous les stades coexistent, la population de l'hylésine est constituée à 62.5% par des œufs vivants, 12.5% des larves vivantes, 8% des nymphes et 16.7% des adultes (Fig.1).

Une telle structure témoigne de la pleine activité de l'insecte en phase de reproduction.

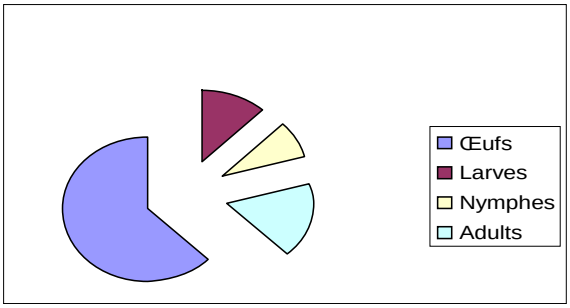

Figure 1: Structure de la population d'*Hylesinus oleiperda* durant l'été 2006 à Boughrara.

3.2. Sensibilité variétale

Le suivi des attaques de l'hylésine sur olivier dans les deux parcelles permet de distinguer deux types d'infestation :

Les anciennes attaques caractérisées généralement par la craquelure de l'écorce au niveau des galeries maternelles et larvaires.

Les nouvelles plaques qui ont lieu au printemps 2006 suite au développement d'une nouvelle génération.

Les niveaux d'attaques sont tout à fait différents entre les deux parcelles. La parcelle I est la moins infestée, avec des maxima ne dépassant pas 70 et 5 plaques anciennes et nouvelles/ arbre. Toutefois, les différentes variétés cultivées ne présentent pas la même intensité d'attaque.

3.2.1. Parcelle I

Les résultats du comptage des anciennes plaques sont illustrés par la Figure 2.

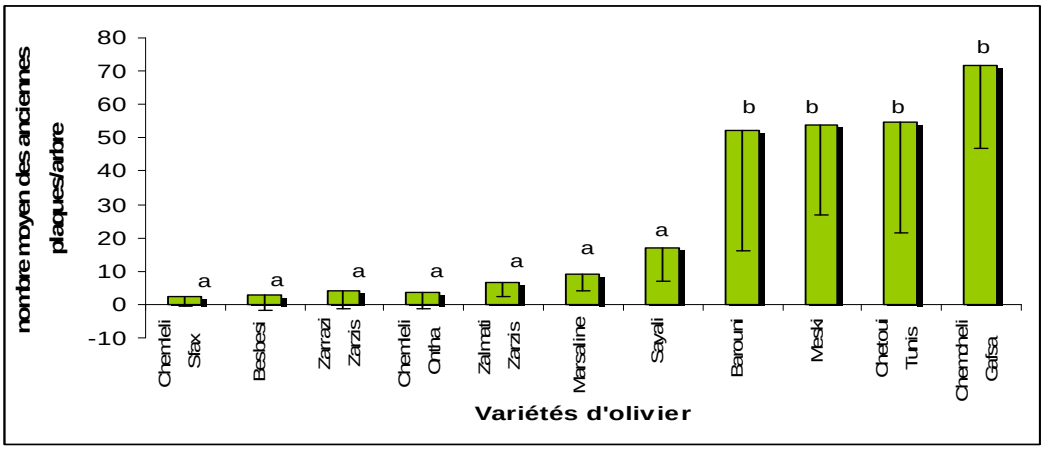

Figure 2: Variation du degré d'infestation par l'hylésine des différentes variétés d'olivier cultivées dans la parcelle I. Les variétés suivies de la même lettre ne sont pas significativement différentes au seuil de 1%.

Il ressort de la Figure 2 que nous sommes devant trois degrés d'infestations:

- Les variétés à faible degré d'infestation : Chemleli Sfax, Besbesi, Zarrazi Zarzis, Chemleli Ontha et Zalmati Zarzis.

- Les variétés ayant un degré d'infestation moyen : Marsaline et Sayali.

- Les variétés ayant un degré d'infestation élevé : Meski, Chétoui Tunis et Chemcheli Gafsa.

Toutefois, l'analyse statistique moyennant le test d'homogénéité (test Duncan, logiciel SPSS 11.0) entre les variétés, a révélé la présence de deux groupes bien distincts:

- Le premier groupe, le moins attaqué, est composé de Chemleli Ontha, Zarrazi Zarzis, Chemleli Sfax et Zalmati Zarzis.

- Le deuxième groupe est composé de Barouni, Chétoui Tunis, Meski et Chemcheli Gafsa.

Les variétés Marsaline, Sayali et Besbesi n'ont pas fait l'objet d'analyse statistique vu la rareté des représentants de chaque variété.

Au champ, les attaques se manifestent par les craquelures présentes sur le tronc des variétés censées être les plus sensibles (Meski, Chétoui Tunis et Chemcheli Gafsa) alors qu'elles sont absentes sur les variétés de moindre sensibilité (Chemleli Sfax, Besbesi, Chemleli Ontha, Zarrazi Zarzis, Zalmati Zarzis, Marsaline et Sayali).

Il en ressort aussi que la variété Chemcheli Gafsa est la plus recherchée par l'hylésine. Ce résultat élargi la liste des variétés sensibles étudiées par Ksantini et Jardak (2004), Jardak et al, (1997), Gouider (1983) et par Touzeau (1957).

La moyenne des plaques anciennes par variété représente le cumul dans le temps de l'ensemble des attaques du scolyte. Toutefois, un organisme vivant (plante, insecte, champignon…) se caractérise par l'évolution est non pas par la stabilité, alors, est ce que l'hylésine a gardé le comportement envers les variétés de l'olivier dans la parcelle I ?

Pour répondre à cette question on s'est référé au nombre des nouvelles plaques. Celles-ci ont eu lieu après l'envol printanier de 2006. Les résultats de ces comptages sont illustrés dans la Figure 3.

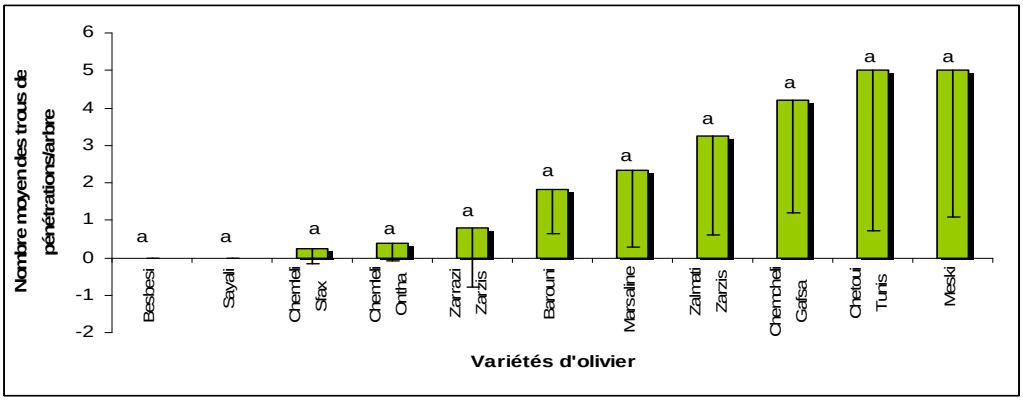

Figure 3: Variation des nouvelles plaques par variété dans la parcelle I. Les variétés suivies de la même lettre ne sont pas significativement différentes au seuil de 1%.

D'après la Figure 3, il apparaît que l'hylésine n'a pas changé de comportement avec le temps. Elle a toujours une préférence plus importante aux variétés Chemcheli Gafsa, Chétoui Tunis et Meski qu'aux variétés Barouni, Sayali, Marsaline, Zalmati Zarzis, Zarrazi Zarzis, Chemleli Ontha, Chemleli Sfax et Besbesi.

Cependant on note une légère variation de la réceptivité entre Chemcheli Gafsa et Chétoui Tunis : il y a plus de nouvelles plaques sur la Chétoui que sur la Chemcheli.

Toutefois, le test de Duncan appliqué aux 8 variétés déjà analysées révèle qu'elles appartiennent à un seul groupe d'infestation (de 0.4 à 5.8 plaques /arbre).

3.2.2. Parcelle II

Les résultats illustrés par la figure 4 montrent une homogénéité de l'infestation entre 7 variétés: Barouni, Oueslati , Picholine, Meski , Chemcheli Gafsa, Zalmati Zarzis, Zarrazi Zarzis, alors que la variété Chétoui Tunis est, cependant, la plus infestée. Ceci est confirmé par le test de Duncan qui révèle deux groupes bien distincts:

- Le premier groupe est composé de : Barouni, Oueslati, Picholine, Meski, Chemcheli Gafsa, Zalmati Zarzis, Zarrazi Zarzis.
- Le deuxième groupe est composé de Chétoui Tunis seul.

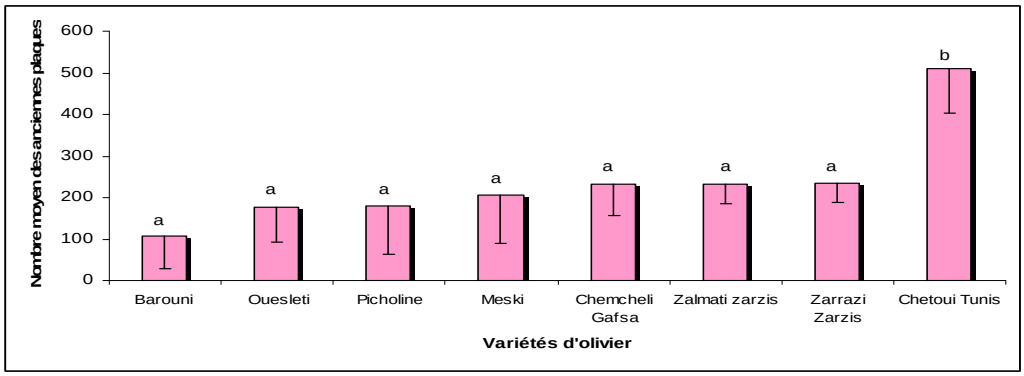

Figure 4: Histogramme des plaques anciennes /arbre/variété dans la parcelle II. Les variétés suivies de la même lettre ne sont pas significativement différentes au seuil de 1%.

En comparant le niveau d'attaque des deux parcelles expérimentales nous remarquons que toutes les variétés de la parcelle II sont beaucoup plus attaquées que celles de la parcelle I.

En effet la moyenne maximale des plaques par arbre dans cette dernière est de 71.8 alors que dans la première, la moyenne minimale des plaques par arbre est de 102. En outre on note des craquelures de l'écorce de toutes les variétés. Ces craquelures sont bien développées au point que le comptage des anciennes plaques devient très difficile.

La différence du degré d'infestation entre les deux parcelles peut être expliquée par les la vigueur des oliviers qui sont greffés sur Chemleli dans la parcelle II et issus de boutures herbacées dans la parcelle I.

Comme pour la parcelle I nous avons tenu à vérifier si l'hylésine a gardé le même comportement préférentiel des variétés de l'olivier dans le temps (Figure 5).

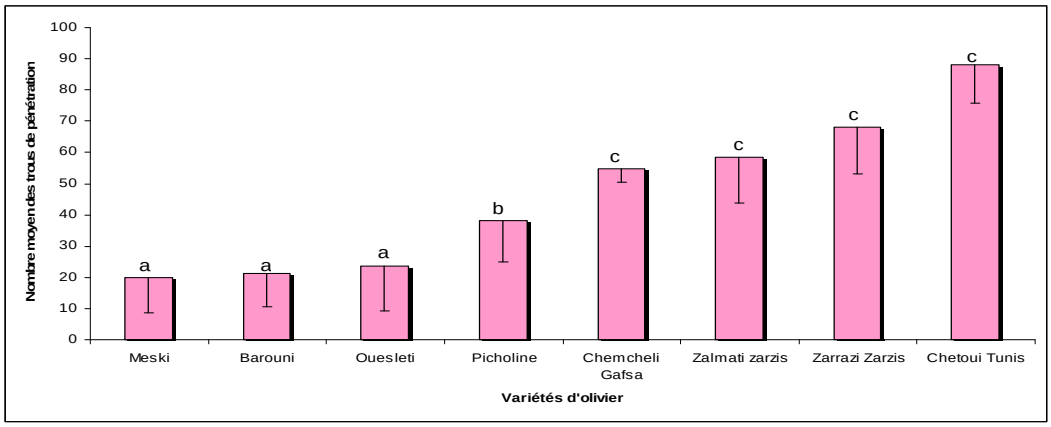

Figure 5: Variation des nouvelles infestations par variété dans la parcelle II. Les variétés suivies de la même lettre ne sont pas significativement différentes au seuil de 1%.

D'après la Figure 5, on constate que la sensibilité des variétés se confirme au niveau des nouvelles attaques. Ce qui conclu que l'hylésine n'a vrai semblablement pas changé de comportement envers les différentes variétés.

En effet, nous constatons, à l'opposé de la parcelle I , que les différentes variétés de la parcelle II comportent des degrés d'infestation variant de 18 à plus de 80 plaques/arbre.
L'analyse statistique, par le test Duncan fait ressortir 3 groupes d'infestation qui se résument comme suit :

- Les variétés fortement sensibles : Chétoui Tunis, Zarrazi Zarzis, Zalmati Zarzis et Chemcheli Gafsa.

- Une variété moyennement sensible : Picholine,

- Les variétés moyennement résistantes : Meski, Barouni et Ouesleti.

Paradoxalement avec la première parcelle, la variété Meski, se trouve la moins infestée. Elle semble avoir développé un mécanisme de défense contre pour les adultes de l'hylésine.

Dans ce contexte, par ailleurs, on a pu isoler quelques réactions développées par certaines variétés qui se traduisent par des sécrétions de résine chez la Marsaline, la Picholine, la Ouesleti. Ce constat fut même observé chez la Chétoui Tunis qui a excrété des taches huileuses et des taches blanches tout autour des plaques de ponte de l'hylésine.

Ces différents aspects de réaction aux attaques de l'hylésine méritent d'être étudiés pour comprendre les mécanismes de résistance développés par ces variétés dans leur aire de culture traditionnelle.

4. 0Conclusion

En conclusion, à tout ce qui a été présenté précédemment, l'hylésine est un scolyte qui s'attaque à l'olivier avec des préférences variétales différentes.

Nous avons pu classer environ 12 variétés autochtones et une variété française la Picholine selon le degré d'attaque par l'hylésine. Toutefois, les résultats obtenus restent préliminaires et méritent d'être approfondis dans une étude plus détaillée et portant sur un nombre de répétition plus important et sur une période d'étude plus longue.

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S3-C15

Rôle des biosenseurs pour une meilleure maîtrise endogène et exogène des systèmes de culture : application à l'olivier

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Résumé

La biodiversité représente aujourd'hui un enjeu scientifique, social, économique et politique considérable. La biodiversité domestique recouvre la biodiversité des espèces cultivées. Pour une espèce donnée plus elle a de diversité, plus elle a de chance de trouver une solution aux différents problèmes qui se poseront (sécheresse, salinité du sol, maladie fongique...). C'est dans ce contexte général que nous allons présenter le rôle des bios senseurs dans la compréhension des stratégies adaptatives et à l'évaluation des réponses aux interventions de l'homme assurant respectivement une stabilité endogène et exogène du système de culture. Nous nous intéresseront particulièrement à l'olivier variété Chemlali une espèce ligneuse clé des agro systèmes semi arides et arides tunisiens. Malgré les nombreux travaux sur son adaptation à l'aridité les études sur son fonctionnement hydrique sont rares. Avec le développement techniques des méthodes d'évaluation du fonctionnement hydrique nous avons adopté une approche d'évaluation quantitative en continue du fonctionnement hydrique global de l'arbre grâce aux méthodes thermo électrique de mesure du flux de sève brute, ce ci nous a permis de quantifier la consommation en eau des arbres et d'identifier les relations entre la dynamique saisonnière de ces flux et les déterminismes majeurs de la production entre autres les échanges gazeux et leur régulation.

Mots clés: *Olea europaea* L., trunk shaker, mechanical beater, pneumatic combs, oil quality.

Biosensors role for monitoring endogenous and exogenous stability of the olive culturing system

Abstract

Today, biodiversity represents a considerable scientific, social, economic and political issue. The domestic biodiversity covers the biodiversity of the cultivated species. The more diversity a given species has, the more chance it has to overcome different problems (drought, soil salinity, fungal illness…). In this general context, we will present the biosensors role in the understanding of the adaptive strategies and the evaluation of the responses to man's interventions ensuring respectively an endogenous and exogenous stability of the culturing system. We are interested particularly in Chemlali variety, a woody species the key of arid and semi arid Tunisian agro systems.
In spite of the numerous works on its adaptation to aridity, the studies about its water functioning are scarce. With the technical development of the evaluation methods of water functioning, we have adopted a quantitative evaluation approach continuous to the global water functioning of the tree thanks to the thermo electrical methods such as brute sap flux, this enabled us to quantify water consumption for trees and to identify the relationships between seasoned dynamics of these fluxes and major determinisms and production between others gas exchanges and their regulation.

Key words: ***Olea europaea*** L., trunk shaker, mechanical beater, pneumatic combs, oil quality.

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1. Introduction

Aujourd'hui, la Méditerranée représente 98% de la production mondiale d'huile d'olive. La production d'huile d'olive joue un rôle déterminant pour les économies et l'emploi, ainsi que pour la biodiversité des régions méditerranéennes. Marqueur du territoire, l'olivier est un capital précieux et un patrimoine à sauvegarder et à enrichir.

Dans les pays du sud de la méditerranée l'évolution du climat montre que le réchauffement climatique est plus important que la moyenne. Si au niveau planétaire la hausse des températures durant le 20ème siècle a été de 0,75 ℃, celle sur le Maghreb s'est située entre 1,5 et 2 ℃ selon les régions, soit plus du double de la hausse moyenne mondiale. Quant à la baisse des précipitations, elle varie entre 10 et 20 %.

Malgré cet environnement aride de plus en plus hostile découlant d'un changement climatique notoire, l'olivier est bien ancré dans le paysage tunisien et constitue le seul arbre cultivé pouvant valoriser cette région de plus en plus fragilisée par des pratiques agricoles erronées. La sauvegarde et la gestion rationnelle de cet arbre sont une des priorités nationales. L'eau étant la contrainte principale à la production, l'étude du fonctionnement hydrique de l'arbre pourrait orienter la conduite culturale rationnelle. Le développement technologique des équipements de contrôle de l'état hydrique de l'arbre a provoqué une révolution dans le domaine de la recherche ecophysiologique.

Un certain nombre de caractéristiques permettent de décrire l'état hydrique du végétal. Plusieurs travaux ont montrés que l'état énergétique de l'eau (ψF) et la transpiration sont très corrélés avec la croissance d'une part et le développement d'autre part. Ces deux paramètres peuvent intervenir dans la détermination du rendement en biomasse et en fruits chez une variété donnée. De plus, le maintien d'un bon état hydrique et une faible transpiration peuvent être des indicateurs d'une bonne adaptation des plantes à la sécheresse (Henchi et al. 1986). Ainsi, le maintien de la production dépend des mécanismes de tolérance physiologiques qui assurent l'hydratation cellulaire et diminuent la perte en eau en maintenant un état hydrique favorable à l'élaboration du rendement. Ce dernier est étroitement lié au rendement photosynthétique qui est un des premiers processus affecté par la sécheresse du notamment à la fermeture des stomates. En effet, cette fermeture qui a lieu pour réduire les pertes hydrique agit aussi sur le flux de carbone en limitant aussi son accès aux sites de carboxylation (Flexas et al., 1998 and Escalona et al., 2002). Ainsi, le suivi du statut hydrique des plantes en plein champ est d'un grand intérêt. Elle permettra de diagnostiquer l'installation d'un éventuel stress et de quantifier sa sévérité et d'orienter les interventions culturales qui ont un rapport avec l'état hydrique de la plante (contrôle de la surface transpirante par la taille, amélioration de l'état hydrique du sol…etc.). Dans ce contexte général s'incère notre travail qui a pour objectif de voir l'efficacité des senseurs biologiques pour le suivi du comportement de l'olivier dans des conditions culturales variées et diagnostiquer des situations de stress hydrique à la quelle il faut intervenir.

2. Matériels et méthode

Le travail est effectué dans une plantation d'olivier conduite en pluvial. Différentes densités de plantation supérieures à celles utilisées dans les plantations traditionnelles sont testées sur les plants agronomiques et biologiques et ce depuis la mise en place de la parcelle. Ces relevés ont montré un recul de la production et de la vigueur par arbre avec l'augmentation de la densité de plantation et ceci malgré une production par ha encore croissante avec la densité de plantation (Boujnah et al. 2006). L'objectif de ce travail est de voir le comportement hydrique et physiologique de l'arbre pour les différentes densités de plantation en vu de comprendre le comportement de l'arbre vis-à-vis de l'eau disponible (à travers les différents saisons et selon les densité de plantation) et de diagnostiquer un éventuel effet de compétition.

2.1. Conditions expérimentales

Le parcelle expérimentale est situé à Jemmel au centre Est de la Tunisie. Elle appartient au domaine du Centre de Formation Professionnelle Agricole de Jemmel (CFPA de Jemmel). La zone fait partie du climat méditerranéen semi-aride à étage supérieur. La pluviométrie moyenne annuelle est de 350 mm. La saison sèche s'étale sur huit mois et l'amplitude thermique est faible. Le sol est profond (50 à 60 cm), argilo- limoneux à accumulation calcaire, la texture est moyenne, le taux de matière organique est faible et le pH est autour de 8 (alcalin).

La parcelle expérimentale s'étend sur 3,5 ha. Elle est divisée en quatre lots. Chaque lot se distingue par une densité de plantation différente. Les densités testées sont toutes inférieures à celle utilisée dans l'entourage qui est de 40 arbres par ha (plantés en carré selon les écartements 16x16). Ces densités se présentent comme suit: 51, 70, 100 et 156 arbre /ha ce qui correspond respectivement aux écartements 14x14 m, 12x12 m, 10x10m et 8x8 m.
La variété cultivée est la Chemlali. Les arbres sont âgés de 20 ans. Cette variété est vigoureuse, productive, et résistante à la sécheresse.

2.2. Mesures réalisées

Le potentiel hydrique foliaire:

Le potentiel hydrique foliaire est évalué par une chambre à pression (Modèle1000, Scholander et al., 1965). Il a été déterminé sur des feuilles adultes prélevées à la périphérie de la frondaison à hauteur d'homme et ce tous les 15 jours durant toute la période de l'essai.

2.2.1. La résistance stomatique:

Ces mesures ont été au poromètre de type « AP4 Delta T Device ». Cet appareil nous permet d'évaluer le degré d'ouverture moyenne des stomates en déterminant la résistance à la diffusion de la vapeur d'eau émise par une portion de feuille insérée dans une chambre de mesure. Ces mesures ont été réalisées tous les 15 jours durant toute la période de l'essai.

2.2.2. Flux de sève

La mesure du flux de sève xylèmique est effectuée selon la méthode thermique de Granier (1985) à chauffage alternatif (Do et Rauchteau, 2002).Le fluxmètre est constitué par deux sondes cylindriques identiques, de longueur 20mm et de diamètre 1,8mm. L une est chauffée d une manière discontinue (20mn sans chauffage et 10mn avec chauffage). Chaque sonde contient un thermocouple Cuivre-Constantan permettant de mesurer l écart de température entre les deux thermocouples. La tension électrique de chauffage est assurée par une batterie de 12 V. La saisie automatique des données est programmée suivant un pas périodique de 60s avec une moyenne enregistrée en mémoire toutes les 5mn à raison d un cycle de chauffage/non-chauffage de 10mn/20mn. Les données enregistrées sont récupérées tous les 15 jours à l aide d une centrale d acquisition de données (Data Loger). Seule la deuxième valeur de la phase chauffage est considérée pour la quantification des flux de sève, suivant la formule suivante : U = J.S

- J (m3.s 1) est la densité de flux estimée par le suivi d un coefficient K qui dépend des différences de température entre les deux sondes du capteur, avec K = [T (0)- T (u)] / T (u) où T (0) est la différence de température à flux de sève nul, T (u) : la différence de température à flux de sève de densité u ;

- S (cm2) est la surface conductrice du bois, elle est déterminée par coloration des coupes de génératrice après l expérimentation, elle est estimée à 70% de la section du bois.
Les relevées se sont étalées sur toute la période expérimentale.

2.2.3. La photosynthèse:

La photosynthèse nette est mesurée à l'aide d'un appareil de type LI-COR LI-6400. Il s'agit d'un système ouvert qui mesure à la fois la concentration en CO2 et H2O de l'air de référence (atmosphère) et de l'air issu de l'échantillon situé dans la chambre. Le taux de photosynthèse nette est exprimé par la même unité de CO2 (µmol CO2. m-2 .s-1). Ce paramètre a été évalué au cour du printemps qui est la période d'activité biologique la plus intense de l'olivier

3. Résultats et discussion

3.1. Potentiel hydrique foliaire

Les courbes de la Figure 1 illustrent l'évolution du potentiel hydrique foliaire de base chez l'olivier Chemlali planté à différentes densités. Ce paramètre mesuré en fin de nuit, alors que la transpiration est négligeable et que la plante a reconstitué ses réserves en eau, renseigne sur la disponibilité en

eau du sol et fournit une information sur l'état hydrique dans lequel se trouve le végétal, en raison d'une plus faible variabilité des conditions de milieu. En effet, on considère que la tension de sève dans le végétal est en équilibre avec le potentiel hydrique du sol dans la zone d'implantation des racines.

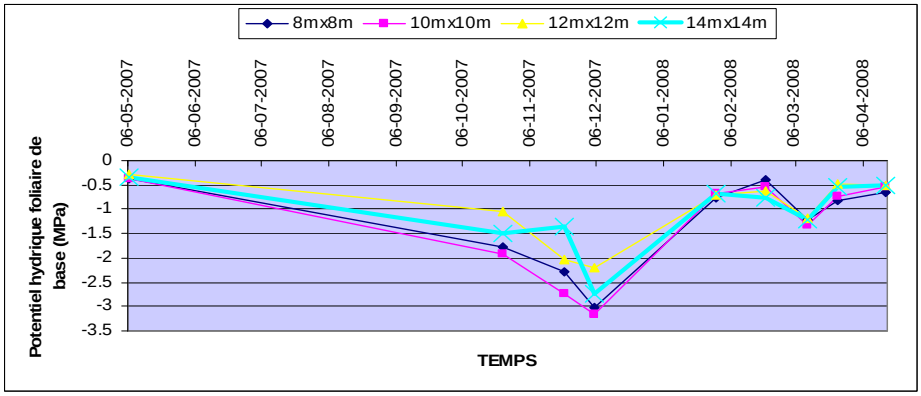

Figure 1: Evolution du potentiel hydrique foliaire de base en fonction du temps pour l'essai de révision des densités de plantation en culture pluviale de la parcelle expérimentale de Jemmel.

En fonction de la densité de plantation nous remarquons des potentiels hydriques plus bas chez les arbres plantés aux densités les plus élevées pour la période s'étalant de juin 2007 à janvier 2008 avec les valeurs les plus élevées pour les arbres plantés aux écartements 70 arbres/ ha.

Par ailleurs, le potentiel hydrique foliaire minimal illustré par les histogrammes de la Figure 2 confirme ces résultats puisque cette densité s'est distinguée par le potentiel hydrique le plus élevé pendant la période estivale où les conditions hydriques sont à leur minimum. Durant la période printaniere, qui correspond à la période d'activité la plus intense chez l'olivier puisqu'au cours de la quelle se déroule simultanément la croissance végétative et le développement fructifère et pour les deux potentiel hydrique (de base et minimum) les courbes se confondent. Ceci témoigne des grandes aptitudes de cette espèce à moduler sont potentiel hydrique en fonction de la disponibilité de l'eau dans le sol. Le maintien de la production dépend des mécanismes de tolérance physiologiques qui assurent l'hydratation cellulaire et diminuent la perte en eau en maintenant un état hydrique favorable à l'élaboration du rendement dans ses différentes phases (organogenèse florale, floraison, fécondation.. etc.). En conditions de déficit hydrique, la baisse du potentiel hydrique des plantes induit une importante perte de turgescence au niveau des feuilles (Boyer 1982 et Sonells et al. 2000). Le maintien d'un potentiel hydrique élevé est lié à l'aptitude à extraire l'eau du sol et à la capacité à limiter les pertes d'eau par transpiration (Turner 1986) Il caractérise une stratégie d'esquive à la déshydratation (Levitt, 1980).

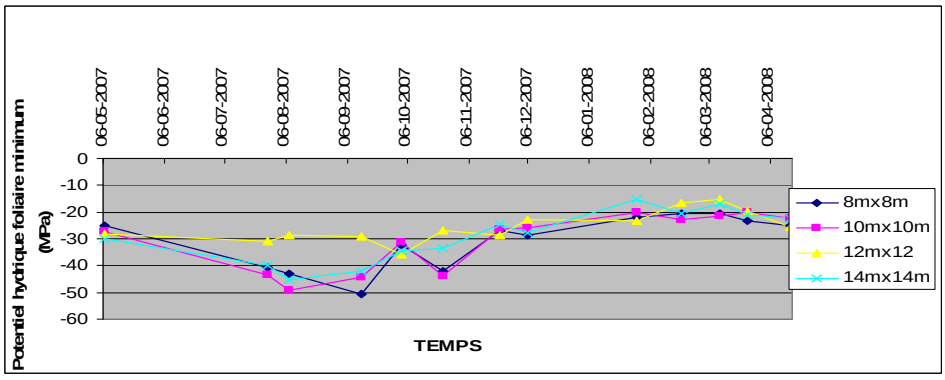

Figure 2: Evolution du potentiel hydrique foliaire minimum en fonction du temps pour l'essai de révision des densités de plantation en culture pluviale de la parcelle expérimentale de Jemmel.

L'absence de différence entre les autres écartements testées peu être attribué au comportement iso hydrique qui mène à des valeurs similaires du potentiel hydrique chez l'olivier (Escalona et al., 2002). Ceci serai du à la régulation stomatique de la transpiration qui pourrait entraîner une baisse importante du potentiel hydrique menant à une un endommagement des feuilles (Naor and Wample, 1994 and Schultz and Matthews, 1988). Les mesures de la conductance stomatique (Fig. 3)

confirment ces constatations puisque l'intensité des pertes hydriques par les stomates a varié en fonction de la situation culturale (densité de plantation). D'après Tyree and Sperry, 1988 la fermeture des stomates est une stratégie efficace qui permet d'éviter les pertes excessives d'eau pouvant mener à une basse importante du potentiel hydrique à des niveaux dangereux pour la plante. Chez l'olivier Natali et al. (1985) montrent que cette espèce semble réduire ses pertes hydriques par la fermeture des stomates jusqu'à un certain niveau par la suite il peut changer de stratégie en accentuant la transpiration ce qui augmente l'absorption par les racines.

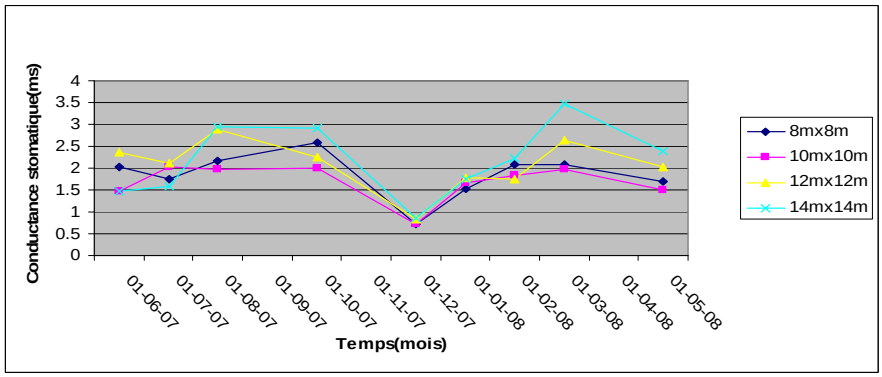

Figure 3: Evolution de la conductance stomatique en fonction du temps pour l'essai de révision des densités de plantation en culture pluviale de la parcelle expérimentale de Jemmel.

Malgré l'efficience et la précision de ces paramètres hydrique pour l'étude du comportement hydrique de l'olivier ces deux méthodes pressentent le désavantage d'être évaluées d'une façon discontinue, ceci peut être résolue par l'établissement d'une relation quantitative entre le statut hydrique de la plante et d'autres paramètres physiologiques qui peuvent être plus facilement relevés. C'est ainsi que nous avons mesuré le flux de sève en vu d'avoir une estimation instantanée et précise de la transpiration foliaire (Las Cano et al., 1992, East End and Gray, 1998, Braun and Schmid, 1999 and Escalona et al., 2002).

Les courbes de la Figure 4 illustrent l'évolution durant deux journées consécutives du flux de sève en fonction de la densité de plantation pour les quatres saisons. Ce paramètre permet de quantifier l'utilisation de l'eau par la plante. Nous remarquons des différences de consommation hydrique en fonction des saisons pour les différentes densités de plantation. Pour la saison automnales les différences entre les différentes densités ne sont pas significatives par contre une nette différence est observée durant la saison estivale où les conditions hydriques sont les plus sévère.

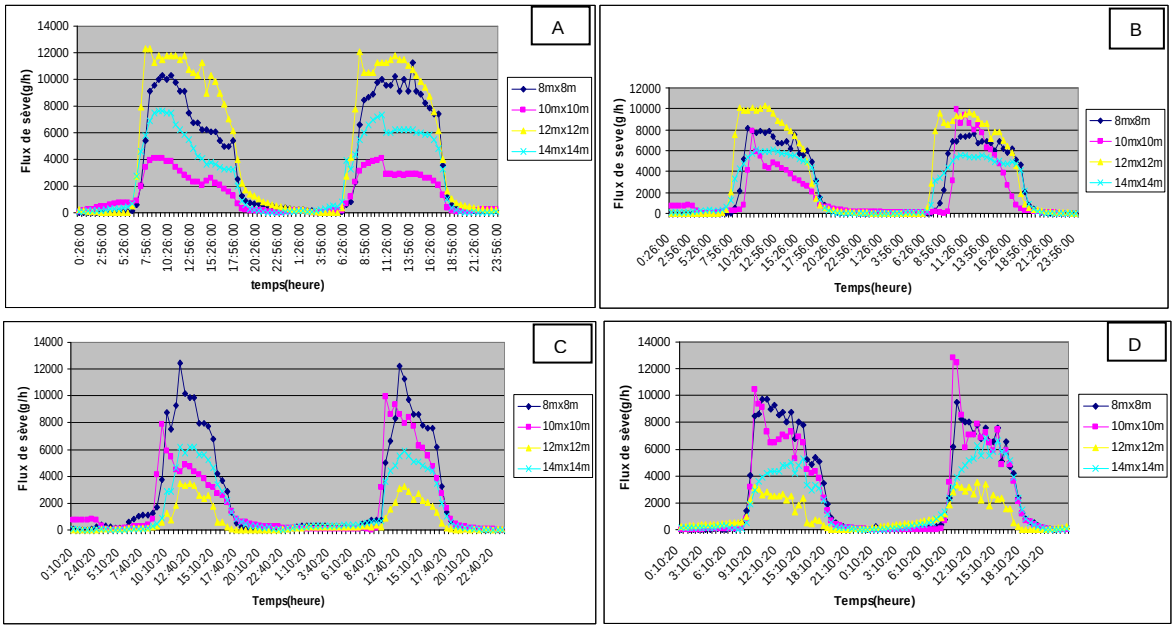

Figure 4: Evolution journalière de flux de sève au cours de deux jours consécutives pendant la saison estivale (A) Automnale (B), Hivernale (C) et Printanière (D) pour l'essai de révision des densités de plantation en culture pluviale de la parcelle expérimentale de Jemmel.

L'estimation des pertes hydrique transpiratoires donne une idée sur l'intensité du stress et permet de palier à ces pertes par des interventions culturales au moment opportun. En effet la détection d'une baisse continue de la consommation hydrique journalière peut être considéré comme un signal de début de stress. Fernandez et al. (2001) ont mis en évidence le double avantage de cette technique pour l'olivier puisqu'elle peut être utilisée comme un outil de recherche pour fournir une mesure robuste de l'utilisation de l'eau et le fonctionnement hydraulique de l'arbre d'un coté et le potentiel d'être utilisé comme un dispositif pour fournir un signal à partir de laquelle les décisions peuvent être prises concernant la nécessité et le calendrier de l'irrigation.

Les courbes de la Figure 5 illustrent les variations de la photosynthèse en fonction de la densité de plantation et ce au cours de la saison printanière, période d'activité biologique la plus intense ce paramètre donne une idée sur le potentiel de croissance des plantes. En fonction de la densité de plantation nous n'avons remarqué au démarrage de l'activité biologique au printemps aucune différence entre les quatre densités de plantation, par la suite le taux de photosynthèse augmente pour atteindre des valeurs maximales au mois d'avril.

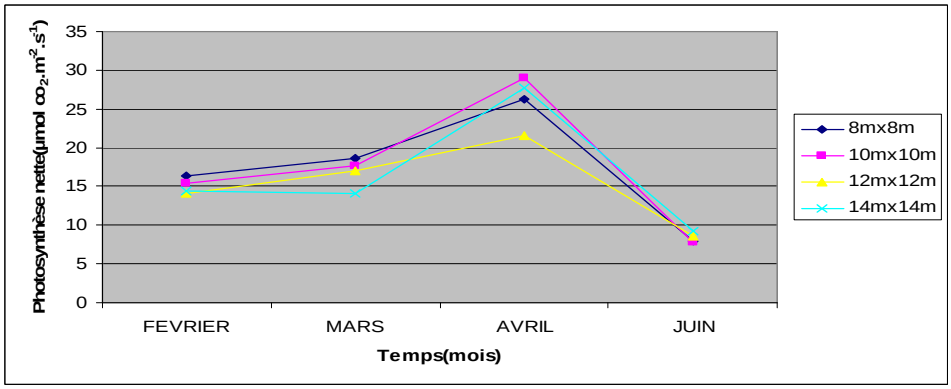

Figure 5: Evolution de la photosynthèse nette en fonction du temps pour l'essai de révision des densités de plantation en culture pluviale de la parcelle expérimentale de Jemmel.

Avec l'augmentation de la température au mois de juin nous assistons à une baisse de la photosynthèse avec des valeurs similaires pour tous les traitements. Pour ce paramètre les seules différences significatives ont été notées chez les arbres plantés aux plus faibles densités. En effet, au mois de mars les oliviers plantés à la densité51 arbre /ha n'a subit aucune augmentation et au mois d'avril les arbres plantés à la densité 70 arbre par ha ont présenté les valeurs la plus faible de la photosynthèse. Etudiant la réponse de quelques espèces végétales, plusieurs auteurs notent que le stress hydrique affecte la physiologie de la plante en diminuant la photosynthèse nette et que ses effets sont variables avec le niveau de stress (Bensari et al. 1990 et Marsal et al. 2000). Chez l'olivier variété Chemlali, Laouar (1978) a montré que l'activité photosynthétique est maintenue maximale pour de légers déficits hydriques.

Plusieurs auteurs signalent que l'un des principaux processus touchés par la sécheresse est la photosynthèse, un fait dû principalement à la fermeture stomatique qui diminue la perte d'eau mais aussi le flux de carbone vers les sites de carboxylation (Flexas et al., 1998 et Escalona et al., 2002).

4. Conclusion

L'utilisation des senseurs biologiques en se référant à la plante pour étudier le comportement adaptatif de l'olivier dans différentes situations culturales où l'eau peut être limitant (utilisations de densités de plantation plus où moins élevées) est très important notamment en plein champs où le contrôle des facteurs du milieu est impossible. Elle nous a permis de nous rendre compte de l'importante capacité du cultivars d'oliviers testé (Chemlali) à mobiliser l'eau en fonction de ces besoins selon sont état biologique et du rôle fondamental de la régulation stomatique dans le contrôle des pertes transpitatoire.

Par ailleurs, la le suivi journalier des pertes transpiratoire grâce aux mesures du flux de sève donne une idée précise sur la consommation hydrique de l'arbre et permet le diagnostic au moment opportun du stress. Il pourrait guider les interventions culturales. Les mesures de la photosynthèse

donne une idée sur le potentiel de croissance des plantes et pourrait servir à l'évalution de l'efficacité des interventions appliquées.

Il ressort de ce qui précède que les senseurs biologiques - déjà important en recherche ecophysiologiques- peuvent devenir très utiles pour la gestion des systèmes de culture de plus en plus fragilisés par le changement climatique car elle permette le dépistage rapide et précis de la contrainte environnementale guidant ainsi le choix variétal adapté et la conduite adéquate au moment opportun.

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S3-C16

Mecanisation de la récolte adaptée pour les oliviers âgés et de trés grand taille

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Résumé

En Italie du sud, en particulier dans de région de la Calabre et de Pouilles, une partie significative des oliveraies se caractérisent par la présence de vieux arbres de très grande taille (taille > 8 m). Habituellement, dans ces vergers, la récolte d'olives se fait par le ramassage périodique des fruits qui tombent naturellement par terre, avec des effets négatifs sur la qualité d'huile. Dans certain cas, c'est difficile de rajeunir ces oliveraies par des nouvelles plantations en raison de leur importance historique et/ou du paysage. Afin de trouver une alternative à cette façon de récolter les olives, en 2006, dans la région de Pouilles, un essai de récolte mécanisée des olives sur des vieux arbres de grande taille a été effectué. Les arbres des cultivars "Cellina di Nardò" et "Ogliarola Salentina" étaient âgés 60-100 ans avec une taille de 8 à 10 m et d'un volume de frondaison d'environ 450-500 m^3/arbre. Dans la première moitié de novembre, la récolte mécanique a été effectuée: 1) en utilisant une batteur mécanique appliqué à un tracteur pour récolter la partie plus haut de la frondaison + les peignes pneumatiques manuels pour récolter les parties inférieures de la frondaison; ou 2) en utilisant un dispositif secoueur automoteur appliqué aux branches principales. Avec le batteur mécanique + peigne pneumatiques tenus à la main, le rendement de récolte (le pourcentage des olives récoltées sur toute la chevelure) était environ 90% et l'efficience de récolte était atour 60 kg d'olives/heur/ouvrier. Avec le dispositif secoueur le rendement était d'environ 75% sur les variétés "Cellina di Nardò" et d'environ 40% sur les variétés "Ogliarola Salentina" tandis que la productivité de récolte était atour 190 kg d'olives/heur/ouvrièr avec la variété "Cellina di Nardò" et atour 150 kg d'olives/heur/ouvrièr avec la variété "Ogliarola Salentina". L'huile obtenue a été de qualité elevée. Les résultats indiquent des possibilités importantes pour la mécanisation de la recolte et la production d'huile de qualité dans les oliveraies caractérisées par des arbres de très grande taille.

Mots clés: *Olea europaea* L., dispositif secoueur de tronc, batteur mécanique, peignes pneumatiques, qualité de l'Huile.

Mechanization of harvesting in olive trees of very large size

Abstract

In southern Italy, particularly in Calabria and Apulia regions, a significant portion of the olive orchards are characterised by old trees of very large size (height > 8 m). Usually, in these orchards olive harvesting is done by periodically collecting the naturally fallen fruits from the soil, with strong negative effects on oil quality. In some cases, these orchards cannot be replaced by young orchards because have a historical and/or landscape importance.
In order to find an alternative to the harvesting of olives from the soil, in 2006, in Apulia region, a trial for the mechanization of olive harvesting in orchards constituted by old, large trees was carried out. The trees, of the cultivars "Cellina di Nardò" and "Ogliarola Salentina", were 60-100-year-old and had a height ranging from about 8 m to about 10 m and a canopy volume of 450-500 m^3/tree. In the first half of November, mechanical harvesting was performed 1) by using a mechanical beater applied to a tractor to harvest the high parts of the canopy + hand-held pneumatic combs to harvest the low parts of the canopy or 2) by using a self-propelled shaker applied to the main branches. With mechanical beater + hand-held pneumatic combs the harvesting yield (percentage of harvested olives with respect to the total present on the canopy, was about 90% and the harvesting working productivity was around 60 kg of harvested olives/h/worker. With the shaker the harvesting yield was about 75% on the cultivar "Cellina di Nardò" and about 40% on the cultivar "Ogliarola Salentina", whereas the harvesting working productivity was around 190 kg of harvested olives/h/worker with the cultivar "Cellina di Nardò" and about 150 kg of harvested olives/h/worker with the cultivar "Ogliarola Salentina". The oils obtained from the mechanically harvested olives always showed a high quality.
The results indicate possibilities for the mechanization of harvesting and the obtainment of oils of high quality in olive orchards characterised by trees of very large size.

Key words: *Olea europaea* L., trunk shaker, mechanical beater, pneumatic combs, oil quality.

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Thème 3

Impacts, enjeux, possibilités et limites de l’olivier en hyper-intensif

S6-C2

Manual and mechanical pruning tests in the super intensive olive growing

Giuseppe Zimbalatti, B. Bernardi

Abstract

In the “super intensive” olive growing, the limited availability of low vigour varieties and the lack of dwarfing graft holders able to control the plants growth efficiently, make the tree-crowns need important pruning interventions in order to guarantee a sufficient illumination level, avoid the arising of competition phenomena and keep plants dimensions which can allow the passing of grape harvesters. This paper concerns the results relative to the first year of test, on manual and mechanical pruning, carried out in super intensive olive cultivation systems, with the purpose of evaluating the performance achieved through two different pruning systems, and making the comparison in terms of productivity and quality of the previously done work.

Key words: Pruning, Mechanization, Super intensive olive growing, Productivity.

Les tests d’élagage manuels et mécaniques dans les cultures en hyper-intensif de l’olivier

Résumé

Dans le cadre de la production oléicole en super intensif, la disponibilité limitée des variétés à basse vigueur et l’absence de porte-greffes capables de contrôler efficacement la croissance des plants, implique la nécessité d’effectuer d’importantes opérations de taille sur la frondaison, dans le but de garantir un niveau suffisant de luminosité, de réduire le phénomène de compétition, et de préserver une dimension des plants permettant le passage des machines à vendanger. Le présent article expose les résultats relatifs à la première année d’expérimentation, sur la taille manuelle et mécanique, effectuées dans le cadre de la conduite en super intensif de la culture d’olivier, en vue d’évaluer les performances effectuées à travers deux différents systèmes de taille, et d’établir la comparaison en terme de productivité et de qualité avec les travaux antérieurs.

Mots clés: Taille, mécanisation, production oléicole en super intensif, productivité.

1. Introduction

During the last ten years a new high density olive-growing (2000 and over plants/ha), called “super intensive” has become popular (Rallo *et al.*, 2006). The success of this model, centred on the high efficiency of the mechanical harvest carried out through the use of grape harvesters, is linked to the mechanization of pruning, since also this operation, just like harvest, has a great influence upon oil yield and production quality.
Tree-crowns, from the 5^{th}-7^{th} year, exceed the total volume of 10.000 m^3/ha (Pastor *et al.,* 2006), so they need important pruning interventions in order to guarantee a sufficient luminosity level, avoid the arising of competition phenomena and keep small plants dimensions which can allow the passage of grape harvesters.

In the olive-grove, the employment of machines facilitates pruning operations, as it consists in a regular external cut of the tree-crown which can be done without particular problems (Giametta *et al.,* 1997). The use of machines for topping and hedging (Lodolini, 2006): operations which adjust respectively the crown height and thickness could be a decisive factor for the success of this model.
The present work has evaluated results relative to the first year of trials on manual and mechanical pruning, carried out in spanish super intensive olive cultivation systems. The aim of this research was to elevate the achieved performance and compare their productivity and quality with previous work.

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2. Material and Methods

The aim of this study was to compare different work productivities achievable by means of manual and mechanical interventions on pruning operations. During the tests, the evaluation of the operational time was carried out according to the formalities provided by C.I.O.S.T.A. classification.

Tests on **manual pruning** were carried out in Andalucía (Córdoba). The plantation, made up of a eight-year-old "Arbequina" variety (selection Agromillora), had a planting distances of 3.75 x 1.35 (about 1,975 plant/ha). It presented a flat position, North-South direction and was provided with drop fertirrigation. The irrigation dose was comprised between 2,000 and 2,300 m³/ha-year. The formation was single axis with 4 metres high plants. The crown was 0.70 m from the ground, with an average volume of 11.85 m^3. 15 rows, each one with an average length of 189 m, and 140 plants, were tested. The work group was made up of three operators, supported by power saws of a light type (model STIHL MS192T, with a volume of 30 cm^3 and power a of 1.3 kW), who had to carry out a hard intervention by means of shears since during the previous years this kind of operation had been done at irregular intervals (Figure 1).

Figure 1: Manual intervention

Tests on **mechanical pruning**, carried out in Cataluña (Reus) concern two different areas of the same olive-grove which has a planting distances of 3.00 x 1.35 meters (2,469 plants/ha), a flat position, East-West direction, and is provided with drop fertirrigation. The "Arbequina" plants (selection Agromillora) are 3.50 metres high and their crowns are 0.50 m from the ground. Two different kinds of interventions have been tested:

- **Thesis A**: cutting of both sides along the rows (hedging on two planes) with a 25° inclination from the vertical. During tests carried out, it was necessary to put a manual final touch to the low part of the tree-crown since the pruning bar could not reach it. The plants presented an average crown volume of 8.19 m^3. 10 rows, each one about 169 m long with 125 plants, were tested (Figure 2).

Figure 2: Hedging carried out with a 25° inclination off the vertical

- **Thesis B**: cutting of a single side, carried out in parallel with the vertical, as close as possible to the trunk and topping 2.20 m from the ground. This scheme is provided by the same operation every year, but on alternate sides. 10 rows, each one about 337.5 m long with 250 plants, were tested. The average crowns volume was 6.34 m^3 (Figure 3).

The work was made up of a single worker operating the pruning machine. The beginning of each survey was the moment when the machine was in front of the row to start pruning, and the final point was the moment when the work in the tested area ended. In both theses, every cut operation, was carried out by a single passage of the machine.

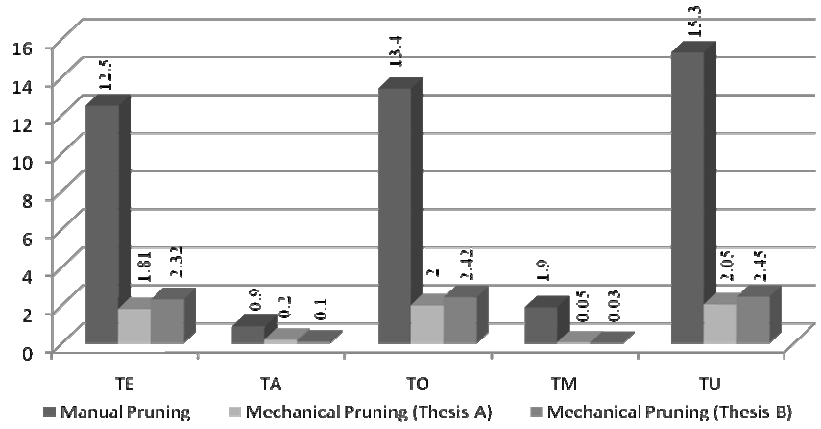
Figure 3: Hedging of a single wall and topping 2.20 m from the ground.

The pruning bar used in the tests, mounted towards the front on a 60 kW New Holland tractor, was comprised of a series of five sharp toothed wheels with the same diameter (0.55 m), placed on the same supporting shaft. These hydraulically operated wheels were made of tempered steel and had teeth with widia plates in order to facilitate the work; they had a rotation speed of 2.000-2.500 cycles/min and were able to cut branches with a diameter of over 0.8 m without problems. The single arm bar, thanks to an oleodynamic control system, can be lifted up vertically over 4 meters of height and

allows all the intermediate cut positions between the vertical one, for the wall pruning (hedging) and the horizontal one for the top pruning (topping). For the manual pruning, power saws of a light type model STIHL MS192T, with a volume of 30 cm^3 and a power of 1.3 kW were used.

3. Results

Results of pruning tests (graphic 1), show for manual interventions, a working effective time (TE) equal to 12.5 h/ha, and a total additional time (TA) of 0.9 h/ha, while the dead times (TM) have an influence of 1.9 h/ha. The total usage time (TU) is 15.3 h/ha. The equipment operating capacity is equal to 0.07 ha/h with a productivity of 0.023 ha/h per operator; the quantity of taking away wood is on the average equal to 17.5 kg/plant, equivalent to 34.5 t/ha. The diameter of 70% of pruned branches is shorter than 0.4 m since plants, in the super intensive model, keep small sizes. The average volume of the crowns is reduced to 4.04 m^3. The width of the path existing between the rows changes from 1.65 m to 2.35 m.



Graphic 1. Comparison between working times collected for interventions of manual and mechanical pruning.

Note: TE = effective time, TA = additional time, TO = operational time, TM = dead time, TU = usage time. Working time expressed in h/ha.

With reference to mechanical pruning interventions, the elaborated data show that the lowest working operating times (TO), equal to 2.00 h/ha, were reported in thesis A, even if a possible manual final touch would have a considerable influence on this value. The additional times, requested only for the turns, have an average influence of 0.15 h/ha while the dead times are out of account. The working operating productivity is 0.50 ha/h in thesis A and 0.42 ha/h in thesis B. The quantity of taking away wood is equal to 3.0 kg/plant in thesis A and 4.5 kg/plant in thesis B, with an average value of 18.6 t/ha. The diameter of 80% of pruned branches is shorter than 4 cm. The average advance speed of the pruning machines along the rows is equal to 1.6 km/h. The average volume of the crowns is reduced to 4.68 m^3 in thesis A and 2.74 m^3 in thesis B.

Mechanical interventions have guaranteed a quite good structure continuity along the row, keeping the balance between geometric form, necessary to allow the passage of grape harvesters over the plants, and functional one, connected with productive aspects.

4. Discussion

The obtained results show a noteworthy economic profit achievable thanks to the employment of mechanical pruning: the working productivity is, on the average, about 22 times higher than manual pruning productivity. The advantage would remain, but considerably reduced, even if, (for thesis A) after the mechanical cut, there was a selective manual intervention of thinning in the low part of the tree crown.

The machine went on quickly with an average speed, as above-mentioned, of 1.6 km/h. However, the presence of a second cut bar (which, if necessary, can carry out the topping) would have allowed a better performance of the work, contributing, in thesis A, to the removal of that part of crown not subject to pruning.

In order to increase work speed and efficiency, it is important to stress that on the market we can find new machines able to remove only the hanging branches of the base crown which cannot be reached by the grape harvesters (Olint, 2006).

However this kind of pruning is not selective since both useful and useless wood is removed; so a reduction of buds, with a consequent fall of the production, can occur. However, since this, is only the first year of experiments, final remarks about productive aspects cannot be expressed yet.

The critical stage of the system is the time that plants take to recover a full production after pruning. This period of lower productivity must be reduced by stimulating the plant to balance the air-root relation as soon as possible. However a proper fertilization and irrigation, typical of the super intensive system, could be a good solution for this problem.
In conclusion, the use of mechanical pruning interventions in the super intensive models, can be an effective solution, taking in consideration the small size that plants must keep (Tous *et al.*, 2006); but further studies are necessary to define the best intervention scheme, the optimal turn and the proper cutting intensity (more or less severe, or rather close or less close to the trunk, taking in consideration the possible vegetative/productive answer of the super intensive olive-grove).

The authors have contributed equally to the present work.

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S6-C3

La fertilisation raisonnée de l'olivier conduit en hyper-intensif

Ben Khelil Malek, Sanaa Mustapha

Résumé

Jeunes Arbéquina oliviers, grandissant dans la région de Mornag, au Nord de la Tunisie, plantés en système hyper-intensif, ont été choisi pour une fertigation annuelle depuis 2006 de N, P2O5, K2O fractionnés selon les stades critiques de développement de l'arbre. Le fruit est choisi à maturité pour déterminer l'effet de la fertilisation combinée sur le rendement, la qualité des fruits et de l'huile d'olive.
Les résultats ont montré une amélioration du poids moyen du fruit et du rendement cumulé des trois années successives de 20%, des arbres qui ont reçu les traitements par rapport au témoin. La teneur en huile a augmenté. Une amélioration d'acide oléique et une diminution de l'acide palmitique ont été constatées. Cependant les teneurs en carotène et en chlorophylle semblent ne pas être affectées par les traitements.

Mots clés : Olivier Arbéquina, hyper-intensif, fertigation, rendement, qualité des fruits

Abstract

Young Arbequina olive tree, growing in North of Tunisia, planted under high density system, were selected to annual fertigation since 2006 of N, P_2O_5, K_2O distributed according to the critical stage of development of the tree. The fruit was chosen at maturity to determine the effect of the combined fertilization in yield, quality of fruit and olive oil.
Results showed an improvement of the fruit mean weight and cumulative yield for three successive years of 20 % of the trees which received treatments with regard to witness. Content in oil was increased. An improvement of Oleic acid and decrease of the Palmitic acid were noticed. However contents in carotene and in chlorophyll seem not to be affected by treatments.

Key words: Arbequina olive tree, high density, fertigation, yield, quality of fruit.

1. Introduction

La Tunisie est classée au premier rang quant aux surfaces consacrées à l'olivier (30% des terres arables). La production nationale de l'huile d'olive représente 9 % de la production mondiale.

En Tunisie, l'intensification de la culture a commencé à naître ces dernières années par la variété espagnole Arbéquina adaptée à ce type de culture qui a été introduite en culture intensive et hyper intensive dans plusieurs régions du pays. Il en existe actuellement 3 000 ha plantés en hyper intensif, avec une production moyenne de 7 à 8 tonnes par ha (Msallem et al., 2004). Cependant cette évolution n'a pas été accompagnée par une amélioration de la fertilisation et les agriculteurs continuent à pratiquer une fertilisation inadéquate par rapport aux besoins nutritionnels de l'arbre.

La fertilisation raisonnée consiste donc à ajuster les apports en éléments fertilisants et les adapter aux besoins de la plante et au bon moment pour d'une part assurer une récolte suffisante et d'autre part limiter les intrants pour minimiser leurs effets secondaires sur le complexe sol-plante –climat.

La pratique de la fertilisation raisonnée se base sur l'analyse des éléments exportés par l'arbre (feuilles, fruits et bois de taille), ceci permet d'apprécier ce qu'il convenait de restituer au sol. Cette méthode permet d'évaluer les besoins globaux de l'arbre et permet d'estimer, au moins, ce qui est perdu annuellement par la culture et d'ajuster les quantités d'éléments fertilisants à apporter par la fumure en fonction de son stade de développement.

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En effet, une amélioration du statut nutritionnel et surtout par une fertilisation raisonnée qui tient compte des caractéristiques du milieu dans lequel on travaille (sol, climat, proximité d'eau de surface ou de zones de captage) permet une amélioration de la productivité de l'olivier. En effet, le statut nutritionnel est le facteur le plus important qui régit le rendement de l'olivier d'après El Fouly (2005)

Cependant ce créneau de la fertilisation raisonnée reste inexploré et peu de travaux se sont intéressés pour améliorer le rendement de l'olivier en culture hyper intensive et surtout la composition chimique des olives (Fernandez- Escobar et al., 2006).

Ainsi, et compte tenu de l'importance de ce type de fertilisation dans le secteur oléicole, nous avons mené ce travail dans le but de déterminer l'effet de la fertigation en N, P_2O_5, K_2O fractionnés selon les stades critiques de développement de l'arbre sur le rendement, la qualité des fruits et de l'huile d'olive Arbéquina conduit en mode hyper intensif.

2. Matériels et méthodes

Des jeunes oliviers de la variété 'ArbéquinaI18' (*Olea europaea* L.) utilisés pour la production d'huile, plantés en système hyper-intensif (Figure1), à une densité de 2x4m dans une sous-parcelle expérimentale de Sadira sise à Mornag au Nord de la Tunisie de 1.5ha sur un sol sablo- argileux ont été choisis pour une fertigation annuelle depuis 2006 des trois engrais essentiels pour l'olivier. L'azote est apportée sous forme d'ammonitre (33.5%) , le phosphore sous forme d'acide phosphorique (50% de P_2O_5) et le potassium sous forme de sulfate de potasse (50% de K_2O). Les doses de fertilisants sont fractionnées selon les stades critiques de développement de l'arbre. Les arbres qui reçoivent le programme adopté par la société Sadira et qui est effectué sur le reste de la parcelle de 21ha sont considérés comme arbres témoins.

Figure 1: Arbéquina cultivée en hyper-intensif (4mx2m).

Pour chaque traitement, 4 blocs sont choisis de manière aléatoire. Chaque bloc comporte 12 arbres d'oliviers de même âge et représentatifs de la parcelle.

2.1. Evaluation des besoins globaux de l'arbre

Cette méthode permet d'estimer, au moins, ce qui est perdu annuellement par la culture et d'ajuster les quantités d'éléments fertilisants à apporter par la fumure en fonction de son stade de développement.

Méthodologie : Elle se base sur l'analyse des éléments exportés N, P et K par l'olivier (feuilles, fruits et bois de taille), ceci permet d'apprécier ce qu'il convenait de restituer au sol.

- Prélèvement des fruits : Un échantillon de 100g / bloc de fruits (le 16/11/2005), de la récolte de l'année 2005 est pris pour la parcelle d'étude, les fruits sont desséchés à l'étuve puis broyés par le mortier en porcelaine, ensuite on détermine les exportations des éléments minéraux dans le fruit.

- Prélèvement de gros bois de taille : On a déterminé les exportations de 5 arbres de chaque parcelle et puis on a extrapolé pour toute la parcelle de densité 1250 pieds /ha. Un échantillon de 60gr de chaque arbre est pris pour analyse des différents éléments minéraux : N ; P, K puis on a calculé le pourcentage en azote en P2O5 et en K2O.

- Prélèvement des feuilles, ceci pour déterminer les exportations des éléments minéraux dans les feuilles. Les feuilles sont collectées à la hauteur d'un homme sur la périphérie de l'arbre et sur les quatre points cardinaux (Est, Ouest, Nord et Sud). Les feuilles matures et saines sont prélevées de la partie médiane des pousses non fructifères d'après Fernandez- Escobar (1997). Un échantillon est pris de chaque bloc, composé de 100 feuilles pour la détermination des éléments minéraux.

Le dosage des éléments minéraux P et K est effectué selon la méthode de Pauwels et al. (1992) et l'azote total N est déterminé par la méthode Kjeldhal.

2.2. Estimation des besoins en fonction du rendement

Nous avons estimé les exportations pour un rendement moyen de 10T /ha pour la variété 'Arbéquina' en ne considérant que les exportations par les fruits, le gros bois de taille et les feuilles. En effet le petit bois de taille est resté sur place dans la parcelle. L'exportation par le gros bois de taille et les feuilles est évaluée en matière sèche de l'ordre de 2000 kg /ha (pour la parcelle de densité 1250 pieds/ha).

On donne les exportations en kg/ha de N, P2O5 et K2O trouvés par Xilloyannis et al. (2002) pour la variété Coratina conduite en mode intensif (Tableau1) et celles trouvées dans la parcelle de sadira à Mornag (Tableau 2).

Tableau 1: Exportations en kg/ha de N, P2O5 et K2O.

Année après plantation	Elément (Kg/ha) N	P2O5	K2O	zone	observation
(2ème année)	9,2	1,3	8,6	sud Italie (lavello)	densité 6x3m
(6ème année)	85,9	30,7	163,1		irrigué, 8000kg/ha

Tableau 2: Exportations en N, P2O5 et K2O pour le fruit, les feuilles et le gros bois de taille des oliviers de Sadira pour une production de 10T/ha d'olives (46% d'humidité).

Exportations en élément fertilisant Kg/ha	fruits	gros bois de taille+feuilles	Totaux des exportations
N	37	80	**120**
P2O5	20	18	**40**
K2O	123	36	**160**

Les analyses physico-chimiques du sol ont montré un niveau de fertilité satisfaisant, ne montrant pas de facteurs limitants et ne nécessitant pas une dose de fertilisation de correction pour les éléments précités, à ajouter aux exportations.

2.3. Fractionnement des doses et périodes d'apport

Le fractionnement de la dose d'ammonitre est donné en tenant compte des périodes critiques du besoin de l'olivier en cet élément qui sont : le débourrement, la floraison, le durcissement du noyau et la veraison.
La quantité de phosphore à apporter est appliquée avec le début de l'activité radiculaire (fin hiver – début printemps), la floraison et le grossissement du fruit.
La quantité de sulfate de potasse est fractionnée pendant la période de grossissent du fruit. Ce fractionnement est déduit d'après la dynamique du potassium dans la feuille (Tableau 3).

Tableau 3: Pourcentage des engrais donnés suivant les stades de développement de l'olivier.

Stade physiologique		% de la dose de fertilisant		
		N	P	K
1	Réveil végétatif et débourrement	10	30	_
2	Début floraison	30	30	_
3	Nouaison	20	15	25
4	Début de grossissement des fruits et sclérification du noyau	20	15	50
5	Veraison	20	10	25

Le Tableau 4: suivant présente les quantités apportées par stade physiologique. La quantité de la dose à apporter par fertigation dépendra de la solubilité du fertilisant utilisée.

Tableau 4: Quantités des engrais donnés suivant les stades de développement de l'olivier.

Stade physiologique		**Quantité d'engrais par stade physiologique (Kg /ha)**		
		N	**P_2O_5**	**K_2O**
1	Réveil végétatif et débourrement	12	12	_
2	Début floraison	36	12	_
3	Nouaison	24	6	40
4	Début de grossissement des fruits et sclérification du noyau	24	6	80
5	Veraison	24	4	40

La récolte a été effectuée pour chaque arbre séparément, pour déterminer le rendement moyen pour chaque bloc (moyenne de 12 arbres) considéré comme une répétition.

Le poids moyen a été déterminé par une pesée de trois sous échantillon de 100 fruits frais à l'aide d'une balance de précision. L'échantillon récolté pèse 2 Kg , puis 960 g d'olives sont triturés . Après centrifugation, le résultant est mis dans une éprouvette gradué de 1000ml pour décantation, puis on lit directement la teneur en huile (sachant que la densité de l'huile =0.920) exprimée en pourcentage de la matière fraîche. L'acidité a été déterminée selon la méthode Wolf (1986). La composition en acides gras a été réalisée par les méthodes décrites In Regulations EEc/2568/91 et EEc /1429/92 dans le (EUC, 1991). La teneur en chlorophylle et la teneur en β carotène exprimées en ppm sont déterminées dans la solution de cyloxane à 470nm, mesurés par spectrophotomètre à différentes longueurs d'ondes 472, 630, 670, 710nm. Les coefficients d'extinction K 232 et K270 sont calculés à des longueurs d'ondes d'absorption respectivement 232 et 270 nm par spectrophotomètrie en utilisant une solution de 1% d'huile en cyloxane.

Les analyses statistiques des données ont été effectuées grâce au logiciel STATITCF (ver.V). L'ensemble des mesures a fait l'objet d'une analyse de variance à un facteur au seuil de risque de 5%.Il est complété par une comparaison multiple de moyennes par le test de Newman et Keuls (au seuil de 5%).

3. Résultats et Discussions

3.1. Effet de la fertilisation raisonnée sur le rendement en olives

D'après la Figure 2 qui représente le rendement final en olives, on note une amélioration du rendement pour les arbres qui ont reçu le programme tri-factoriel (N, P, K) par rapport au témoin (le programme de fertigation adopté par la société) pour les trois années d'étude. En effet le rendement des arbres du traitement NPK pour l'année 2006 a été de 10,54 Kg/arbre par rapport au témoin 9,2 Kg/arbre. Cependant, on note une diminution du rendement pour les deux traitements pour l'année suivante.

Les rendements obtenus sont de 7,07 Kg /arbre pour le Témoin et de 8,32 Kg /arbre pour le traitement NPK. On note que la diminution du rendement est plus faible pour le traitement NPK par rapport au témoin relatif à une amélioration de la nutrition minérale de l'arbre apportée au stade critique de développement. Pour l'année 2008, on note aussi une diminution du rendement des arbres témoins égal à 6,3 Kg /arbre, cependant le rendement est maintenu presque stationnaire pour le traitement NPK (8,13 Kg/ arbre) par rapport à l'année précédente. L'analyse de la variance ne montre pas de différence significative entre le traitement NPK par rapport au témoin pour les trois années d'étude.

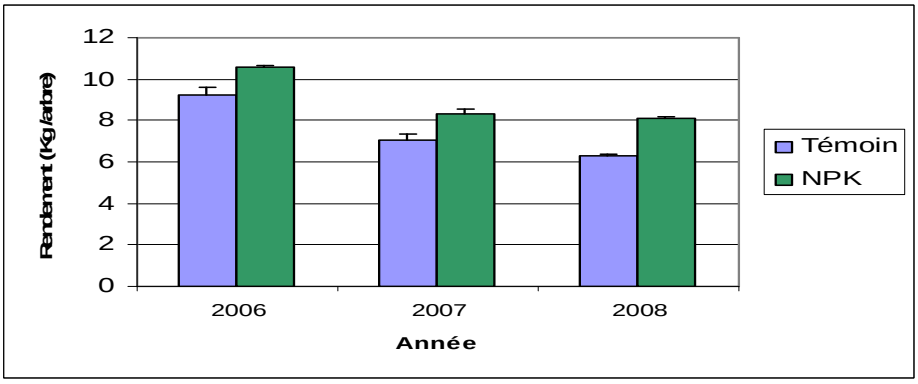

Figure 2: Le rendement des olives à la récolte pour les arbres ayant reçus les traitements NPK et le témoin effectués pour les trois années d'étude.

D'après la Fgure 3 qui donne le rendement cumulé des trois années pour les deux traitements, on note une amélioration de 20% du rendement pour le traitement NPK par rapport au témoin.

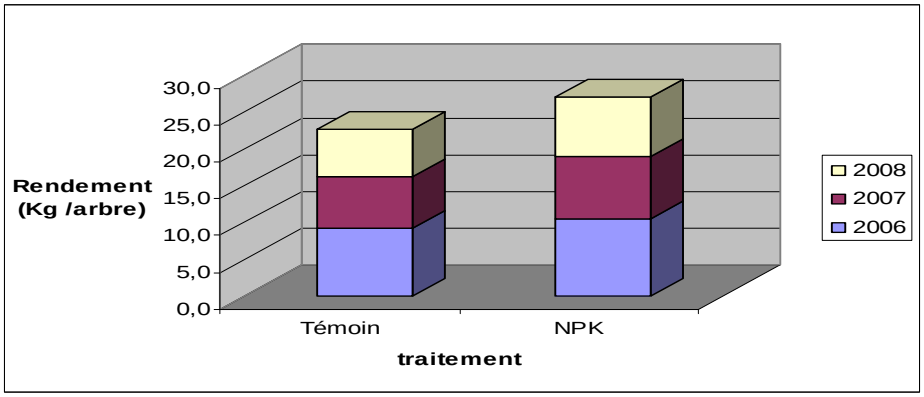

Figure 3: Le rendement cumulé des olives à la récolte des trois années pour les arbres ayant reçus les traitements NPK et le témoin

3.2. Effet de la fertilisation raisonnée sur la teneur en huile

La teneur en huile pour les oliviers du traitement NPK pour l'année 2006 a été de 17,2 % par rapport au témoin égal a 16,54%. Cependant, on note une diminution de la teneur en huile pour les deux traitements pour l'année suivante. Les teneurs obtenues sont de 15,7 % pour le traitement NPK par rapport au Témoin qui était de 15,2% pour l'année 2007 (Fig. 4).

Cette diminution est due aux conditions de croissance et des conditions climatiques au moment du processus de la lipogenèse qui sont essentiellement les pluies torrentielles de septembre et d'octobre qui ont engendré une augmentation du poids des fruits au dépends de la lipogenèse. D'après (Lavee, 1997) la quantité potentielle de l'huile qui s'est accumulée dans l'olive à la récolte est déterminée par la variété, mais elle peut varier sensiblement selon les conditions de croissance, du climat et de la charge de l'arbre en fruits.

A la troisième année d'étude la teneur en huile a été aussi plus élevée pour le traitement NPK (16,51%) par rapport au Témoin (16,07%) (Fig. 4). Cette amélioration de la teneur en huile pour les trois années d'étude est due à l'amélioration du statut nutritionnel azoté et phospho–potassique de l'olivier constatée par les traitements apportés à la parcelle de Sadira surtout au moment de la croissance végétative améliorant la capacité photosynthétique de l'arbre et des fertilisants apportés au cours du développement du fruit et de la lipogénèse. En effet, l'accumulation de l'huile dans l'olive est un processus qui dépend de la quantité des carbohydrates qui alimentent les fruits au moment de la lipogenèse. Ces carbohydrates proviennent principalement des feuilles mûres, mais aussi du fruit lui-même (Conde et al., 2008). Cependant l'analyse de la variance ne montre pas de différence significative entre le traitement NPK par rapport au témoin pour les trois années d'étude.

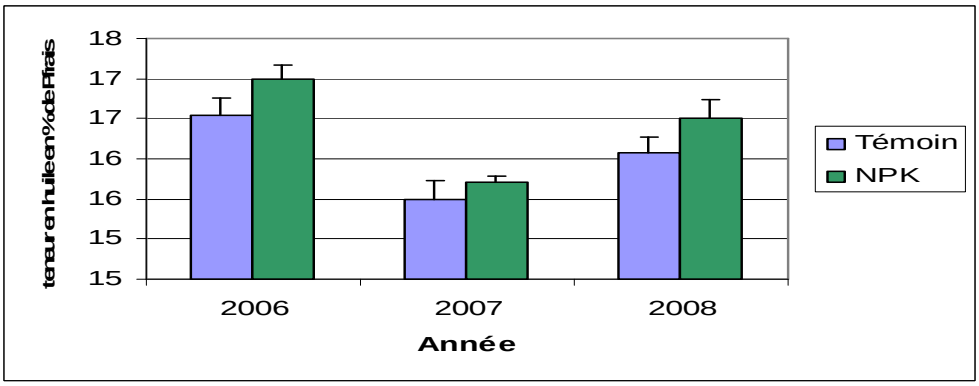

Figure 4: La teneur en huile (% du poids frais) pour les traitements NPK et le témoin pour les trois années d'étude.

3.3. Effet de la fertilisation raisonnée sur le poids moyen des olives

Le poids frais des olives à la récolte a été égal à 2,05 g pour le traitement NPK et était plus élevé par rapport au témoin égal à 1,9g au cours de la première année, cependant il a diminué à la deuxième année pour le traitement NPK égal à 2,86g par rapport au témoin égal à 2,97g. Quant à la troisième année, les teneurs ont été presque similaires et égales à 2,25g pour le témoin et 2,28 g pour le traitement NPK (Fig. 5). Toutes ces valeurs sont supérieures au poids frais moyen de la même variété cultivée en Espagne, qui est de 1,86g (Tous et Romero, 2002). Cette différence peut être expliquée par l'influence du milieu et par l'apport des intrants (eau et fertilisants) (Aouini, 2006). Cependant l'analyse de la variance ne montre pas de différence significative entre le traitement NPK par rapport au témoin pour les trois années d'étude.

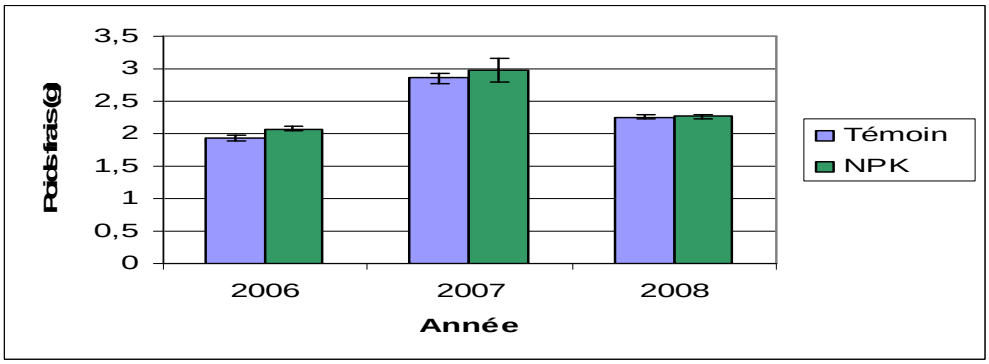

Figure 5: Le poids frais des olives à la récolte pour le traitement NPK et le témoin pour les trois années d'étude

3.4. Effet de la fertilisation raisonnée sur les critères de qualité de l'huile

3.4.1. Composition acidique

L'analyse chromatographique de l'huile d'olive a permis de donner la composition en acides gras totaux de l'huile allant de l'acide palmitique (C16:0) à l'acide eicosénique (C20:1). Tous les échantillons du traitement NPK et du témoin sont dans les normes proposées par le COI (1988) et sont riches en acide oléique (C18:1) et faibles en acide palmitique. Cependant, on note une légère augmentation de l'acide oléique et linoléique (C18:2) pour le traitement NPK par rapport au témoin pour les trois années d'étude et qui se rapproche du pourcentage de l'acide oléique de l'Arbéquina en Espagne qui est de 65,7% et linoléique 14,8% (Tous et al., 1999). Cette augmentation est significative au seuil de 5%.

Aussi, on note une diminution significative de l'acide palmitique pour le traitement NPK par rapport au témoin constatée pour les trois années d'étude (Tableau 5). La variété 'Arbéquina' semble avoir une meilleure adaptation dans le nord du pays.

Tableau 5: Composition en acide gras totaux de l'huile de l'olivier de la variété 'Arbéquina' selon les traitements effectués pour les trois années de l'étude.

Année	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1
Témoin 2006	18,8a	2,87a	0,10	0,21	1,67a	58a	15,7	0,62	0,29	0,22
NPK	17,1b	2,48b	0,12	0,21	1,73b	61,7b	15,20	0,62	0,28	0,21
Témoin 2007	16,77a	1,81a	0,08	0,21	1,68a	67,33a	10,56	0,43a	0,24	0,14a
NPK	15,70b	1,91b	0,08	0,22	1,88b	68,5b	10,53	0,40b	0,26	0,21b
Témoin 2008	18,53a	2,52a	0,08	0,18a	1,75a	62,28a	14,13	0,47	0,25	0,19a
NPK	18,16b	2,31b	0,07	0,16b	1,71b	63,8b	14,02	0,47	0,24	0,17b
Norme (COI)	**7.5-20**	**0.3-3.5**	**-**	**-**	**0.5-5.0**	**55.0-83.0**	**3.5-21.0**	**<1.0**	**<0.6**	

Les moyennes suivies de lettres différentes pour chaque année sont significativement différentes au seuil de 5% selon le test de Newman et Keuls

3.4.2. Caractéristiques physico-chimiques

L'acidité de l'huile a été variable, en diminuant pour le traitement NPK pour les années 2006 et 2008 par rapport au témoin alors qu'elle a augmenté pour l'année 2007. L'acidité la plus élevée a été observée pour l'année 2008, où elle était égale à 0,28% pour le traitement NPK. Elle ne semble pas être affectée par les traitements effectués malgré les différences significatives observées vu que parfois elle augmente et tantôt elle diminue entre les années d'étude (Tableau 6).

La teneur en chlorophylle a varié entre 0,73 ppm à 1,17 ppm pour les trois années de l'étude. Ces teneurs ont été légèrement plus élevées pour le traitement NPK par rapport au témoin pour les deux dernières années alors que l'inverse est observé pour l'année 2006 où elles ont augmenté significativement (Tableau 6).

Les teneurs en carotène semblent ne pas être affectées par le traitement NPK, où on note parfois une faible augmentation, et tantôt une diminution des teneurs en carotènes du NPK par rapport au témoin (Tableau 6).

L'extinction spécifique mesurée à 232nm et 270 nm, montrant l'état d'oxydation de l'huile, a augmenté significativement pour le traitement combiné NPK pour les deux premières années pour 232 nm par rapport au témoin. En effet ceci est du à l'augmentation de la teneur en acide oléique constatée, sujet de cette oxydation qui favorise la formation d'hyperoyde linoléique, cependant aucune variation est obtenue pour ceux de K270 nm.

Tableau 6: Caractéristiques de l'huile selon les traitements effectués pour les trois années de l'étude.

Année	Acidité (%)	Chlorophylle (ppm)	Caroténoïde (ppm)	Extinction Spécifique K 270	K 232
2006 Témoin	0,28a	1,18a	14,33	0,12	1,71a
NPK 2007	0,24b	1,01b	14,49	0,11	1,78b
Témoin	0,17a	0,73	26,83a	0,11	1,45a
NPK	0,19b	0,75	28.49b	0,11	1,49b
2008 Témoin	0,3a	0,84	15.33a	0,12	1,93
NPK	0,28b	0,87	13,66b	0,11	1,94

Les moyennes suivies de lettres différentes pour chaque année sont significativement différentes au seuil de 5% selon le test de Newman et Keuls.

4. Conclusion

Nous pouvons conclure que la fertilisation raisonnée a permis l'amélioration de certaines caractéristiques des olives (poids moyen, rendement en olive) et de l'huile d'olive (la teneur en huile, augmentation de l'acide oléique, baisse de l'acide palmitique..). Cette amélioration de la qualité en huile et du rendement pour les trois années d'étude est due aux traitements NPK, donnés à juste dose et au bon moment.

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S6-C4

Comparative study of Tunisian and foreign olive cultivars suitability for high density planting system

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Abstract

In 2000, a new concept of olive orchard based on high densities was introduced in Tunisia. Limited cultivar choice is considered as the main inconvenient of high density planting system. In deed, only Arbequina and Arbosana are used in these types of orchards. Therefore, two comparative trials were set up in 2003 and 2004, respectively in Takelsa (north of Tunisia) and Sfax (south of Tunisia), to evaluate the suitability of 4 olive cultivars (Arbosana, Arbequina, Chemlali and Chetoui) to this planting system (1250 trees ha^{-1}).

Results obtained here showed that local varieties (Chemali and Chetoui) are more vigorous than Arbequina and Arbosana. However, the latest varieties presented the highest yield in comparison to the other varieties. Arbosana had the highest accumulated olive yield after 4 harvests in Takelsa while in Sfax; Arbequina presented the highest accumulated olive yield after 3 harvests. However, the highest yield efficiency was obtained by Arbosana variety in the two locations. The Chetoui variety, despite its lower production and accentuated alternate bearing, showed some interesting agronomic characteristics related with the high density planting system like the lower width canopy and the erected port.

Keywords: High density planting system, olive trees.

Etude comparative de l'aptitude des cultivars d'oliviers Tunisiens et étrangers au système de plantation en hyper-intensif

Résumé

Un nouveau concept de la culture de l'olivier basé sur l'emploi de hautes densités a été introduit en Tunisie l'an 2000. Le choix limité de variétés est considéré comme étant l'inconvénient principal de ce système de culture. Pour ceci, deux essais ont été installé en 2003 et en 2004 à Takelsa (le nord de la Tunisie) et Taoues (Sfax, sud de la Tunisie) pour évaluer le comportement de 4 variétés (Arbequina, Arbosana, Chemlali et Chetoui) à la culture super intensive (1250 arbres/ha).

Les résultats obtenus dans ce travail indiquent que les variétés locales (Chemlali et Chetoui) sont plus vigoureuses que les variétés Arbequina et Arbosana. Toutefois, les variétés Arbequina et Arbosana présentent une productivité plus élevée. En effet, la variété Arbosana a présenté la production cumulée la plus importante à Takelsa après 4 récoltes alors qu'à Taoues, c'est la variété Arbequina qui a présenté la production cumulée la plus élevée après 3 récoltes. Cependant, l'efficience de production la plus élevée a été obtenue chez la variété Arbosana dans les deux sites sujets de notre étude. Malgré la faible production et l'alternance de la production de la variété Chetoui, elle a présenté des caractéristiques agronomiques intéressantes en relation avec le système de plantation hyper intensif tels que la faible largeur de la frondaison et son port érigé.

1. Introduction

The high density system developed in Spain 13 years ago was introduced in Tunisia in 1999 by some private investors. Since this date, this system of plantation was a matter of debate concerning the choice of cultivar and the choice of planting density. The high density planting system was developed to facilitate the use of the over-head mechanical harvester which may reduce costs relative to hand harvesting.

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The life time of this new planting system as compared to other planting density remains the basic question by the detractors of this new planting system. Higher densities may allow higher productions during the first years. Later, the vigor will be difficult to be controlled which may reduce the light interception with the consequent significant decrease of production. So, the cultivar choice is considered as the main criterion when deciding to establish a high density orchard. Actually, Arbequina and Arbosana are considered as the most suitable cultivars for high density planting (Tous et al., 2007, De la Rosa et al., 2007). However, little is known about the behavior of these varieties under Tunisian conditions. Furthermore, no work has been done on the behavior of Tunisian varieties conducted under high density planting system, which limit the variety choice in high-density olive orchards.

The aim of this work was to study the behavior of Arbequina, Arbosana, Chemlali and Chetoui varieties grown under high density planting system in two different regions (Takelsa in the north and Sfax in the south).

2. Material and Methods

The olive tree orchards were located in Takelsa (North of Tunisia, 36° 47'; 10° 37') and Sfax (South east of Tunisia, 35° 02'; 10° 28'). Olive varieties planted in these orchards are Chemlali, and Chetoui from Tunisia, and Arbequina and Arbosana from Spain. Olive trees are 6 and 5 years old, respectively and planting density is 1250 trees/ha with a frame of 2 x 4 m. After planting, a trellis system was placed and each tree was tied to a stake, for central leader training. During the first's years, trees were lightly pruned to maintain the central leader. The statistical design was randomized complete blocks with two rows of ten trees per variety in Takelsa and 4 rows of five trees in Sfax. All measurements were carried out on the totality of trees composing each block.

Vigour (height and canopy volume), tree yield and estimated yield per ha, yield efficiency (yield/canopy volume), oil content, and oil characteristics (fatty acid composition) were determined. It must be pointed out that for studying oil characteristics, 3 fruit samples (3 kg) per block and per cultivar were taken each year from 2005 in Takelsa and 2006 in Taoues.

3. Results and Discussion

Results obtained in this work indicated that all studied cultivars bear in the second year after planting except for Chetoui in both localities (Table 1). In deed, Chetoui variety bears in the third and the fourth year after planting in Takelsa and Taoues (Sfax), respectively. Furthermore, Chetoui variety showed a tendency to an accentuated biennial bearing from the third year in Takelsa (Table 1). The most productive variety in the second year after planting was Arbosana in both localities. In the third year after planting Arbosana was also the most productive variety in Takelsa followed by Arbequina, Chetoui and Chemlali. However, in Taoues, Arbequina was the most productive followed by Arbosana, Chemlali and Chetoui. Without taking into account the production obtained the fifth year after planting in Takelsa, only "Arbequina" planted in Taoues showed a highest accumulated yield as compared to the same variety planted in Takelsa. With respect to the other varieties, similar accumulated yield were obtained in both localities. It must be pointed out that trees were severely damaged by *Spilocea oleaginae* during the fourth year after planting in Takelsa which reduced significantly the olive yield.

Estimated yield per ha for the first three harvests for Arbequina I18 and Arbosana in Taoues and Takelsa were inferior to those obtained with the same varieties in Cordoba (Spain) for the same period after planting (De la Rosa et al., 2007). This result may be due mainly to the differences in trees density which were about 1975 trees per ha in Cordoba. However, when compared to the production obtained by the same varieties in Tarragona (Spain), productivity in Tunisia was much higher; beside the tree density employed in Tarragona was 2 fold superior to that employed in Tunisia (Tous et al., 2007).

Table 1: Average yield (Kg of olives/tree) and accumulated yield of studied varieties from the second to the fifth and the fourth year after planting in Takelsa and Taoues.

	Takelsa					Taoues			
	Years after planting				Accumulated	Years after planting			Accumulated
	2	3	4	5		2	3	4	
Arbequina	2.06	6.73	2.23	9.4	20.42	1.83	3.21	11.6	16.6
Arbosana	3.62	7.13	2.7	13	26.48	3.06	2.18	7.88	13.12
Chemlali	2.42	5.28	1.18	7.7	16.57	1.1	0.16	9.82	11.07
Chetoui	0	7.07	0	6.4	13.5	0	0	7.55	7.55

With respect to the vegetative characteristics, results obtained in both trials indicated that Arbequina I18 and Arbosana are low vigorous as compared to Tunisian varieties (Figure 1). In the fifth year after planting in Takelsa, Chetoui showed the highest vigour followed by Chemlali, Arbosana and Arbequina. However in Taoues, the highest vigour was obtained by Chemlali followed by Chetoui, Arbequina and Arbosana. Canopy volume of Arbequina reached in the third year after planting in Taoues (4.97 m^3 per tree) were superior to that obtained in Takelsa (3.59 m^3 per tree). Arbosana was the less vigorous variety in both localities. Tous et al. (2007) reported that Arbequina I18 and Arbosana were less vigorous than Koroneiki, Jonanenca and Fs17 planted in high density planting system.

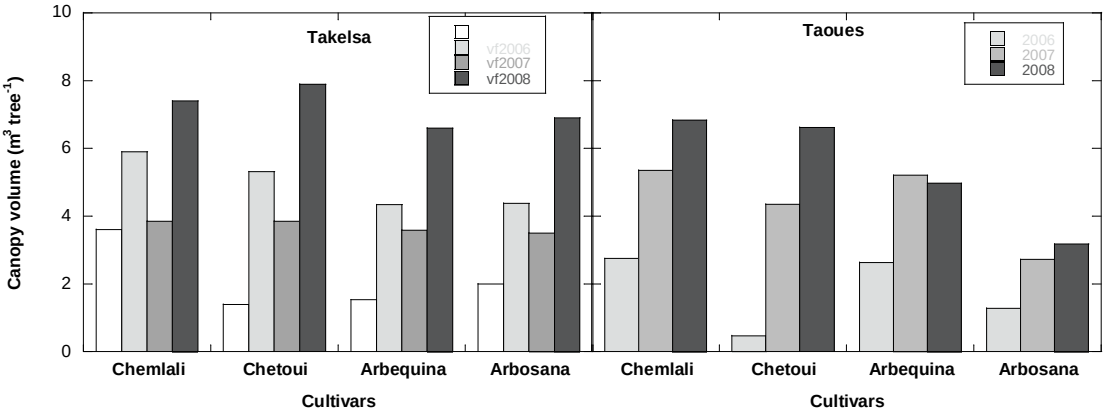

Figure 1: Canopy volume of studied varieties for the years under study.

The highest yield efficiency (Kg per m^3 of canopy) was obtained with Arbosana followed by Arbequina, Chemlali and Chetoui in both localities (Fig. 2). Similar results were obtained by Tous et al. (2003) indicating the highest yield efficiency of Arbequina I18 and Arbosana. The highest yield efficiency is mainly due to the lower vigour of these varieties as compared to Tunisian studied cultivars (Fig. 1).

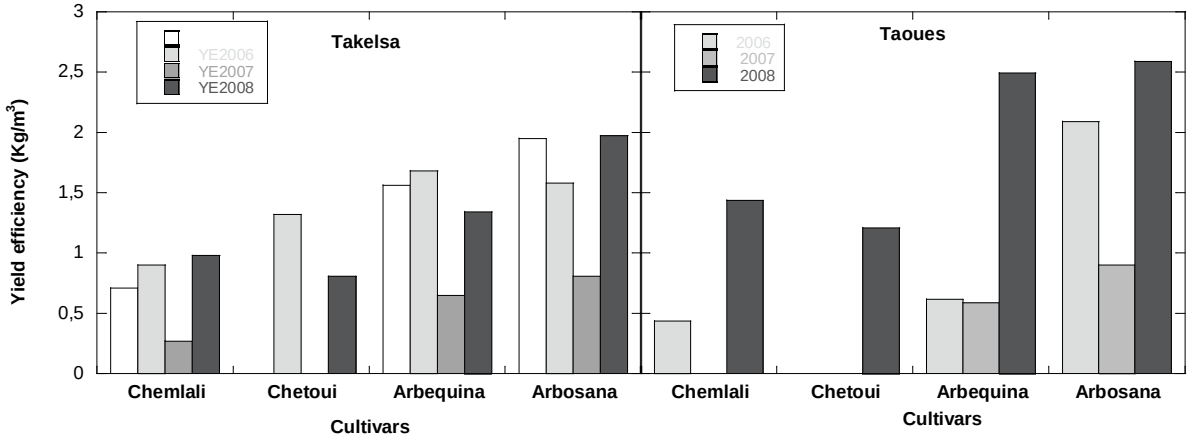

Figure 2: Yield efficiency for studied varieties for the years under study.

All studied varieties planted in Taoues showed higher oil content on dry weight basis as compared to those planted in Takelsa, except for Chetoui which showed similar oil content in both localities (Table 2). In contrast to the oil content, moisture content was higher in olives harvested from Takelsa for all studied varieties except Arbequina. Chetoui showed the highest fruit weight in both localities followed by Arbequina, Arbosana and Chemlali.

Table 2: Average fruit characteristics for the years under study.

Varieties	Takelsa			Taoues		
	Oil content (NMR)	Fruit weight (g)	Moisture (%)	Oil content (NMR)	Fruit weight (g)	Moisture (%)
Arbequina I18	47.6	1.94	47.64	51.79	2.07	56.42
Arbosana	46.24	1.22	57.08	52.5	1.55	53.34
Chemlali	44.98	0.89	54.97	48.6	0.85	50.73
Chetoui	52.6	2.62	62.34	51.26	2.28	55.93

Arbequina I18 and Chemlali were characterized by low oleic acid content and a high plamitic and linoliec acid (De la Rosa et al., 2007; Grati-Kammoun and Khlif, 2001). Results obtained here indicated that oleic, palmitic and linoleic acid content of Arbequina planted in Takelsa were similar to those obtained in Cordoba (De la Rosa et al., 2007). While, in Taoues, the oleic acid content decreased and the palmitic and linoleic acid increased as compared to those obtained with the same variety in Takelsa. By contrast, Chemlali and Chetoui showed highest and lowest oleic acid content in Taoues, respectively in comparison with those obtained with the same varieties in Takelsa. These results may be explained by the origin site of each variety which was the north for Chetoui and the south for Chemlali. Contrary to these varieties, Arbosana showed similar oil characteristics in Taoues and Takelsa (Table 3). Several authors reported that olive oil composition in fatty acids varies according to the geographical origin (Ben Temime et al., 2004, Ryan et al., 1998).

Table 3: The fatty acid composition of the oil of the different studied varieties. Values are the average of three harvests in Takelsa and two harvests in Taoues except the chetoui.

Varieties	Takelsa			Taoues		
	C16:0	C18:1	C18:2	C16:0	C18:1	C18:2
Arbequina I18	17.63	63	14.21	18.53	58.8	16.5
Arbosana	16.37	68.77	9.57	16.93	67.12	10.56
Chemlali	18.19	60.15	15.9	16.07	63.8	14.57
Chetoui	16.95	66.77	12.81	12.47	60.91	22.14

In summary, Arbosana showed a good suitability for high density orchards mainly due to its lowest vigour when compared to Arbequina and Tunisian varieties, high productivity and stable oil characteristics in different environments. Furthermore, Arbosna were characterised by its high oleic acid content. Arbequina seem to be better adapted in the north than the south of Tunisia due to the decrease of oleic acid content recorded in the south in comparison with the north (Takelsa). However, it must be pointed out the high productivity of this variety in both localities. The employment of Tunisian cultivars (Chetoui and Chemlali) in high density planting system seem to be difficult due to the high vigour of these varieties, the alternate bearing of Chetoui and the instability of fatty acid composition when these varieties are planted in non original sites.

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S6-C5

Effect of topping pruning on pollen viability and germination of Arbequina and Arbosana varieties grown in high density planting system

Ameur Teycir, **Larbi Ajmi**, Msallem Monji, and BenDhiab Ali

Abstract

The main objective of this work is to study the effect of topping pruning on pollen quality, into the canopy of Arbequina and Arbosana varieties conducted under HDP. For this purpose an experiment was conducted in Mornag (North of Tunisia, 450 nm; 24.2° C; 36.42 North), planted in 1999 with a density of 1250 trees/ha. Trees were divided into three layouts: the bottom layout (0-1 m), the medium (1-2 m) and the top one (up to 2 m). The pruning was carried out mechanically at a height of 220 cm above the soil at February 2007. The pollen sampling was carried out at white button stage (May 2008). In the same time, the light interception by the canopy (PAR) and branch growth were measured. Our results show that the highest rates of pollen viability were recorded at the more lighted parts of the canopy of the control trees of Arbequina and Arbosana varieties. The germination rate followed the same trend in both varieties, with a decrease of 30.27 and 12.87% respectively at the bottom layout (0-1m) and the medium one (1-2 m) as compared to the upper layout of Arbosana variety. In the second year after the "Topping", pruning improved significantly the viability and germination rate in the lower parts of the canopy of the two studied varieties. However, a significant decrease of pollen viability and germination was recorded in the pruned part of the canopy, as compared with controls.

Keywords: high density, « Arbequina », « Arbosana », quality, pollen, pruning, «Topping ».

Résumé

La lumière constitue un élément essentiel pour l'olivier surtout pour les plantations hyper intensives. La qualité du pollen est fortement influencée par son microenvironnement. La taille « Topping » est appliquée comme moyen de correction en haute densité. A cet effet un essai été réalisé dans un site expérimental à Morneg (Nord de la Tunisie, 450 nm ; (13,9 et 24,2 ℃); 36,42 Nord) à une densité de 1250 arbres/ha en Arbequina et Arbosana (plantation 1999). Les arbres ont été subdivisés en trois étages : le premier étage (0-1 m), le deuxième étage (1-2 m) et le troisième étage (supérieur à 2 m).
La taille est réalisée mécaniquement à une hauteur de 220cm parallèlement au sol (au mois de Février 2007). Les prélèvements des échantillons du pollen, le suivi de l'interception lumineuse et de la croissance végétative ont été effectués au stade bouton blanc (mai 2008). Nos résultats montrent qu'au niveau des arbres non taillés, la qualité du pollen ainsi que la croissance végétative sont meilleure au niveau des zones les mieux éclairées de l'arbre, avec une diminution de 30,27 et de 12,87%, respectivement au niveau des étages 0-1m, 1-2 m par rapport à l'étage supérieur chez la variété Arbosana. A la deuxième année de son application, la taille a contribué à l'amélioration significative de la fertilité du pollen au niveau des étages inférieurs des arbres taillés pour les deux variétés, mais pas celui de l'élongation végétative. Au même temps, la qualité du pollen a été négativement affectée au niveau de la zone de coupe, en comparaison avec les témoins.

1. Introduction

The olive production sector in Tunisia has known a marked intensification and mechanization from 1999 (Msallem et al., 2004; Msallem et al., 2008; Caballero, 2009). This intensification was based mainly on the use of the high density planting (HDP) system developed in Spain since 1995. The HDP system orchards cover actually 4500 ha in Tunisia (Msallem et al., 2008) and is based on a high production per hectare, early fruit-setting cultivars, rapid recovery of investment and a low cost of production through mechanization of the harvest (Msallem et al., 2004; Tous, 2007; Léon et al., 2007). In these orchards, the production distribution is a function of the location, its cardinal directions and its height all over the olive tree (Acebedo et al., 2000). In fact, the light penetration in the canopy of the tree is under the influence of inter-tree and intra-tree shading in HDP system (Faust, 1989, Connor, 2006).

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In the HDP system, it is necessary to define an optimal density and ensure a good control of the dimensions of trees to avoid the negative effects of the shading (León et al., 2007; Pastor et al., 2007). The "Topping" pruning is applied as a correction technique in HDP (Pastor, 1989; Dag, et al., 2006; Dias et al., 2009). Depending on this pruning types (frequency, period of application, position, and severity), the physiological reaction and the reproductive performance of the olive tree will change (Faust, 1989, Cronje et al., 2004).

Phenological behaviors of olive tree are also largely influenced by the micro-environmental factors (temperature and exposure) (Defni et al., 2000, Delphi et al., 1997). The influences of these factors are explained by the probable relation of heat accumulation with bud's location by the canopy (Cesaraccio, 2005). Viability has been defined as "having the capacity to live, grow, germinate or develop" (Lincoln *et al*. 1982 cited by Dafni et Firmage, 2000). So, the evaluation of pollen viability is the first step in understanding the chances of the pollen to germinate on the stigma as a crucial stage toward fertilization.

A pollen grain quality is determined by pollen viability, germinability and/or ability to fertilize flowers. The main objective of this study is to carry out first the effect of HDP system and second the reaction to the application of the "Topping" pruning on pollen potential's performance of "Arbosana" and "Arbequina" olive oil varieties, and the relationship with the vegetative growth and the light distribution by the canopy.

2. Material and Methods

This work was carried out in Mornag in a high density planting orchard (1250 trees/ha) planted at 1999 with Arbequina and Arbosana varieties. The dormant pruning trial was carried out in this site in February 2007. The experimental design consisted of two randomized complete blocks with 5 trees each one for every variety. Three trees / variety / treatment (Topping and control) were considered. Each tree was divided into three layouts: the bottom one (0-1 m), the medium one (1-2 m) and the top one (up to 2 m). On each layout, three branches for each quadrant (North, East, West and South) and three from the interior were selected and labeled in March 2008. In total, 15 twigs for each layout were observed during the reproductive season until fruit set stage. The pollen sampling was carried out at white button stage (May 2008) from this five locations/layout/variety/treatment. At the same time, the light interception by the canopy (PAR) and branch growth were measured. Statistical analyses were performed using the SPSS 13.0 for Windows.

3. Results and discussion

Results obtained here showed that photosynthetic active radiation (PAR) decreases significantly from the top to the medium and low parts of the tree (Figure 1). In fact, PAR measured above vegetation (up to 3m) was 1220.33µE/m²/s and 1376.52µE/m²/s for Arbosana and Arbequina varieties respectively. From the upper part to the lower one of the tree, PAR decreased continually by 10-19%, 39-31.5% and 70.5-59% respectively, for Arbosana and Arbequina. León and al. (2007) reported that trees planted in high density are likely to suffer from the mutual shading problems by the seventh years.

The topping pruning did not affect significantly the PAR interception by the canopy (Fig.1). In deed the light interception only increased (10.7 and 23.7%) in the upper part of the canopy of pruned trees as compared to the control trees (Figure 1). However, a small decrease of light interception was recorded in the medium and the bottom layout in pruned trees in comparison with control trees. Porpiglia and Barden (1981) found that light intercepted by the canopy increased during the first years, but tend to decrease the following years, depending to the pruning severity level.

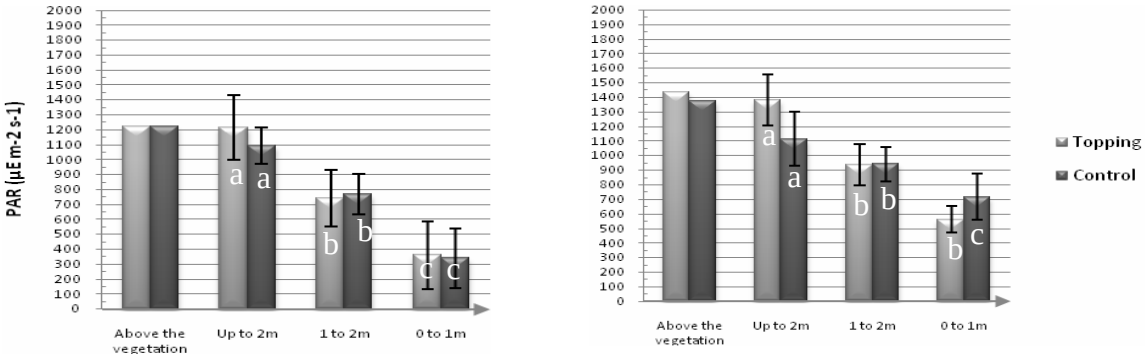

Figure 1: Par intercepted by the 'Arbosana' (**A**) and 'Arbequina' (**B**) olive oil canopy of 'pruned' and 'unpruned' trees conducted in high density planting system (Means with different superscript letters (a,b,c).

Results show that Pollen viability varied significantly with the location by the canopy and the cultivars. The topping pruning application did not influence significantly pollen viability rate in the medium and lower parts of the Arbosana, but a significant increase in the lower layout in case of Arbequina was noted. In contrast with the upper and lower layout of the canopy of Arbosana, a significant increase of pollen viability was found in the upper part of the canopy of pruned trees in comparison with the controls (Figure 2).

Viability rate was explained by pollen grain origin, its position in the inflorescence and its location in the branch, but also the microenvironment during pollen formation (Villemur, 2006; Dafni and Firmage, 2000).

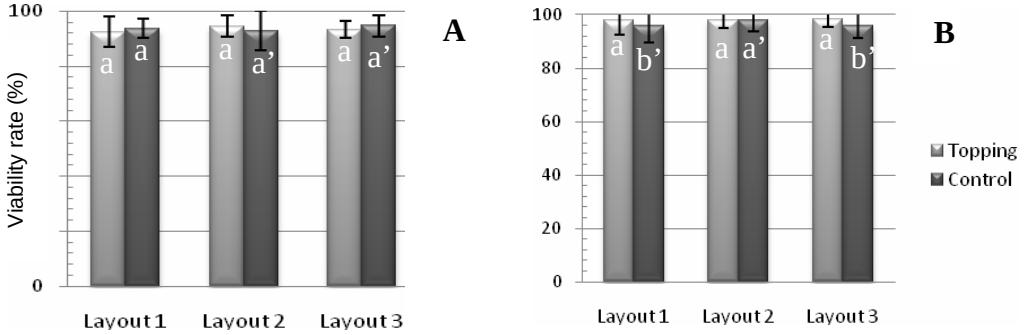

Figure 2 (A and B): Viability rate distribution by the canopy of 'pruned' and 'unpruned' trees 'Arbosana' (**A**) and 'Arbequina' (**B**) varieties conducted in high density planting system (Means with different superscript letters (a,b,c) are significantly different at $P < 0.05$ (Duncan's test)).

Considering germination rate, our results showed that it varied significantly by the treatment and the employed variety and the combined effect. In fact, germination rate was closely associated with the PAR levels intercepted by the canopy. It was higher in the branches localized up to 2m with 43.9%-30.2% for Arbosana and Arbequina. In the medium and lower parts of Arbosana and Arbequina, the potential of pollen to germinate was 38.3%-31.2% and 30.6%-24.6%, respectively.

Similar results were found with Frangivento and Frantoio olive cultivars (Cimato 1980; 1982 cited by Acebedo et al. 2000).

The "Topping" pruning application, led to the improvement of pollen germination rate in the lower part of the canopy by 6.7% and 39% in comparison with control trees, for Arbosana and Arbequina cultivars, respectively. In contrast with the lower part of the canopy, a significant decrease of germination rate was observed in the upper layout for two varieties.
This result could be explained by the stress effect of the pruning technique which was done in the upper part of the canopy.

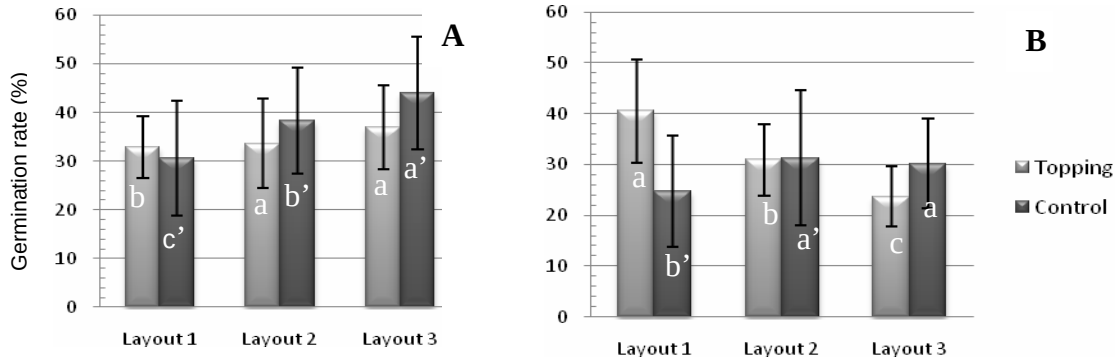

Figure 3 (A and B): Germination rate distribution by the canopy of 'pruned' and 'unpruned' trees 'Arbosana' (A) and 'Arbequina' (B) varieties conducted in high density planting system (Means with different superscript letters (a,b,c) are significantly different at P < 0.05 (Duncan's test)).

With respect to vegetative growth, results obtained in this work indicated that the top of the tree grew significantly more than other parts of the tree for pruned and controls trees. However, it must be pointed out that the difference observed between the two higher layouts in control trees was not significant.

Results shown in Fig.4 indicated that the length of branch in the upper layout was higher than the other parts of the canopy. The length of new shoots varied significantly with the same trend under the combined effect of the layout and variety.

Faust (1989) suggested that the growth in the top of the tree would shade the rest of the tree at the same time; growth at the base of the canopy would diminish. The reductions in the resources available for pollen production could be explained by the limited growth vegetative (Delphi et al., 1997).

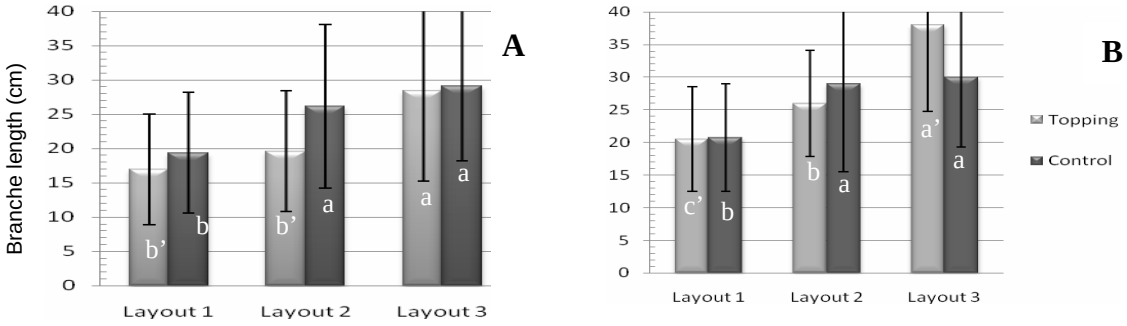

Figure 4 (A and B) : Shoot growth according to the three layout by the canopy of 'pruned' and 'unpruned' trees 'Arbosana' (**A**) and 'Arbequina' (**B**) varieties conducted in high density planting system (Means with different superscript letters (a,b,c) are significantly different at P < 0.05 (Duncan's test)).

The topping pruning increased the number of flowers per inflorescence in the different layouts of the canopy of the two studied varieties except the upper layout of the pruned Arbequina. The decrease of the number of flowers per inflorescence in the upper layout of the pruned Arbosana may be explained by the highest flowering intensity (data not shown).

Similar results were found by Acebedo (2000) indicating that flowering intensity was highest in parts of unpruned trees receiving the highest levels of radiation. Furthermore, Cesaraccio et al. (2005) indicated that the branch exposures have certain effect on reproductive cycle and phenological behavior of olive tree in term of timing of reproductive stages.

The fruit set percentage of Arbequina and Arbosana varieties were higher in the top layout as compared to the medium and bottom ones (table 1). Pastor et al. (2007) found a linear response between the number of inflorescence per shoot and the relative photosynthetic active radiation. For Arbequina cultivar, Acebedo et al. (2000) concluded that the effect of shoot location on fruit setting was quit significant between the top and the lower part of the tree.

The topping pruning decreased the fruit set percentage of Arbequina and Arbosana. Decreases were more pronounced in Arbosana. Fruit set decrease in Arbequina variety may be due to the stimulation of vegetative growth after pruning topping application. With respect to Arbosana variety, the higher decrease of fruit set in pruned trees, may be due to either stimulation of vegetative growth and to the higher percentage of flowers buds in comparison with control trees.

Contrary to our results, several authors have indicated that pruning increased fruit set which might result from increased water to the tree and nitrogen supply to the leaves (Chandler, 1919 cited by Faust, 1989). Aldrich and Grim (1938) cited by Faust (1989) found an increased N supply in fruiting spurs of pruned trees.

Table 1: influence of topping treatment on flowers numbers per inflorescence and fruit set for Arbequina and Arbosana varieties.

Treatment	Layout	Number of flowers/inflorescence		Fruit set (%)	
		Arbosana	Arbequina	Arbosana	Arbequina
Control	(0-1 m)	11,42 a ±3,88	11,79 a ±3,35	20,75 b ±14,22	14,50 b ±8,96
	(1-2 m)	11,59 a ±3,30	11,94 a ±3,97	22,50 ab ±16,03	15,52 b ±9,57
	(up to 2 m)	12,93 a ±5,65	11,26 a ±3,94	24,30 a ±14,00	23,83 a ±16,30
Topping	(0-1 m)	12,17 a'b' ±7,07	11,40 a' ±4,47	12,74 b' ±8,04	13,43 b' ±8,85
	(1-2 m)	14,12 a' ±6,23	13,00 a' ±4,67	12,92 b' ±7,77	15,22 b' ±9,98
	(up to 2 m)	9,97 b' ±7,73	12,43 a' ±4,39	21,35 a' ±15,87	22,99 a' ±12,59

4. Conclusion

In conclusion our preliminary results indicate that the high density planting system limit the potential of pollen grain to fertilize in the lower part of the canopy.

The application of the topping improves pollen potential to have great number of fertilized flowers.

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S6-C6

Mechanical harvesting of table and oil olives

Louise Ferguson Vossen Pm*, Rosa U**, Guinard Jx***, Burns J****, Krueger Wh*, Glozer K*

Abstract

Mechanical harvesting must be developed for successful table and olive oil production in California. Both canopy contact shaking head and trunk shaking harvesters can produce processed black ripe olives that trained sensory panels and consumer panels cannot distinguish from hand-harvested olives. However, both types of harvesters remove and capture less than the 80% efficiency required. No successful abscission compounds to decrease fruit removal force have been identified. Therefore, as with oil olives the tree shape must be modified for successful Mechanical harvesting must be developed for successful table and olive oil production in California. Both canopy contact shaking head and trunk shaking harvesters can produce processed black ripe olives that trained sensory panels and consumer panels cannot distinguish from hand-harvested olives. However, both types of harvesters remove and capture less than the 80% efficiency required. No successful abscission compounds to decrease fruit removal force have been identified. Therefore, as with oil olives the tree shape must be modified for successful table olive harvesting. Recent results demonstrate training to an espalier shape with and without a trellis does not decrease yield and the tree that can be harvested with both canopy contact and trunk shakers. However, the harvesters differ in their removal patterns, efficiency, and tree damage.

Key words: ***Olea europaea****;* flower development; SSH; gene expression, Gene Ontology

Résumé

La récolte mécanique des olives doit être développée aussi bien pour les oliviers à tables ou à huiles. Des tests de dégustations effectué par des panels de dégustateurs ou par des consommateurs n'ont pas réussit à détecter les olives de tables noirs récoltés mécaniques par vibration au niveau de la frondaison ou du tronc des olives récoltées manuellement. Cependant les deux types de récolteurs mécaniques ont une efficience de récolte inférieure au 80% demandé à ce genre d'opération. Aucun produit d'abscission efficace n'a pu être identifié pour augmenter cette efficience. Par contre la forme de la frondaison des oliviers à tables doit être adaptée pour la récolte mécanique comme cela a été fait pour les oliviers à huile. Les résultats récents montrent qu'une forme en mur fruitier avec utilisation ou non de structure ne diminue pas le rendement. Les arbres peuvent être récoltés en utilisant une vibration au niveau du tronc ou au niveau de la frondaison. Néanmoins les deux types de récolteur différent d'un point de vue de leur efficience et des dégâts occasionnés sur les arbres.

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Thème 4

Changement climatique, gestion de l'eau et développement durable de l'olivier

S7-C1

Tendances de floraison des oliviers et caractéristiques climatiques dans deux régions Méditerranéens (Italie et Tunisie)

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Résumé

L'influence de la latitude sur les dates de floraison chez olivier a été étudiée à travers quinze stations de culture de cette espèce en méditerranée: quatre en Tunisie et onze en Italie (de Zarzis, 33° au Sud, jusqu'à Perugia, 43° au Nord). La floraison ét é suivie grâce à la technique de captage pollinique sur une période de 10 ans (1999 à 2008). La tendance du déroulement de la floraison a mis en évidence une étroite relation entre la phénologie de l'espèce, les températures et les caractéristiques géographiques, même si les variations météorologiques annuelles portent à considérer que la précocité ou le retard phénologique sont des événements locaux.

Mots clés : Olivier, floraison, latitude, climat.

Flowering trends in olive and climate features in two mediterranean countries (Tunisia and Italy)

Abstract

Research on the influence of latitude on flowering phenology has been realized to investigate the different magnitude of biological response. This type of analysis was conducted considering one of the more important plant species of Mediterranean shrub, the olive, in fifteen monitoring stations, four of which located in Tunisia and eleven in Italy, from the southern area of Zarzis (33°) to the northern Perugia (43°). The flowering was studied utilizing an aerobiological monitoring method through pollen traps inside olive groves from 1999 to 2008. The flowering trends during the study period evidenced very close relationships between plant's phenology, temperature trends and geographical features even if the yearly mesoscale meteorological variations force to consider phenological advances or delays as local events.

Key-words: Phenology, Olive, Climate, Latitude, Mediterrany.

1. Introduction

The investigation efforts on the theme of climate change have necessarily to consider the importance of plants, since they are very sensible to climate and manifest with their phenological developments its variations. In these terms we can consider plants as bio-indicators so as biological systems used for evaluation of deviations from the stable environmental condition, independently of its organization level and its function in the ecosystem (Menzel 2002; Osborne et al. 2000; Schwarz 2000; Parmesan et al. 1999). The climate variability and the climate change can influence the vegetative-reproductive cycle of a plant species (Menzel et al. 2006). Plants can highlight the alterations caused by different factors; a response to any kind of disturbance must thus be interpreted and evaluated because it summarizes the synergic action of all environmental components. The possible consequences are numerous, from risks on a small scale, like late spring frost, to more drastic consequences, likes a change of growth area. The actual climatic change could have the sufficient impact to determine a modification in area of distribution of the most sensible natural species and a temporal shifting of phenophase manifestations. Obviously, phenological responses to climatic variables are rather complex and varied in different species; nevertheless, some simple empirical temperature-based models have shown good forecasting results for different phenological phases. As a consequence of the present hyphotesis, if phenological developments are highly influenced by climate, the same climate depends on geographical characteristics (latitude, altitude, closeness to water surfaces and so on) which may variously influence it.

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In particular one of these characteristics, the latitude, was considered through phenological response to climate changes (Parmesan 2007; Kleunen and Fischer 2008) in order to investigate the possible different magnitude of biological response in relation to lower or higher latitudes. The present study, although at a lower scale, permitted to collect phenological data on olive flowering through the aerobiological monitoring in study areas located on a close interval between the longitude of 9°50' and 16°00' but at various latitudes, aproximatively from 33° of the southern (Zarzis) to 43° of the northern (Perugia). The two stations represent the geographical limit of olive cultivation, considering that Zarzis is situated in a Tunisian very dry climatic area (the desert side), while Perugia with its continental climate constitute the north Italian limit, especially during winter, to the productive olive grove cultivation. In this manner the investigation permitted to compare the obtained flowering dates to evidence a possible relationship between latitude gradient and plant response. Moreover, another aim of the research was to investigate on the presence of flowering trends in the different geographical areas and on the hypothetical relationships with the same latitude.

2. Materials and methods

In the present investigation 15 olive monitoring stations were evaluated, considering that beside the two cited Zarzis and Perugia, other 13 olive cultivated areas were monitored: Taous, Mornag, Jammel in Tunisia, Agrigento, Trapani, Reggio C., Catanzaro, Cosenza, Lecce, Salerno, Brindisi, Benevento and Foggia in Italy (Fig. 1). All these areas were evaluated because their significance by a olive productive point of view. Moreover, in all these areas different olive cultivars were cultivated, in particular in the four Tunisian ones the principal cultivars are Chetoui and Chemlali, in Sicily the cultivars Biancolilla and Nocellara are the most famous while in the other Italian areas the more important cultivated varieties are: Frantoio, Carolea and Coratina.

The olive flowering phenomenon was monitored utilizing two aerobiological methods principally used in this scientific field of research, one developed by Hirst (a volumetric method) and the other by Cour (Cour 1974; Fornaciari et al. 2000a, b; Galan et al. 2004). Really in all the monitoring stations the quantitative evaluation of pollen emission was utilized to determine the start of flowering SF, defined as the day in which one pollen grain/m3 was reached wherever five subsequent days contained one or more pollen grains/m3, the maximum of flowering (peak of flowering) MF, defined as the day of maximum pollen concentration and the end of flowering EF, defined as the last day with pollen concentration.

The phenological data (flowering dates) were collected from 1999 to 2008 in every monitoring station and intra-inter variability analyses were carried out.

The presence of monotonic positive or negative trends of the flowering dates during the 10-year study (1999-2008) in all the monitoring areas were at first tested with the nonparametric Mann-Kendall test and secondly the slope of a linear trend was estimated with the nonparametric Sen's method (Gilbert 1987).

The meteorological variables considered were obtained both in Tunisia and in Italy by the Stations of the National Meteorological centres located near the pollen monitoring areas. They include the minimum, maximum and average temperatures, the range of temperature between the maximum and the minimum levels, precipitations intended as quantity of rainfall, the number of days with precipitations and the evapotranspiration. The daily values of temperature accumulation in Growing Degree Days (GDD) were calculated with different threshold temperatures (7, 9, 11, 13° T), while the GDD amounts were considered, for each one of the monitoring station, from the first day of January to the mean flowering date during the 10-year study period.

Up to three different GDD calculation methods were utilized to determine the daily GDD in each area; Single Triangle and Sine Methods; "Cesaraccio" Method.

The determination of the most suitable Threshold Temperature between those utilized for heat accumulation and the evaluation of the different GDD calculation methods for the different study years and monitoring areas were carried out through the method of the RMSE considering its reliability in the threshold temperature estimation (Snyder et al. 1999).

To obtain specific results, about the relationships between flowering dates and the most suitable GDD amounts for each one of the monitoring areas, 15 singular regression analyses were carried out utilizing the temperature summations as independent variable.

3. Results

The relationship analyses between flowering dates and GDD amounts for each one of the monitoring areas are shown in the 15 singular regression analyses carried out and reported in Table 1. All the statistical analyses evidenced good percentages of explained variability (R^2 higher than 0.35) and high significances of the independent variables (prob level < 0.05). The main results evidenced by this type of analysis were represented by the specific slopes of flowering related to the GDD amounts calculated for each one of the monitoring areas. The slope value permitted to consider mathematically the ratio between variation in days and GDD amount, all the slopes were negative indicating that the increase of one GDD unit provokes the flowering advance of a day portion. The average slope of –0.05 defines the advance of about 1.2 hour (24h * 0.05) in relation to the increase of a GDD unit. In this sense the slope calculated in relation to the latitude of the respective monitoring stations evidenced as the biological response of olive plants are quite constant independently from the different study areas.

In Figure 2 the relationships between full flowering dates, temperature amounts (GDD amounts) and latitude are explicitated by linear regressions considering contemporarily the biological, meteorological and geographical data of all the monitoring areas. The statistical results evidenced the high positive influence of latitude on flowering dates and on the other hand, its negative influence on temperature amounts.

Taking in consideration the first regression, the slope of full flowering dates per latitude degree is 4.38 with a standard error of 0.45. The value of R-Squared is 0.88 while the correlation between the two variables is 0.94. A significance test that the slope is zero resulted in a t-value of 9.75. The significance level of this t-test is 0.0000. Since 0.0000 < 0.0500, the hypothesis that the slope is zero is rejected. The estimated intercept is -32.34. In the regression that considers as dependent variable the GDD amounts the slope of temperature summation per latitude degree is -26.23 with a standard error of 5.98. The value of R-Squared, is 0.60. The correlation between variables is -0.77. A significance test that the slope is zero resulted in a t-value of -4.39. The significance level of this t-test is 0.0007, the hypothesis that the slope is zero is rejected. The estimated intercept is 1815.16.

4. Discussion and conclusion

We could conclude that averagely the relationships between plant's phenology, temperature trends and geographical features are very close. However, the yearly mesoscale meteorological variations avoid the possibility to forecast flowering dates of northern areas on the base of southern ones, considering that the meteorological and consequently the phenological advances or delays are local events.

Finally, the regression analysis between full flowering dates and GDD amounts at the different latitudes permitted us to evidence the response of olive species in geographic regions subjected to different rates or patterns of climate change. The magnitude of biological response at different latitude was investigated and the slope results of respective monitoring stations evidenced as the flowering phenomenon of olive plants are quite constant, practically the same temperature increase provokes similar olive flowering advance in the different areas. As reported in a recent study (Parmesan 2007) while an effect of latitude is present (justified by complessive regression analysis) the same latitude is not so important as predictor of the magnitude of olive phenological response to climate change in the Mediterranean area investigated.

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S7-C2

Climatic change impacts on olive orchards in Tunisian arid area: the resort to the irrigation with brackish wastewater

Bechir Ben Rouina, Chedlia Ben Ahmed & Saïd Jilani

Abstract

Since the beginning of 1980, Tunisian arid climate has not stopped to show a worrying evolution. In comparison with the period extended between 1950 and 1980, the rise of the average annual temperatures exceeded 1,5°C ; whereas the averages of precipitations regressed of at least 30 mm $year^{-1}$ and the episodes of one year dryness and more became numerous. In corollary with these two phenomena, the already excessive evapotranspiration rate raised at least to 35 mm of water per degree Celsius, which makes the atmosphere hotter and less saturated with water vapour.

Under these hostile conditions the soil is the basic element in the life of plants by playing a great role as a source of water and nutrients. The rains sustain the water reserves which will be restored at the plant when they will be in need. To face this climate effects: increasingly more hostile and drier with the plants and in order to ensure a better productivity of the olive trees, the resort to the irrigation of the olive groves is developed. However, confronted with fresh water scarcity, the use of treated wastewater water is extended and risks of generating serious problems threatening at the same time the soil and the plant, and consequently, the fragile ecosystem sustainability following grounds salinization and plant toxicity by salts.

Impacts du changement climatique sur l'oléiculture en milieu aride tunisien : le recours à l'irrigation avec les eaux non conventionnelles

Résumé

Depuis le début des années 1980, le climat aride tunisien ne cesse de montrer une évolution inquiétante. En comparaison à la période étendue entre 1950 et 1980, la hausse des températures annuelles moyennes a excédé 1,5°C ; alors que les m oyennes des précipitations ont régressé d'au moins 30 mm an^{-1} et les épisodes de sécheresse d'une année et plus sont devenues nombreuses. En corollaire à ces deux phénomènes, l'évapotranspiration potentielle déjà excessive, se trouve majorée d'au moins 35 mm d'eau par degré Celsius, ce qui rend l'atmosphère plus chaude et moins saturée en vapeur d'eau.

Dans ces conditions hostiles à la vie des végétaux, le sol est l'élément de base dans la vie des plantes en jouant un rôle de source d'eau et de nutriments. Les pluies entretiennent les réserves hydriques de celui-ci qui vont être restituées à la plante au fur et à mesure qu'elle aura besoin lors de ces différentes phases de développement. En culture pluviale sous des conditions de précarité hydrique, ce rôle de puits devient très important puisque l'olivier est en deçà de ses besoins en eaux estimés à 450 mm de pluie par an et la qualité du sol devient un facteur capital de régulation de l'alimentation hydrique de celui-ci.

Pour faire face à ce climat de plus en plus hostile aux cultures et en vue d'assurer une meilleure productivité des arbres, le recours à l'irrigation des oliveraies est développé. Cependant, confrontés à la rareté de l'eau douce, l'utilisation tout azimut des eaux non conventionnelles est en extension et risque de poser de sérieux problèmes menaçant à la fois le sol et le végétal et par voie de conséquence, la durabilité de l'écosystème fragile suite à la salinisation des terrains et à la toxicité des sels.

Mots clés : Olivier, climat, sol, aridité, eaux salines, eaux usées traitées.

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1. Introduction

The Middle East and North Africa are considered as the driest region in the world and freshwater availability in those areas is very low (World Bank, 1996). According to several authors (Ben Ahmed et al., 2006; 2008; 2009a; 2009b; 2010; Bedbabis et al., 2009; 2010a; 2010b; Ben Rouina et al., 2006; 2010) the use of brackish water or recycled wastewater in agriculture may help in reducing the problem of the limited availability of freshwater. In Tunisia, 106 wastewater treatment plants generated approximately 238 Mm^3 $year^{-1}$ of treated wastewater (TWW) in 2009 (ONAS, 2010). About 65 millions cubic meters (30%) of these TWW quantities were recycled on agriculture and supply to irrigate 9600 hectares. Many studies (Ben Ahmed et al., 2009a ; Bedbabis et al., 2009; 2010a ; 2010b ; Rejeb, 1992) showed that the application of treated wastewater at a reasonable rate improved growth and productivity of some crops and trees. However, the main problem that can arise from excessive and continuous application of TWW is phytotoxicity due to high content of salts and heavy metals, which may pose a health risk for human beings or livestock (Rejeb, 1992). In Sfax, TWW is from domestic provenance (88%) and industrial (12%) sources and is typically reclaimed at the secondary level by using biological processes. These processes consist of eliminating biodegradable material by transforming it into microbial residues. Secondary treatments remove dissolved and colloidal organic matter by using an aerobic-biological treatment. The aerobic-biological treatment is performed in the presence of oxygen by microorganisms which metabolize the organic matter, thereby producing more microorganisms and inorganic end-products (CO_2, NH_3 and H_2O). Studies on the effect of TWW on olive's growth and productivity are scarce. The topic should be better investigated in countries like Tunisia where olive trees play an important role for the economy but water availability for agricultural purposes is very limited. The objective of our study is to verify if irrigation with TWW can be considered a good source of water and fertiliser's source for "Chemlali" olive trees with no phyto toxicity effects.

2. Materials and Methods

2.1. Experimental plots

Olive trees (*Olea europaea* L.), planted in a sandy soil at 'El Hajeb' experimental station at Sfax, Tunisia (34◦43N, 10◦41E) were used from February 2004 to 2009. Eighteen-years 'Chemlali' olive trees spaced 24 m × 24 m were selected for this experiment (17 trees ha^{-1}). A randomized block design with three blocks and two treatments (TWW and WW irrigation) were used. Both plots contained twenty four olive trees (8 x 3 replications) irrigated by submergence at an annual rate of 5000 m^3 ha^{-1} with treated wastewater (TWW) or well water (WW). Irrigation with TWW started in February 2003 and extended until now (2010). The region is characterized by an arid climate of Mediterranean type. The annual rainfall and temperature averages over 52 years were 250 mm and 23 ◦C, respectively.

2.2. Water sampling and nutrient analyses

The characteristics of TWW and WW were reported in table (1). The pH of the TWW and WW were 7.6 and 7.9, respectively. These were within the pH 6 - 9 range appropriate for irrigation reuse (ONA, 2010). EC values were 6.3 and 4.6 mS cm^{-1} for TWW and WW, respectively. These values are indicators of a high level of salinity.

2.3. Soil sampling and mineral analysis

Soil samples were collected in plastic bags and stored in a portable cooler. Composite soil samples were collected quarterly from each plot at both 0 - 40 cm and 40 - 80 cm depth twice by year (July and December). The samples were air-dried at room temperature, ground and crushed to pass a 2 mm sieve, and mixed thoroughly for analysis. Soil pH was measured in suspension (soil: water = 10: 25) by a pH meter. Electrical conductivity (E. C.) was measured in a saturated paste using a conductivity meter. Organic matter was determined using volumetric method. Further, 10 soil samples were selected and analysed for texture using the Robinson pipette method. For mineral nutrients and heavy metals analyses, the soil samples were extracted with 100 ml ammonium acetate and analysed following the same methodologies as for the same water. For P analysis a soil samples were dissolved in 100 ml of calcium carbonate, agitated for one hour, and filtered. The assimilated phosphorus was determined using a JENWAY colorimeter.

Table 1: Characterization of Irrigation Waters (Mean Values).

Elements	Unit	Well Water	Treated Wastewater	Tunisian standards
pH (H_2O)		7.9	7.6	6.5 – 8.5
E. C.	mS cm^{-1}	4.6	6.3	7.0
Salinity	g L^{-1}	3.50	4.66	-
P total	mg L^{-1}	0.8	10.3	0.05
K^+	mg L^{-1}	35	38	50.0
Na^+	mg L^{-1}	355	470	300.0
Cl^-	mg L^{-1}	1580	1999	600.0
Ca^{2+}	mg L^{-1}	184.5	95.8	-
Mg^{2+}	mg L^{-1}	126.2	83.8	-
SO_4^{2-}	mg L^{-1}	162.3	787.0	1000
Zn^{2+}	mg L^{-1}	0	0.42	5
Mn^{2+}	mg L^{-1}	0	0.5	-
DCO	mg L^{-1}	0	73	90
DBO	mg L^{-1}	0	22	30

2.4. Leaf mineral analyses and growth control

Thirty-two fully developed leaves were detached from the middle portion of the current year's shoot from the external and internal canopy in the four cardinal directions (8 shoots / tree). They were collected in paper bags and stored in a portable cooler at monthly intervals during the two years. Successively, the leaves were dried in an oven (60 °C) and analyzed according to the methods described by Pauwels et al. (1992). Mineral elements (P, K, Na, Cl, Mn, Zn, Cd and Pb) analysis was carried out after dry - ashing at 450 °C in a muffle oven (HEROTEC) and digestion of the ashes with 1 M HNO_3. N was determined by using the Kjeldahl method; K and Na were determined by atomic emission spectrophotemetry (JENWAY PFP7, Milan, Italy); heavy metals (Mn, Zn, Fe, Cu, Cd and Pb) by atomic absorption spectrophotometry (Perkin Elmer A Analyst 300, PerkinElmer Inc., Willesley, MA, USA). P in leaves was determined by a vanado-molybdate colorimetric procedure with a JENWAY 6405 UV / Vis spectrophotometer (Milan, Italy). Finally, Cl was determined titrimetricaly with $AgNO_3$.

Eight shoots of the current season were tagged, two shoots for each cardinal direction (8 shoots/ tree). Data on shoot length were collected yearly from February 2003 and the number of flowers/ inflorescence was recorded on the representative chosen shoots. Olives were harvested manually to guarantee the accuracy and weighed to obtain the yield.

2.5. Statistical analysis

Statistical analysis (General Linear Model) was used to study the effect of treated wastewater on mineral nutrients and heavy metal content in soil. Data were analysed using the Super anova statistical software SPSS for Windows 14.0. The means were separated by Fisher's protected univariate least significant difference (LSD) test at $p < 0.05$.

3. Results

3.1. Environmental effects on olive tree growth

The olive tree development depends on the interaction of major ambient environmental conditions (rainfall pattern, air temperature, solar radiations intensity, atmospheric humidity, air CO_2 concentrations, etc…). Several researches have studied the relationship between olive tree activity and environmental conditions; they have showed that drought and heat are the most important environmental stress factors that disrupt the plant activity.

Water availability is the principal cause conditioning crops growth. It interacts directly in the plant activity via photosynthesis and transpiration process and affects its growth and fruit set up. In arid area, climate change affects average temperatures and temperature extremes (fig. 1a), timing and spatial patterns of precipitation (Fig. 1b), soil moisture, runoff, and the frequency of disturbances, such as drought. Between 1981 and 2009 global warming is twice more important in arid area of Tunisia than the world average (1.5 - 2 ° C against 0.75 ° C). This climate warming is accompanied by a regression between 10 and 20 % of average annual precipitation.

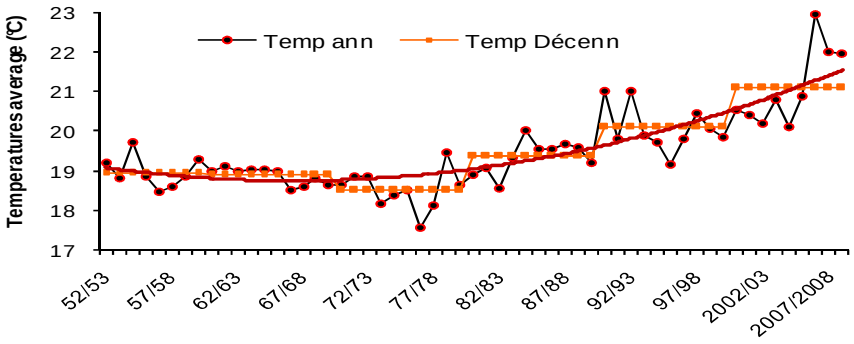

Figure 1: Yearly average of temperature (a) and rainfall (b) at the Chaâl farm between November 1952 and August 2009.

3.2. Soil texture

Soil samples from both experimental plots were classified as sandy-silty soils. Mechanical analysis of soil samples are descried in Table 2, with means sand content varying from 68.3 to 67.6%, silt content varying from 22.8 to 25.0%, and clay content varying from 7.4 to 8.9% in both TWW irrigated and WW irrigated plots, respectively.

Table 2: Characterization of soil texture (depth of 0 to 80 cm).

Treatment	Treated wastewater irrigated soil				Well water irrigated soil			
Element	Clay	Silt	Sand	$CaCO_3$	Clay	Silt	Sand	$CaCO_3$
Mean (%)	8.9	22.8	68.3	12.9	7.4	25.0	67.6	10.3
S. D .[a]	4.8	0.9	5.5	4.9	2.2	0.8	14.5	4.5

[a] Standard deviation

3.3. Effect of treated wastewater reuse in soil

At the beginning of the experimental period, soil organic matter contents were similar for both treatments: 0.42% and 0.45%. Soil macronutrients concentrations were comparable and no statistical differences were shown. For both soil irrigated treatments, 2.6 g kg^{-1} for N, 143 mg for K and 70 mg for P (Table 3). After 6 years of irrigation, soil O.M, N, P and K concentrations increased due to TWW effects (table 3). In particular, the increase of O.M, N and K was highly significant ($p < 0.001$). These finding are in the agreement with previous study [7, 8, 10]. However, the increase of Na (315 mg kg^{-1} vs. 180 mg kg^{-1}) and Cl (650 mg kg^{-1} vs. 212 mg kg^{-1}) concentrations was detected after 6 years of irrigation with TWW with respect of WW treatment, as reported previously (Ben Ahmed et al., 2010; Bedbabis et al, 2009; 2010a). At the beginning of experiment, heavy metals concentrations ranged from 25 to 28 mg kg^{-1} for Zn and from 120 to 123 mg kg^{-1} for Mn. Significant increase of Mn (168 mg kg^{-1} vs. 105 mg kg^{-1}) and Zn (44 mg kg^{-1} vs. 26 mg kg^{-1}) were found, after 6 years of irrigation with TWW. This might be attributed to cumulative addition of these metals to the soil thought irrigation, as reported in previous investigations (Rejeb, 1992; Bedbabis et al, 2009; 2010a; 2010c). However, significant increase of all organic and mineral elements was detected after six years of irrigation with TWW and denotes the fertilizer values of this kind of water.

3.4. Effect of treated wastewater reuse in leaf nutriment concentrations

Irrigation with TWW caused a significant increase ($p \leq 0.001$) of leaf N, P, K, Na, Mn and Fe concentrations compared to WW treatment (Table 3). Furthermore, the comparison between these mineral concentrations between February 20003 and February 2009, showed a significant high differences. The increase was a consequence of the larger amount of these mineral nutriments supplied by TWW compared to WW. Olive trees generally exhibit significant variations in the seasonal and the annual levels of leaf nutrients which depend on the biological cycle and the annual yield status. In fact, at spring, an important decrease of N, K and P concentrations was found in high (On)

or low (Off) yield periods. This decrease indicated the mobility of these nutriments in order to allow the development of vegetative and reproductive structures.

After six years of TWW irrigation, high significant differences have been observed ($p \leq 0.001$ for Na and $p \leq 0.01$ for Cl) for leaf Na and Cl concentrations between the two treatments after six years (Table 3). In particular, Na varied between 0.03% at the beginning of experimentation and 0.12% in 2009. The same tendency was observed with Cl which concentrations increase from 0.30% to 0.47% in the same periods. TWW irrigated trees had a higher leaf Na and Cl concentrations than WW irrigated trees, as a consequence of higher NaCl concentration in the TWW which is basically from domestic provenance (88%) sources. Leaf Fe, Mn, Cu and Mn concentrations were statistically different between the two treatments (Tab. 3) and amounted to the values of 10 – 50 ppm for Mn, Zn and Cu and between 50 - 150 ppm for Fe, usually reported for olive tree [16].

Table 3: Effects of irrigation treatments (well water, WW and treated wastewater, TWW) on some soil and leaves chemical properties.

Element		Unit	Chemical composition of the soil (depth 0 – 80 cm)			Chemical composition of olive tree leaves			
			WW	TWW	Signifi-cance	Unit	WW	TWW	Significance
2003 February (Initial)	O. M.	(%)	0.42	0.45	ns	-	-	-	-
	N	(g Kg^{-1})	2.6	2.6	ns	(%)	1.25	1.99	***
	P	(ppm)	70.3	69.9	ns	(%)	0.08	0.01	*
	K	(ppm	143.3	142.1	ns	(%)	0.72	1.03	***
	Na	(ppm)	106.0	105.3	ns	(%)	0.04	0.03	ns
	Cl	(ppm)	62.2	62.5	ns	(%)	0. 27	0. 30	ns
	Zn	(ppm)	25.0	28.0	ns	(ppm)	15.9	18.6	*
	Mn	(ppm)	120.2	123.0	ns	(ppm)	26.4	27.2	ns
	Cu	(ppm)	-	-	-	(ppm)	7.0	8.5	ns
	Fe	(ppm	-	-	-	(ppm)	50	65	*
	Cd	(ppm)	<0.004	<0.004	ns	(ppm)	<0.004	<0.004	ns
	Pb	(ppm)	<0.004	<0.004	ns	(ppm)	<0.004	<0.004	ns
2009 February (after 6 years)	O. M.	(%)	0.74	1.30	***	-	-	-	-
	N	(ppm)	3.1	12.1	***	(%)	1.53	2.15	***
	P	(ppm	61.1	82.3	**	(%)	0.09	0.14	***
	K	(ppm)	215	493	***	(%)	0.89	1.42	***
	Na	(ppm)	180	315	***	(%)	0.06	0.12	***
	Cl	(ppm)	212	650	***	(%)	0.32	0.47	**
	Zn	(ppm)	26.3	43.9	*	(ppm)	14.3	21.5	**
	Mn	(ppm)	105.2	168.7	*	(ppm)	22.5	52.1	***
	Cu	(ppm	-	-	-	(ppm)	12.1	20.0	**
	Fe	(ppm)	-	-	-	(ppm)	65.5	112.0	***
	Cd	(ppm)	<0.004	<0.004	ns	(ppm)	<0.004	<0.004	ns
	Pb	(ppm)	<0.004	<0.004	ns	(ppm)	<0.004	<0.004	ns

Data represent mean values of all observations. Significance referred to the comparison between treatments (WW and TWW) within the same year. Differences between means: non-significant (ns), significant at $p \leq 0.05$, (*) at $p \leq 0.01$(**) and at $p \leq 0.001$ (***) according to LSD test.

In the low yield period (2003), olives production was 1469 kg ha^{-1} in TWW treatment compared with 2469.2 kg ha^{-1} in WW treatment ($p \leq 0.01$). In the high yield period (2006), olives production was again significantly higher for TWW with 2918 kg ha^{-1} compared to 1535.1 kg ha^{-1} of WW plot. The high yield obtained in TWW irrigated plot was probably a consequence of the presence of nutrient elements such as N, P and K, and the irrigation treatment worked as fertirrigation. After six years of study, the mean values of yields were respectively 1843. Kg ha^{-1} and 1002.2 Kg ha^{-1}, respectively on TWW and WW plots (Table 4). Based on these results, an efficient fertilization schedule, in particular with N, P and K, may provide a good tool to decrease biennial bearing. Although it is reported in other studies that saline water reduces yield (Ben Ahmed et al., 2009a; Bedbabis et al, 2009; 2010a; Ioannis, 2009), in the present study the fertilization effects seem to balance the saline effects resulting in a higher yield.

Table 4: Shoot growth (cm) and olive yields (kg ha^{-1}) in well water (WW) and treated wastewater (TWW) irrigated plots.

	Yearly shoot growth (cm)		Yearly yield importance (kg ha^{-1})	
	WW	**TWW**	**WW**	**TWW**
High value (Max)	38.1 ± 5.0	42.5 ± 7.9	1535.1	2918.5
Low value (Min)	10.7 ± 2.2	12.5 ± 2.6	269.2	368.8
Mean value (Six years)	25.4 ± 3.3	27.6 ± 4.1	1002.2	1843.4

4. Conclusion

Continuous application for six years of TWW irrigation increased soil fertility; with high significant improves in N, P, K and Fe soil concentrations. Results showed that TWW can be considered as a complementary source of fertilization. However, significant increases in E.C, Na and Cl levels might lead to soil salinization over the long term, and menace to causes soil degradation and plant phytotoxicity.

In conclusion, our data showed that irrigation with TWW caused a high significant improve of shoot growth and olive yield increasing. TWW worked as fertirrigation supplying N, P, K, and Fe in large amounts. Finally, the application of TWW caused an increase of MN and Zn in soil and leaves but within the usual range detected in plants.

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S7-C3

Automatic irrigation scheduling of olive orchard from sap flow records with the heat dissipation method. 1- Physiological validation

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Abstract

We evaluated the potential of sap flow values estimated from records with the heat dissipation method for automating irrigation scheduling in an olive orchard (*Olea europaea* L, cv. Meski) near Enfidha, Tunisia. Two drip irrigation treatments were imposed with an automatic irrigation controller, T1 (100% sap flow) and T2 (60%ET_o). Data on sap flow, predawn, midday and stem water potential, net photosynthesis, stomatal conductance, transpiration rate and chlorophyll fluorescence were recorded in representative trees of both treatments, during the 2008 irrigation season (April to August). Results showed that through the irrigation dose calculated from sap flow records we saved 30% of water delivered for irrigation. But, water potentials were decreased and noted that olive tree were not stressed especially stem water potential, leaf gas exchange parameters were affected significantly by about 15%. An automatic irrigation controller can be easily used in olive orchards to save water, extend irrigation areas with little decreased in plant physiology behaviour.

Keywords: Sap flow, Gas exchange, Automatic irrigation, Olive.

Planification d'irrigation automatique des vergers d'oliviers basée sur le flux de sève en utilisant la méthode de dissipation thermique dans un espace de climat méditerranéen

Résumé

Nous avons évalué le potentiel du flux de sève estimées à partir de la méthode de dissipation thermique pour programmer et automatiser l'irrigation dans un verger d'olive de table (Olea europaea L, cv. Meski) à l'Enfidha, Tunisie. Deux traitements d'irrigation par le système goutte à goutte ont été imposés avec un contrôleur automatique d'irrigation, T1 (100% de sève) et T2 (60%ETo).
Les données sur le flux de sève, le potentiel hydrique de base, midi et celui xylémique, la photosynthèse nette, la conductance stomatique, la transpiration et la fluorescence chlorophyllienne ont été enregistrés dans les arbres représentatifs des deux traitements, pendant la saison d'irrigation (avril à août). Les résultats ont prouvé que l'irrigation par la dose calculée à partir du flux de sève, nous avons économisé plus de 30% de l'eau délivrée pour l'irrigation. Mais, les potentiels hydriques foliaires particulièrement xylémiques ont été diminués notant que l'olivier n'était pas soumis à une contrainte hydrique. Les paramètres d'échange gazeux ont été affectés d'environ de 15%.
Un contrôleur automatique d'irrigation peut être facilement employé dans les vergers d'olivier en intensif pour gérer l'irrigation avec peu d'effet sur le comportement physiologique des plantes.

1. Introduction

Approaches for irrigation scheduling take into account physiological variables related to the water use by the tree (Nicolas et al., 2005 ; Tognetti et al., 2009). Among them, sap flow and trunk diameter variations are considered by many as two of the most promising plant-based indicators for the automatic control of irrigation in fruit tree orchards (Ortuno et al., 2005). Sap flow readings in branches, trunks and roots are being widely used to determine both water consumption and the dynamics of transpiration, as well as hydraulic conductance and water uptake by main roots (Fernández et al., 2001; Nicolás et al., 2005). Sap flow records have been reported as useful to determine actual olive water needs (Fernandez and Moreno, 1999 ; Fernandez et al., 2001) to the point that they have been used to develop an automatic irrigation controller, AIC (Fernández et al.,

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2008b). For the olive tree, Karray et al., (2006) studied the transpiration in agro-system of olive tree with the heat dissipation method, and horticultural species under rainfall condition. Recently, Rousseaux et al. (2009) have estimated olive water needs by the heat balance method developed by Sakuratuni et al. (1981). The heat dissipation method (Granier, 1987) has been used successfully to study transpiration and some physiological aspects of both forest and fruit trees (Sellami and Sifaoui, 2003).

The main objectives of this study were to evaluate the heat dissipation method for scheduling irrigation automatically in an intensive olive orchard under arid climate in Tunisia. Then to test and validate this method using some physiological behaviours of olive tree especially leaf water potential, photosynthesis, transpiration and stomatal conductance

2. Material and method

2.1. Site description and environmental measurements

This experiment was conducted at the irrigated olive orchards Enfidha, Tunisia (36°08'N, 10°22'E, 23 m). Our study site within the olive trees was a 27-year-old olive (*Olea europaea* L. cv. Meski) plantation, with approximately 7m x 7m trees spacing with a density of 204 trees per hectare. The soil was classified as sandy-loam. Soil water content at wilting point and field capacity were 11% and 26% respectively.

2.2. Irrigation management and experimental design

Water for irrigation was delivered daily using a localized irrigation system with two lines of nozzles at 1.0 m from the trunk each. Each tree was equipped by eight nozzles, with four nozzles per side of 8 L h^{-1} each (at 0.5 m and at 1.0 m from the trunk per side).

T1: Nineteen olive trees that received a daily irrigation amount of 100% of transpiration loses measured by sap flow meters ;
T2: Nineteen olive trees full irrigated that received a daily irrigation amount of 60% ET_0.

2.3. Sap flow measurements

The measurement of sap flow by the method of Granier (1985, 1987) is based on two cylindrical probes, 2 mm in diameter and 20 mm in length inserted radially in the xylem and spaced vertically by 8 cm. The upper probe is continuously heated, whereas the lower probe is unheated and the resulting temperature difference is measured with copper constantan thermocouples placed in each probe. Under zero flow conditions, the temperature around the heated probe increases to the point where heat dissipation by xylem conduction is in equilibrium with the heat energy supplied, which gives a maximum temperature difference. When sap is flowing, heat dissipation increases by convection and the temperature difference decreases.

2.4. Leaf water potential

During the irrigation season, leaf water potential was measured using a portable pressure chamber according to Scholander et al. (1965) following the recommendations of Turner (1981). The data were collected during sunny days at predawn and midday. Leaf and xylem water potentials were established according to Nicolas et al. (2005) by sampling a total of 9 fully sunlit and covered leaves of each treatment.

2.5. Photosynthesis, stomatal conductance and transpiration

Leaf CO_2 assimilation rates, stomatal conductance and transpiration was measured using a portable system IRGA (LI-COR, LI-6400). Data were collected other month from April to August. From each tree, three two-year old leaves from different vegetative shoots were measured. All measurements were carried out in the morning between 11:00 and 13:00 h on cloudless days. Photosynthetic photon flux density was fixed at 1500 µmol m^{-2} s^{-1}. External CO_2 concentration was fixed at 400 µmol m^{-2} s^{-1} and flow rate of air passing through the chamber was 200 µmol s^{-1}. Temperature and humidity rate through the chamber were 25 °C and 60 %. After the data were collected, the leaves were cut and leaf area was estimated with a digital camera system (LI-COR, LI-3200).

3. Statically analysis

Data were statistically analysed using the SPSS statistical software Version 16.0 (SPSS Inc., Chicago). Standard errors (S.E.) of the means were calculated. Duncan's multiple range test was used to compare the means.

4. Results

4.1. Climatic parameters

Maximum daily air humidity and minimum temperatures were 85% and 11℃ during the spring measurement period (i.e., April), while maximum daily temperature and minimum air humidity values were 33℃ and 30% respectively during the summer me asurements (i.e. August, Fig. 1a). Minimum and maximum solar radiation were 6 and 16 w m^{-2} at first April and mid-May, they were stabilized with high values during summer (i.g., July and August, Fig. 1b). Evapotranspiration showed two periods; during first April to first June was approx between 1.6 and 2.9 mm day^{-1}. During the second period (mid June to end August), ET_0 ranged between 2.3 and 3.7 mm day^{-1} (Fig. 1c). During the full irrigated season from April to August total evapotranspiration was 420 mm, with a daily mean value of ET_0 approx 2.8 mm day^{-1}.

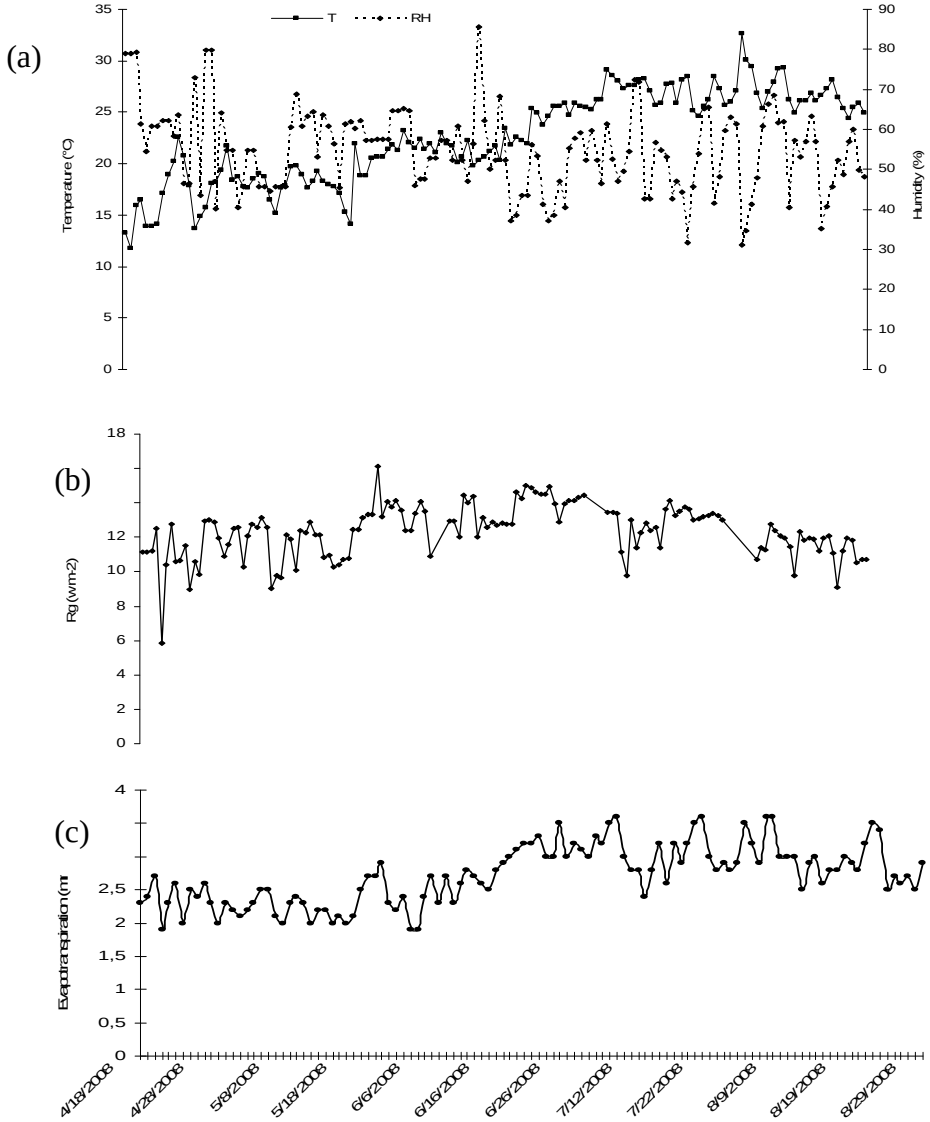

Figure 1: Daily climatic parameters' (a) temperature and air humidity, (b) global radiation, (c) evapotranspiration.

4.2. Irrigation doses and sap flow measurements

Irrigation dose in T2 presented similar values during first April until mid-June approx 490 L Tree Week^{-1}. They increased to reach a maximum value of 700 L Tree Week^{-1} at first August (Fig. 2a). Similarly with significant differences, irrigation dose from sap flow measurements increased from 180 L Tree Week^{-1} in April to reach a maximum level approx 400 L Tree Week^{-1} at August (Fig. 2a). During the full irrigated season from April to August total irrigation dose were 6300 and 11300 L Tree^{-1} in T1 and T2 respectively. The mean daily irrigation doses were 42 and 75 L Tree day^{-1} in T1 and T2 respectively. For the sap flow treatment, maximum soil evaporation measured by lysimetry method was approx 10 % of ET_0. Then total irrigation dose in T1 was about 7800 L Tree and the mean daily dose was 52 L Tree day^{-1}. Average weekly values of canopy transpiration estimated by sap flow were at their minimum during spring (April) and at their maximum during the warmest summer period

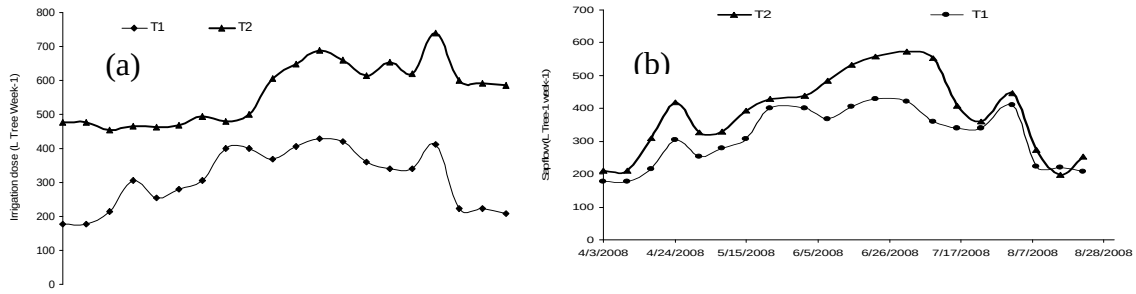

Figure 2: Weekly evolution of (a) irrigation dose and (b) sap flow of irrigation treatments T1 and T2.

4.3. Leaf water potential

Pre-dawn leaf water potential (Ψ_{pd}) increased progressively and continuously during the full irrigation season, reaching the highest values at first May about − 0.45 and − 0.35MPa in T1 and T2 respectively. Ψ_{pd} increased slightly to -0.6 and -0.4 MPa in T1 and T2 respectively with significant differences between the two treatments (Fig. 3a). In all treatments, xylem and leaf water potentials have similar seasonal pattern, high water potentials values were observed in full irrigated treatment T2 with significant differences (Fig. 3b and Fig .3c).

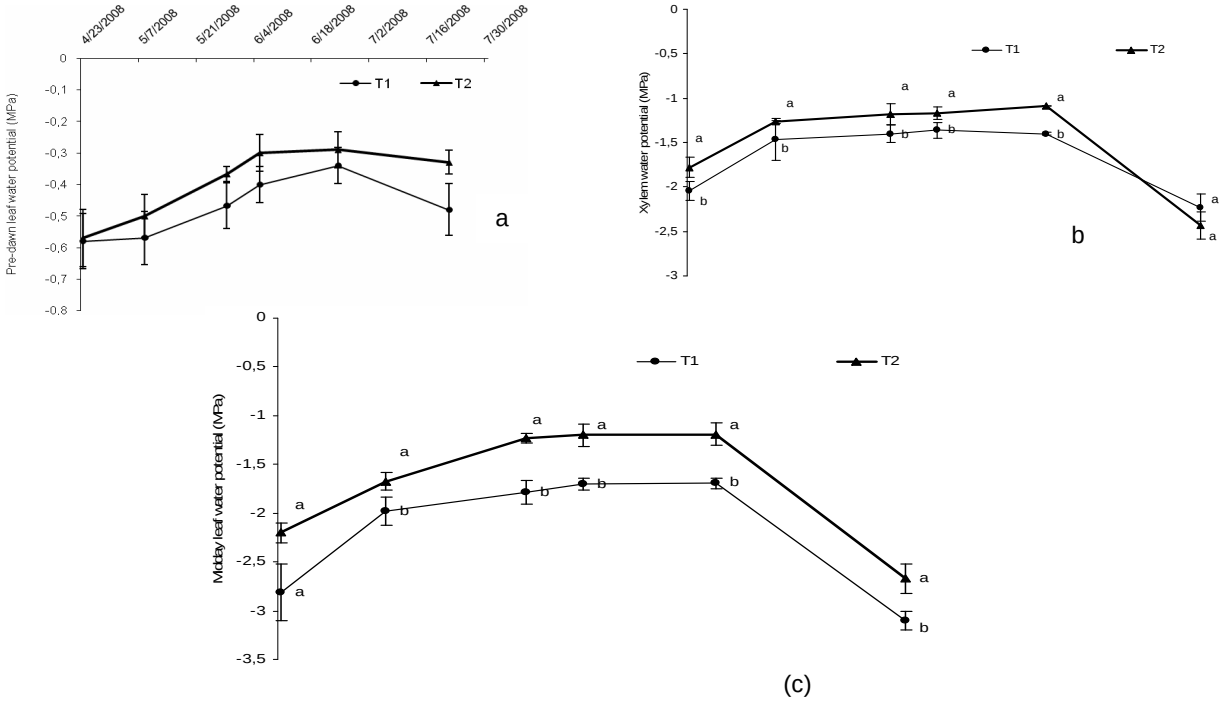

Figure 3: Seasonal pattern of leaf water potential (a) predawon leaf water potential, (b) xylem water potential, (c) midday leaf water potential of irrigation treatments T1 and T2.

4.4. Photosynthesis, stomatal conductance and transpiration

Seasonal patterns of photosynthesis rate measured during the full irrigated period were similar with high values observed in T2 (10-16.8 µmol m^{-2} s^{-1}) then in T1 (10-14µmol m^{-2} s^{-1}). CO_2 assimilation rate were lower in April, increased by about 60% in May and reached a maximum in June and then increased in August (Fig. 4a). In all photosynthesis measurements full irrigated treatment T2 had significantly higher CO_2 assimilation rates throughout the season by about 15%. Stomatal conductance followed similar trends with CO_2 assimilation rates (Fig. 4b). The gs increased from April to the end of June, then decreased during August. The maximum gs values were 0.24 and 0.17µmol m^{-2} s^{-1} in T2 and T1 respectively. Similarly to photosynthesis and stomatal conductance patterns, transpiration rate (E) increased from April to June, and then decreased in August. The maximum E values were 7.3 and 5.3 mmol m^{-2} s^{-1} in T2 and T1 respectively (Fig. 4c).

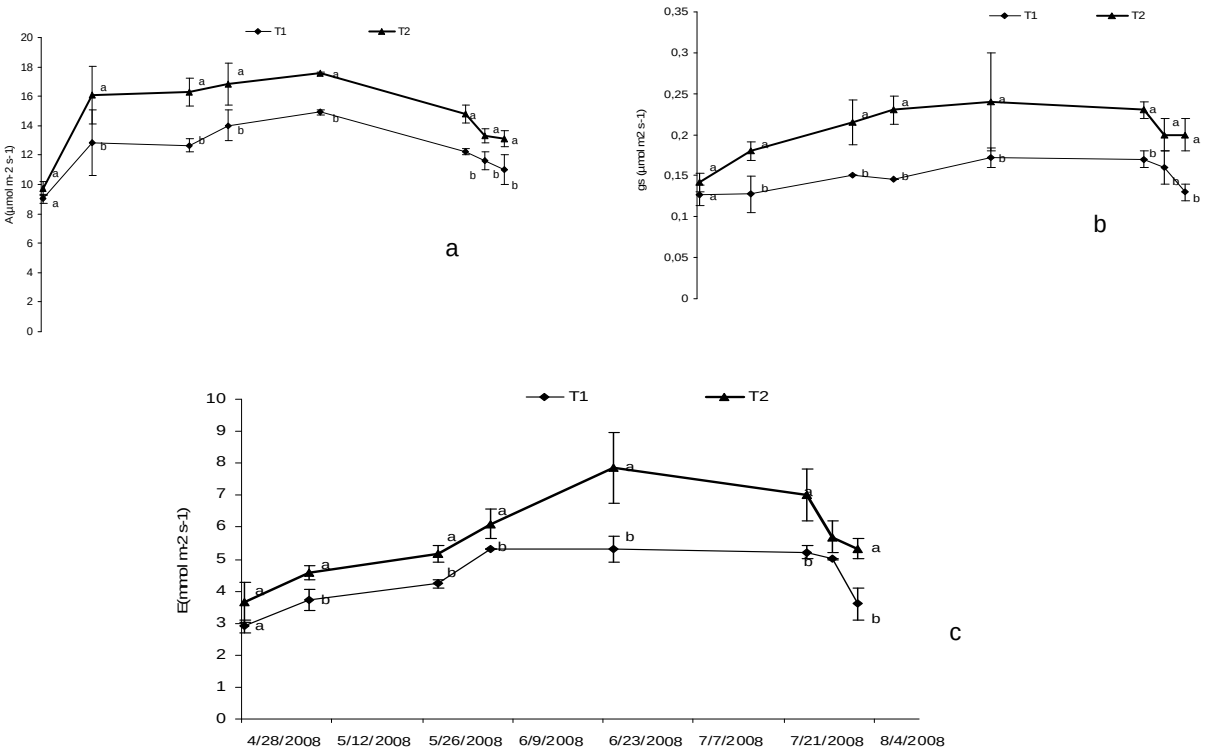

Figure 4: Seasonal pattern of leaf gas exchange (a) Photosynthetesis, (b) Stomatal conductance (c) Transpiration of irrigation treatments T1 and T2.

5. Discussion

We introduce here and testing physiologically the heat dissipation of sap flow technique as a tool for an automatic irrigation control (AIC) and to calculate the irrigation dose (ID) of olive orchards. Compared to the potential irrigation dose estimated by 60%ET_0, we can increase by the sap flow technique the irrigation dose by 30% with high agreement with those published by Fernandez et al. (2008b). The reduction of water delivered for irrigation affected soil water content and acted as a drought stress on olive tree physiological behaviour. Similarly Fernandez et al. (2008b) show that the relative extractable water under AIC by HPV decreased by 20 to 55% during the full irrigated season. Consequently, stem water potential decreased markedly in olive trees irrigated by AIC, indicating that the soil water content was too low to prevent a significant increase in the trees water stress. Our results that predawn, leaf and xylem water potentials decreased significantly under AIC irrigated olive tree. But levels of water potentials showed that theses olive trees were not stressed during the irrigation season. Mature olive trees irrigated with 100% ETc showed higher ψ_{pd} values than all other water treatments together (66 and 33% ETc). However, minimum values of ψ_{pd} never exceeded -1.5 and hardly reached -2 MPa, regardless the water treatment (Moriana et al., 2007 ; Tognetti et al., 2009). During 90 days of experimenting, olive trees irrigated daily near to the field capacity stem water potential reached between - 0.9 and - 1.5 MPa (Lopez et al., 2007 and 2008). The decrease of

irrigation dose and leaf water potential affected directly and significantly photosynthesis, stomatal conductance and transpiration rate during the irrigation season (Ben Ahmed et al., 2008). Hagidimitriou and Pontikis (2005) reported that the high leaf CO_2 assimilation rate value observed during spring were probably due to favourable air temperatures and air humidity. The decrease in A_n in August is probably due to high temperature and low air humidity. Our results showed that under AIC, photosynthesis decreased only by 15% compared to the fully irrigated olive trees by 60%ET_0 with water saving by about 30%. The water use efficiency estimated in our experiment by photosynthesis and transpiration rate demonstrated the ability to make heat dissipation method to scheduling irrigation on olive orchards. It's very clear that transpiration rate was closed correlated to sap flow (R^2 = 0.96), whereas stomatal conductance and photosynthesis were lower correlated (R^2 = 0.75 and 0.66) (Fig. 6) because of the high active regulation of these two parameters under environmental stress especially water and saline stress (Centritto et al., 2005 ; Fernandez et al., 2006; Tognetti et al., 2009). Compared to irrigation scheduling methods based on the atmospheric demand, such as the crop coefficient method (Allen et al., 1998), plant-based measurements could increase the resolution of the calculated ID, which is certainly an advantage for precise high-frequency irrigation.

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S7-C4

Estimation of the impact of cultivation practices on desertification risk at olive growing areas in western Crete, Greece

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Abstract

Land degradation in Mediterranean countries due to topsoil loss can be a significant problem in hilly cultivated land when inappropriate agricultural practices are applied. The predicted increase of extreme meteorological events due to climate change is expected to further accentuate the desertification risk in these areas. In the present work a GIS modeling approach was used for estimating desertification risk, based on specific desertification indicators for olive orchard environment. The selected study area at Kissamos, Crete, Greece, was a hilly landscape with 90% of the land covered with olive orchards. Under the current cultivation management practices 29% of the area is under low to moderate desertification risk. In the worst case scenario, when improper tillage, reduced maintenance of the infrastructure (terraces) and reduced parceling of land were introduced, the area under desertification risk increased to 50%, indicating that good agricultural practices should be followed in the future in order to avoid land degradation and desertification in a significant part of the study area.

Keywords: Desertification, Olive groves, Good agricultural practices, GIS.

Estimation de l'impact des pratiques culturales sur le risque de désertification à la culture de l'olivier en zones de l'ouest de Crète, Grèce

Résumé

La dégradation des terres dans les pays méditerranéens en raison de perte de terre arable peut être un problème important dans les collines où les terres cultivées quand des pratiques agricoles inadaptées sont appliquées. L'augmentation prévue de phénomènes météorologiques extrêmes en raison des changements climatiques devrait accentuer encore le risque de désertification de ces régions. Une approche de modélisation des GIS a été appliquée pour estimer le risque de désertification en utilisant des indicateurs, spécifiques, de la désertification pour un oliveraie environnement. La zone d'étude, qui a été choisie, se trouve à Kissamos, Crète, Grèce, est un paysage vallonné avec 90% des terres couvertes des oliveraies. Dans les conditions courants de gestion, 29% des terres cultivables sont sous faible jusqu'à modérée risque de désertification. Dans le pire des cas quand inconvenant travail du sol, maintenance réduite de l'infrastructure actuelle (terrasses) et réduit morcellement des terres ont été introduits, la zone sous risque de désertification a augmentée a 50%, ce qui indique que les bonnes pratiques agricoles doivent être suivies à l'avenir afin d'éviter la dégradation des terres et la désertification dans une partie importante de la zone d'étude

Mots-clés: Désertification, Olivier, Bonnes pratiques agricoles, GIS.

1. Introduction

Olive farms in the EU countries range from very small (<0.5ha) to very large (>500ha) and from traditional, low-intensity groves to intensive, highly mechanized plantations. Olive groves in Greece occupy an area of about 786,700 hectares with about 154 million trees cultivated by about 686,000 families. Olive oil production in Greece represents about 23% of the European Union production. In the island of Crete 65% of agricultural land (318.394 ha) is covered by olive plantations (NSSG, 2006). Olive groves are found in areas ranging from flat to very steep (slope >35%). In relatively flat areas, intensive cultivation is practiced with dense planting, cultivation of soil and irrigation. On the other hand, the orchard management in hilly areas is closer to the traditional olive cultivation practices, with lower density, no soil cultivation and use of terraces in areas with higher slopes.

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The olive (*Olea europaea* L.) tree is extremely long-lived (up to 1000 years), tolerant to drought and moderately tolerant to salinity. In olive groves located in hilly areas, soil erosion depends on cultural practices, soil type and climatic conditions. The diversity of farming and farm structures in olive groves in Greece leads to many different ways of interaction with the environment. At the same time the relationship between agriculture and the environment can be positive, as well as negative, depending on the adopted agricultural practices.

Soil erosion is probably the most serious environmental problem associated with olive farming. Inappropriate weed-control and soil-management practices, combined with the inherently high risk of erosion in many olive farming areas, is leading to desertification on a wide scale in some of the main producing regions, as well as considerable run-off of soils and agro-chemicals into water bodies.

Desertification can be analyzed at different spatial scales, ranging from local to national and international levels. It is important to know which indicators are appropriate at different scales, and which of them can be obtained directly and in a cost-effective way at the desired scale (Landres, 1992). Some indicators can be locally analyzed and must be determined over smaller space units, even when they can be aggregated at a national level. These indicators require a precise definition of the areas in which they are meaningful, and may not be applicable at larger mapping scales (Kosmas et al, 2000). Local indicators derive from data collected in areas, which must be selected as representative of larger areas. Several indicators have been proposed for determining the desertification risk. Some of them are the most significant in estimating desertification risk in olive groves on the Greek islands. In the present study, selected indicators have been used for analysis at farm level and were combined with GIS for analysis at landscape level. The desertification risk in olive orchards in selected areas of Crete, Greece was estimated for different regimes of cultural practices and different levels of policy enforcement, based on the DIS4ME model (DESERTLINKS, 2004).

2. Materials and Methods

The selected study area was 22.5 km^2, located in the west part of Chania Prefecture and included parts of Kissamos and Mythimna municipalities. The landscape in the area is hilly, in general, with existing plains only close to the coast. The terrain is characterized by moderate to steep slopes, which have led to the creation of an expanded drainage network. The main parent material of the soils in the study area is marl. The mean annual rainfall usually ranges from 650 to 750 mm. The climate is typical Mediterranean with a 5–month-long raining season and practically dry during the growing season.
The economy of the area is based on agriculture and tourism. The most important agricultural activity is olive growing. More than 90% of the area is covered by olive groves (Fig.1). The dominant olive cultivar grown in the area is “Koroneiki” for oil production.

The DIS4ME model was used, for estimating the status of land degradation and the desertification risk. Many indicators have been proposed for determining the desertification risk, some of them are the most significant in estimating desertification risk under olives on the Greek islands (Kosmas et al., 1999).These indicators are mainly related to the specific local characteristics at farm level such as soil depth, soil texture, drainage, slope gradient slope exposure, rainfall, present and previous types of land use, period of existing type of land use, application of fertilizers and pesticides, tillage operations, tillage depth and direction, sustainable farming, soil erosion control measures etc.

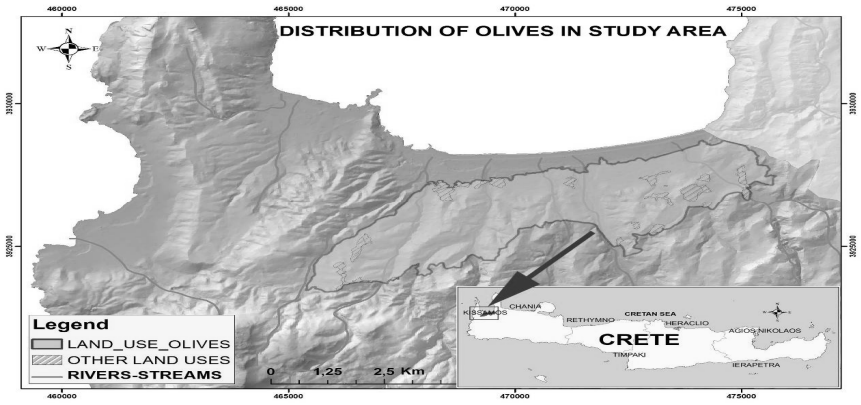

Figure 1: More than 90% of the study area is covered by olive groves.

Based on existing classification systems such as the geo-referenced database, classes have been defined for each indicator and presented in a tabulated form, where numbers have been assigned for each class according to its importance on desertification (Stocking and Murnaghan, 2001). The main components of the model were related to the following factors: climate, soil, topography and farm management (Fig. 2).

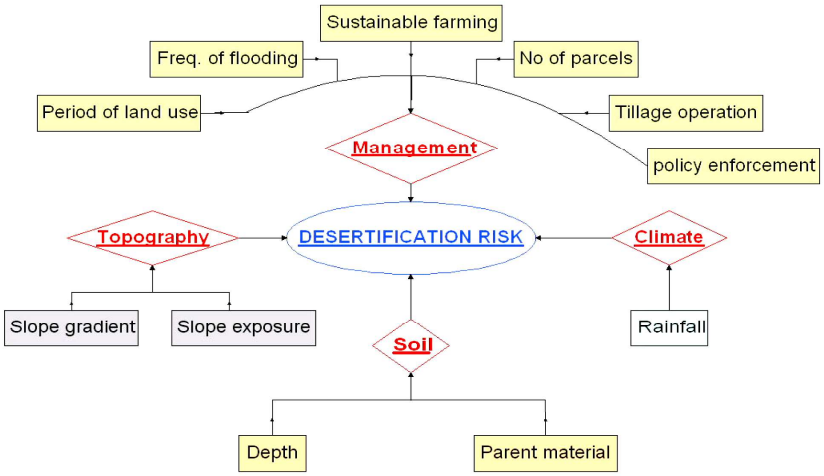

Figure 2: Factors of the model .

About 47% of the study area represent steep slopes (>18%) (Table 1). In such areas the main process of land degradation is soil erosion.

The algorithm used for estimating Desertification Risk (DR) for olive cultivated areas was:

DR = (4.32) - (0.68*number of parcels) + (1.57*tillage operations) - (0.68*period of existing land use) - (0.56*soil depth) + (0.44*slope gradient) + (0.19*parent material) - (0.79*rainfall) + (0.67*aspect) + (0.65*frequency of flooding) + (0.69*sustainable farming) + (0.44*policy enforcement)

Since all the above indicators have spatial – temporal distribution, a grid based model was used in combination with GIS to model the desertification risk at landscape level (Dunio Denti, 2004). For this purpose satellite images interpretation, Digital Terrain Model (DTM) and field-survey techniques were used to recognize these indicators along the study area.

The incorporation of a modeling component to a GIS was required for the prediction of potential outcomes and evaluating alternatives scenarios. The GIS model was created by integrating the mathematical equations of these processes in the ArcGIS software (ESRI, California), which combines GIS tools and spatial modeling. The run of the GIS was individually performed on a one-cell basis (30X30 m) per each indicator in the study site.

Table 1: Slope categories of olive orchards in the study area.

Slope Category	Area (Km^2)	Area %
<6%	3.54	17.3
6-18%	7.32	35.8
18-35%	7.05	34.5
>35%	2.53	12.4
Total	20.44	100.0

The cartography units were identified by interpretation of satellite images in combination with the contours of the area. In an extended olive covered area, like the above described, in which each farm is next to the other, there are a lot of indicators such farm management practices, parent material age of trees and other indicators which could be grouped and analyzed in landscape level. So, we identified cartography units with some common indicators. With the use of DTM the data of slope and aspect of the study area were obtained. A grid with the rainfall pattern in the area was build, using 10-

year-long historical data from 5 meteorological stations within and around the study area. By field survey, the cartography units were validated or modified, and all the other indicators of the model were recorded, giving emphasis on soil depth, number of parcels, tree age, and management practices. All the required information were investigated and integrated to the GIS by field survey with a GPS connected to a laptop. During the field survey, topography, a hydogeological map and the interpretation of satellite images of the study area were taken into account in order to recognize areas with same values of the indicators. Farmer interviews were used to find out or validate indicators like number of parcels and sustainable farming.

The use of the GIS model provides the ability to investigate easily different scenarios, involving single indicator changes or combinations of several indicator changes. During the present study, apart from the current situation analysis (scenario A: actual case), a hypothetical future situation was also analyzed, including the adoption by farmers of intensive cultivation practices (tillage, etc), the lack of policy enforcement in the area and the reduction of land parceling by public intervention (scenario B: worst case scenario). Therefore, the second scenario involved negative, but possible changes in indicators that could be altered in the future, and might have the most negative effects in increasing the desertification risk in the study area.

3. Results and discussion

By testing the two cases it turned out that there is no high risk of desertification for none of them despite the fact that the area was hilly (Table 2). This is probably due to some inherent characteristics of olive growing in the area, including soil depth, no frequent changes in land use, and the limited opportunities for large deviations from good agricultural practices in the future, since the slope of the area does not allow the extensive adoption of practices like, for example, soil cultivation. The analysis of data for scenario A showed that although the area is mainly hilly the desertification risk is low as a result of land management policies (Table 2).

The incorporated changes related to the farming system in scenario B, significantly affected the desertification risk. Therefore, the no risk percentage of 70.9% for scenario A, was reduced to 50.5% in scenario B, thus increasing about 20% the land area that could be subjected to low or moderate desertification risk (Fig. 4).

Table 2: Summarized results for desertification risk under different farming conditions.

Desertification risk	A: Actual case (%)	B: Worst case (%)
No risk	70,9	50,5
Low risk	23,8	34,2
Moderate risk	5,3	15,3
Total	100	100

The current use of retaining walls (terraces) contributes significantly in limiting soil erosion - land degradation. The present practice of using herbicides instead of cultivating the orchards is another factor that protects soils with extreme gradients. Finally, the existence of small fields in the area, contributes in minimizing the desertification risk. However, although high desertification risk is not evident in the area even under the worst case, about 50% of the area can be subjected to low or moderate risk, if current management practices and policy enforcement are not the appropriate ones in the future. This indicates the importance of retaining or improving the parameters that could be altered by human activities in the study area.

Apart from the above studied cases, a wide range of alternative scenarios may be developed with the GIS model, based on policies of particular relevance to the region, providing a useful tool for evaluating the potential desertification consequences of these policies. As a conclusion, based on the two cases tested through the model, the importance of the adoption of good agricultural practices was evident for protecting from land degradation and reducing the desertification risk.

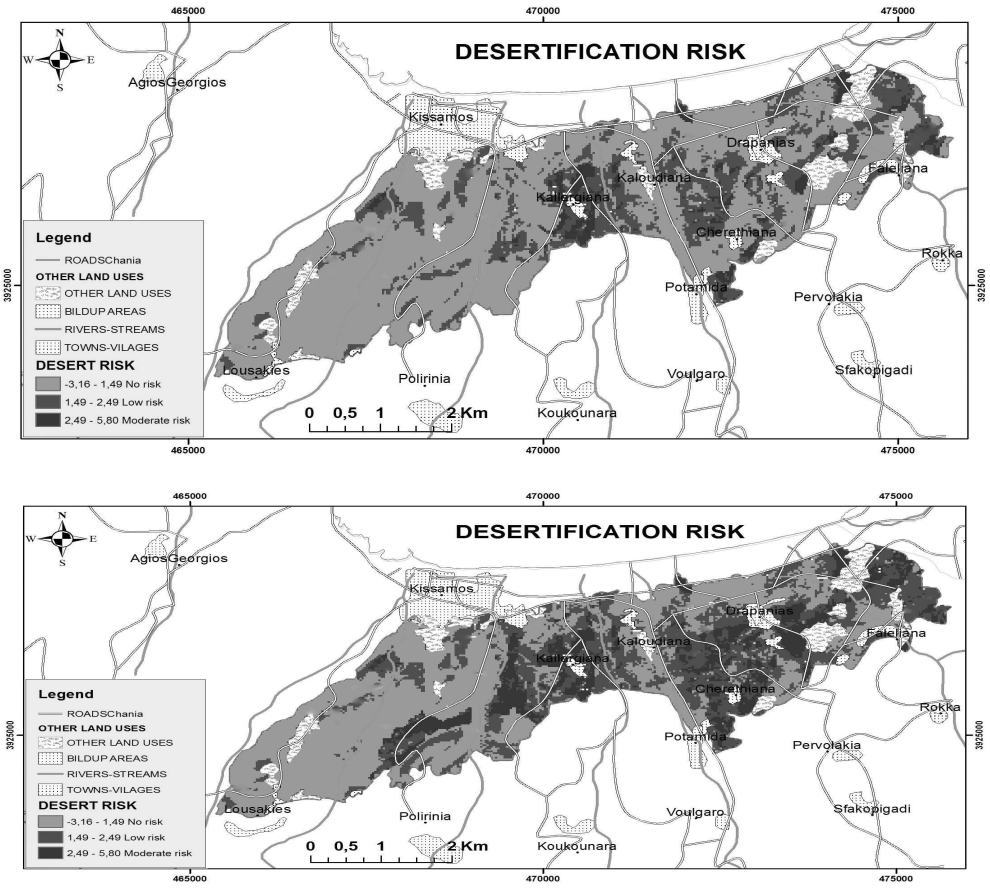

Figure 4: The distribution of Desertification Risk (DRI) in the study area under scenarios A and B.

Acknowledgments

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S8-C1

Saline water irrigation effects on oil composition and quality of field grown olive cv. Chemlali

Ben Ahmed Chedlia B. Ben Rouina, F. Ben Abdallah & M. Boukhris

Abstract

Traditionally, olive was cultivated under rain-fed conditions. In recent decades, the olive plantation has been extended to irrigated lands. However, in arid and semi arid regions as those in Tunisia, the limited water availability and the increased need for good water quality for urban and industrial sector use have led to the use of large quantities of marginal water, as saline water, for olive tree irrigation. Fruit development characteristics, yield and oil quality were investigated during two successive crop seasons (2004 / 2006) on fourteen - year - old -Chemlali Sfax olive trees subjected to the following treatments: FW and SS corresponding, respectively to the irrigation with fresh water (EC = 1.2 dS m^{-1}) and saline water (EC = 7.5 dS m^{-1}). The irrigation with saline water has led to the decrease of fruit weight, olive yield and oil content. The mean olive yield was of 42.5 and 27 Kg /tree /year, respectively in FW and SS treated plants with a relative reduction of 36% in salt stressed plants. The oil content was of 27.85 and 25.7 % Fw during 2005/2006 crop season in FW and SS irrigated plants, respectively. However, major phenolic compounds (tyrosol, hydroxytyrosol, vanillic, oleuropein, syringic, o-coumaric) and total phenols have increased under saline water irrigation regime. During 2005/2006 crop season, total phenol content was of 198 and 223 mg /Kg in FW and SS treatments, respectively with an increment of 11% under greater salinity conditions. Furthermore, SS treated plants showed higher contents of oleic, linoleic, linolenic and heptadecanoic acids than FW ones. The oil samples of both treatments were classified as “extra virgin”. Despite the fact of the decrease of fruit weight and oil content in SS plants, the improvement of their phenolic compounds and total phenols contents assumes that the use of such water quality for olive cultivation could be enhanced, at least in the case of the Chemlali cultivar grown under described experimental conditions.

Key words: Irrigation, oil quality, phenolic compounds, saline water, olive yield.

Effets de l’irrigation à l’eau saline sur la composition chimique et la qualité de l’huile de l’olivier Cv. Chemlali de plein champ

Résumé

D’une culture traditionnellement pluviale, l’oléiculture irriguée connaît ces dernières décennies une extension notoire, malgré l’insuffisance des quantités d’eaux de qualité, sous les climats semi aride et aride de la Tunisie. Cette extension des oliveraies irriguées est basée essentiellement sur l’utilisation des ressources non conventionnelles d’eaux telles que celles salines. Au cours de cette étude réalisée durant deux saisons (2004 / 2006), le développement des fruits, la production d’olives et la qualité de l’huile produite sont examinés. Deux qualités d’eau sont utilisées pour l’irrigation de l’olivier Chemlali, âgés de 14 ans : FW désigne la parcelle irriguée à l’eau douce (C. E = 1,2 dS / m) ; alors que SS correspond à celle irriguée à l’eau saline (C. E = 7,5 dS / m). L’irrigation à l’eau saline a induit une régression du poids moyen d’une olive, de la charge totale de l’arbre en fruits et de la teneur en huile. Les productions moyennes d’olives ont enregistré une régression de 36 % pour passer de 42,5 Kg / arbre / an, chez le traitement FW à seulement 27 Kg / arbre / an chez celui SS. Pour ces deux traitements, le contenu des fruits en huile a respectivement baissé de d’une moyenne de 27,85 % à 25,7 %. Cependant, la concentration des composés phénoliques majeurs (tyrosol, hydroxytyrosol, acide vanillique, euleropeine, acide syringique et o-coumarique) ainsi que celle des phénols totaux se trouvent améliorées par l’irrigation à l’eau saline. Durant la saison 2005/2006, la concentration des phénols totaux dans l’huile de la parcelle SS s’est accrue de 11 % ; puisque égale à 223 mg / Kg en comparaison à 198 mg / Kg chez le témoin (FW). De surcroît, l’huile produite par les arbres SS présente des teneurs en acides oléique, linoléique et heptadécanoïque plus élevées en comparaison à celle des arbres FW. L’huile produite par les arbres des deux traitements est classée dans la catégorie « extra vierge ».

Mots clés: irrigation, qualité d’huile, composés phénoliques, eau saline, production d’olives.

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1. Introduction

Traditionally, olive tree was cultivated under rain-fed conditions. In recent decades, the olive plantation has been extended to irrigated lands. However, in arid and semi arid regions as those in Tunisia, the limited water availability and the increased need for good water quality for urban use restrict the use of fresh water for irrigation. So, large quantities of marginal water, as saline water, are used for olive tree irrigation (33% of irrigated lands are saline water irrigated (16000 ha)).

Most papers dealing with the assessment of olive water needs reported that olive tree is characterized by its limited water requirements and its tolerance to salinity [1, 2, 3]. Existing data on the effects of salinity conditions on yield, quality and phenolic composition of virgin olive oil are few and sometimes contradictory. In 1994, [4] have signalled that salinity alters pollen germination and fruit set, and that it causes the increase of both aliphatic and triterpenic alcohol contents in oil and the percentage of linoleic acid. According to [5], the olive irrigation with saline water (ECe = 6.5 dSm^{-1}) increased the fruit dry weight and oil percentage. The same authors stated that no effects were recorded when irrigation was made with water less than 4.5 dSm^{-1}. Similarly, [6] did not record any effect of irrigation with brackish water (up to 4g/l of solid residue) on yield and oil percentage of some olive varieties grown intensively in the central part of Tunisia.

Besides, it has been shown that olive yield response to salinity is planting density dependent [8]. The effects of salt treatment at ECe of 4.5 dSm^{-1} were a 12% increase in oil yield for the 830 trees/ha planting density, and an 18% decrease for the 410 trees/ha one. However, at high salinity (ECe = 7.5 dSm^{-1}), the decrease of oil yield was at 89 and 74% of the control in the high and low planting density, respectively. Concerning the oil quality, many studies [8, 9, 10, 11] showed that olive oil produced under high water salinity level has higher amounts of total phenols. In addition, the ratio of unsaturated/saturated fatty acids decreased significantly at moderate and high salinity levels.

Nowadays, the controlled use of marginal water (saline water, treated waste water) to improve the qualitative characteristics of horticultural products is becoming more and more important [8, 9, 10, 11], particularly under actual conditions of limited water resources and rainfall scarcity in arid regions.

The objectives of this study were to determine the effects of saline water used for irrigation on fruit development and the quantitative and qualitative parameters of VOO obtained from trees of the cv. Chemlali grown at a high density orchard. In particular, we are interested in the fatty acid composition and concentrations of phenols of VOO and the evolution of soil moisture and salinity with soil depth and around the irrigation source. Our experimental approach allows us to improve the understanding of the qualitative response (oil quality) of field-grown Chemlali olive tree to saline water irrigation in arid region in Tunisia.

2. Materials and Methods

2. 1. Plant Material and Treatments

Olive trees (*Olea europaea* L. cv. Chemlali), planted in 1992 in a sandy soil at a density of 625 trees ha^{-1} (4 x 4 m layout) at Sfax, Tunisia (34°43N, 10°41E) , were used in 2004 and 2005. The sandy soil of the experimental orchard (90.5% sand, 4.5% clay and 5% silt) was characterized by an organic matter of 1.1%, 13.4% $CaCO_3$, 1.3% N, pH of 7.6, a field capacity (measured at 33 KPa) of 11.8% and a wilting point (measured at 1500 KPa) of 5.9%.

In 2004, twenty trees with four replications of 5 trees each were selected to be similar in potential yield and canopy. The Chemlali olive trees were subjected to the following treatments: irrigation with fresh water, 1.2 dS m^{-1} ECe (FW); and saline water, 7.5 dS m^{-1} ECe (salt stress, SS). The water used was either that supplied by the Tunisian National Water Carrier (FW), or saline water (SS) from the local reservoir situated in the area of the Olive Tree Institute in Sfax. The fresh and saline water used with amount of 4000 m^3 / ha / year were characterized by 145 and 600 mg/l Na^+, 326 and 1169mg/l Cl^-, 280 and 520 mg/l K^+, 94 and 261 mg/l Ca^{2+}, 57 and 102 mg/l Mg^{2+}, respectively [1]. The irrigation was delivered using a drip system with 4 drip nozzles (two per side), of 4 l h^{-1} per tree set in a line along the rows (at 0.5 m from the trunk). The plants were subjected to the same olive cultivation practices in the area.

2.2. Fruit Growth Characteristics and Yield

Control of fruit weight (FrW), fruit diameter (FD), fruit volume (FV) and fruit water content (FWC) of harvested olives were made, during both crop seasons, four times per month from June to December. In every measurement, 60 olives from four plants per treatment (15 olives per plant) were collected for characterization (fresh weight, water content, fruit diameter and volume). For the oil analyses, three samples of 4 Kg of fruits each were harvested for each treatment at maturation. For olive yield determination, 10 trees per treatment were chosen and the harvest was made in mid-December of each year manually to guarantee accuracy.

2.3. Oil Quality Indices

Extinction coefficients K_{232} and K_{270} were measured at 232 and 270 nm, respectively. Free acidity and peroxide value expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2 / kg) were measured following the analytical method described in the European Regulation EEC 2568/91 [12]. Fatty acid composition was determined based on the European Regulations EEC 2586/91 method. The chlorophyll fraction at 670 nm and the carotenoid fraction at 470 nm were evaluated from the absorption spectrum of each virgin olive oil sample (7.5 g) dissolved in cyclohexane. Oxidative stability is evaluated using a 679 Rancimat apparatus (Metrohm, Switzerland) at 120 ℃ and 20 lh^{-1} air flow. The concentration of total polyphenols was estimated with Folin-Ciocalteu reagent at 725 nm. The different phenolic compounds analysed were determined by HPLC system. The standards used in the quantification of phenolic substances are: Tyrosol, hydroxytyrosol, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, o-coumaric acid, oleuropein, glycoside oleuropein and ferulic acid.

2. 4. Statistical Analysis

Statistical analyses were performed using the SPSS 10. Windows and treatment means were compared using least significant difference (LSD) test at $p < 0.05$. At least three replicates were used for each laboratory test.

3. Results and discussions

3.1. Soil Salinity

The soil salinity variation was greater in soil irrigated with saline water than in that irrigated with fresh water. For both treatments, there is a slight decrease in ECe through the soil depth. Furthermore, the higher salt accumulation was registered at soil layer of 0-0.3 m. In all soil depths, the salts were more accumulated during summer season (July) and salt distribution through the soil depth was affected by autumn-winter rainfall leaching (December). The seasonal variation of soil salinity in the 1.2 m depth showed that it was lower during autumn-spring period than that of the summer one (Table 1).

Table 1: Distribution of soil salinity (E. C dS m^{-1}) measured at 0.6 m from nozzle at Different Layers in FW and SS treatments during 2004 and 2005 crop Seasons.

Treatmt / Period	FW				SS			
	H1	**H2**	**H3**	**H4**	**H1**	**H2**	**H3**	**H4**
	Soil salinity (dS m^{-1})							
July 05	1.9	1.75	1.52	1.4	7.7	6.9	6.5	6.1
December05	1.30	1.25	1.23	1.10	6.7	6.0	5.8	5.5

H1, H2, H3 and H4 represent the different soil depths from the surface (30, 60, 90 and 120 cm, respectively). Values are the means of three soil samples measurements (n = 3).

3.2. Fruit Characteristics and Yield

During both crop seasons, salinity has altered fruit diameter (FD), fruit volume (FV) and fruit weight (FrW). For both salt treatments, FrW averages increased markedly with time. In June 2004, their values were of 0.38 and 0.28 g in FW and SS treated plants, respectively; and they reached 1.33 and 1.05 g, respectively in December. In 2005, these values were of 0.44 and 0.36 g in FW and SS treated plants and reached 1.38 and 1.22 g, respectively for the respective periods. These results showed that the increase was more important under irrigation with fresh water. Furthermore, the fruit fresh weight in SS plants was statistically lower than that in FW ones ($p = 0.0056$). In 2004, FW plants showed higher fruit water content values than the SS ones (53.39 and 50.88% in FW and SS, respectively).

However, in 2005, the two irrigated treatments showed almost similar values (51.43 and 52.27%, respectively) for which differences were not statistically significant. The non significant differences in FWC values between the two crop seasons for both treatments revealed the role of the active root zone of Chemlali olive in the upholding of a suitable hydration level for its tissues.

Table 2. Olive Yield (Kg tree^{-1}) of olive trees (cv. Chemlali) grown under FW and SS irrigation.

treatment	FW	SS	Relative reduction in SS (%)
2004	38±2.9$^{a, w}$	22±2.2$^{b, w}$	-42,1
2005	14±2.5$^{a, x}$	9±2.8$^{b, x}$	-35
Mean	27^{a}	15.5^{b}	-42.6

Values represent the means of ten samples ± standard deviations. Different letters (a, b) indicate significant differences ($P < 0.05$) between saline water irrigation treatments within each year. Different letters (w, x) indicate significant differences ($P < 0.05$) between crop seasons within each treatment.

The average olive production of SS plants during the experimental period (15.5 kg tree^{-1}) was much lower (42%) than that of FW ones (27 kg tree^{-1}). For both treatments, the first crop season was marked by higher olive yield (Table 2). The yield variation in both treatments could be due to the alternate bearing phenomenon characterizing the olive tree production and/or to the effects of climatic conditions characterizing the experimental site.

3.3. Oil Quality Indices

During both crop seasons, the irrigation treatments did not affect the oil accumulation in the Chemlali olive tree as no statistically significant differences were observed between total oil content of the two treatments ($p > 0.05$). It was of 27.8 and 30.5 % Fw in FW and of 25.7 and 28.3 % Fw in SS during 2004 and 2005 crop seasons, respectively (Table 3).

Table 3: Total oil content, Free Acidity, Peroxide Value, Extinctions Coefficients of total chlorophyll and carotenoids contents of Virgin Olive Oils from Olive Trees (Cv. Chemlali) grown under FW and SS Irrigation.

Treatment	Total oil content (% Fw)	Free acidity (%)	Peroxide value (meq O_2 / kg)	K_{232}	K_{270}	Total chlorophyll (mg / kg)	Carotenoids (mg / kg)
2004							
FW	27.85±2.6	0.25±0.02	3.2±0.31	1.06±0.02	0.05±0.01	9.5±0.07	0.38±0.07
SS	25.7±2.05	0.24±0.02	2.9±0.12	1.05±0.04	0.05±0.02	9.2±0.09	0.41±0.04
2005							
FW	30.56±3.0	0.34±0.04	4.6±0.34	1.62±0.06	0.11±0.02	10.19±0.05	0.42±0.06
SS	28.32±2.2	0.32±0.05	4.4±0.28	1.76±0.06	0.16±0.018	10.22±0.04	0.44±0.075

Values are the means of three different VOO samples (n = 3) ± standard deviations.

In contrast with previous reports [5, 7, 8, 9, 10, 11], the high saline treatment SS we applied to Chemlali olive tended to decrease oil content relative to FW treated plants (though not significantly so). Furthermore, the non statistically significant differences in oil content between both saline water treatments, in comparison to olive yield, is certainly an advantage for the use of saline water for Chemlali olive cultivation, being extended to saline irrigated lands in arid regions in Tunisia. Chlorophyll concentrations in the virgin oils ranged from 9.5 to 10.2 and from 9.2 to 10.2 mg / kg in FW and SS, respectively (Table 3). Differences between the treatments were not significant ($p > 0.05$) in either season. As well for the chlorophylls contents, during both crop seasons, the carotenoid contents were not influenced by saline irrigation regimes.

During 2004 crop season, the free acidity ranging from 0.24 to 0.25 % and peroxide value, ranging from 2.9 to 3.2 meq O_2 kg^{-1} of the different olive oils samples, respectively in SS and FW, were considerably lower than the upper limit of 0.8 % as oleic acid and 20 meq O_2 kg^{-1} as the peroxide

value established by the EU legislation for extra virgin olive oil. Moreover, these two quality indices were not influenced by water quality treatment, since no statistically significant differences were observed between both treatments ($p > 0.05$). In the second crop season, the free acidity and peroxide value have increased in both treatments, if compared to those recorded during the first one (Table 4). The free fatty acid level of both olive oil samples we analysed was lower than those recorded in Barnea olive oil subjected to similar salinity treatments [*7*], and differences between FW and SS treated plants were not significant.

Table 4: Fatty Acid Composition (%) of Virgin Olive Oils from Olive Trees (Cv. Chemlali) Grown Under Fresh Water Irrigation (FW) and Saline Water Irrigation (SS) in 2004 and 2005.

Fatty Acids	2004		2005	
	FW	**SS**	**FW**	**SS**
Palmitic acid	19.82±0.25^{a,w}	16.1±0.35^{b,w}	16.7±0.32^{a,x}	15.51±0.42^{b,w}
Palmitoleic acid	2.57±0.15^{a,w}	2.1±0.17^{a,w}	1.76±0.12^{a,x}	1.65±0.11^{a,x}
Heptadecanoic acid	0.12±0.01^{a,w}	0.11±0.02^{a,w}	0.14±0.03^{a,w}	0.13±0.03^{a,w}
Heptadecenoic acid	0.24±0.02^{a,w}	0.22±0.03^{a,w}	0.27±0.05^{a,w}	0.26±0.08^{a,w}
Stearic acid	2.19±0.015^{a,w}	2.01±0.016^{a,w}	2.83±0.016^{a,x}	2.25±0.025^{a,x}
Oleic acid	55.58±1.05^{a,w}	59.3±2.03^{b,w}	60.73±2.45^{a,x}	64.59±2.56^{b,x}
Linoleic acid	16.14±0.65^{a,w}	17.96±0.52^{a,w}	16.52±1.01^{a,w}	17.26±0.96^{a,w}
Linolenic eicosanoic acid	0.56±0.08^{a,w}	0.63±0.078^{b,w}	0.56±0.08^{a,w}	0.55±0. 09^{a,x}
Eicosanoic acid	0.34±0.03^{a,w}	0.41±0.08^{b,w}	0.4±0.06^{a,x}	0.43±0.07^{a,w}
Eicosenoic acid	0.16±0.02^{a,w}	0.14±0.04^{a,w}	0.18±0.05^{a,w}	0.16±0.07^{a,w}
Uns/ Sat ratio	3.34^{a,w}	4.31^{b,w}	3.98^{a,w}	4.61^{b,w}
Mono/ Poly ratio	3.5^{a,w}	3.32^{a,w}	3.68^{a,w}	3.74^{a,w}

Values are the means of three different VOO samples (n = 3) ± standard deviations. Different letters (a, b) indicate significant differences ($P < 0.05$) between saline water irrigation treatments within each year. Different letters (w, x) indicate significant differences ($P < 0.05$) between crop seasons within each treatment.

The comparison of spectrophotometric absorption characteristics in the UV region at 232 and 270 nm between oils from the two saline irrigated treatments did not show significant differences ($p = 0.433$). Taking into account the values of free acidity, peroxide value and K_{232} and K_{270}, the oil samples obtained from both treatments met the European Union requirements for the extra virgin olive oil category. The distribution of fatty acid composition of the oil samples of both saline water treatments covers the normal range expected for virgin olive oil (Table 4). For both treatments, the most abundant acid was the oleic one with values recorded in oil obtained from SS treated plants were statistically higher ($p = 0.0024$) than those in oil of FW ones. However, the oil obtained from high saline water irrigated plants would be nutritionally better than that obtained in the case of fresh water irrigated ones.

Table 5 reports the concentrations of the major phenolic, total phenols and oxidative stability of VOO samples in both treatments. Total phenols contents of VOO were significantly influenced by the salinity treatments. During 2004 crop season, the total phenols contents were of 181 and 214 mg / kg, respectively in FW and SS treatments. During the second crop, these values reached 198 and 223 mg / kg, respectively. The use of saline water at 7.5 dS m^{-1} has reinforced the phenols accumulation and did not altered the oil quality. The three phenolic compounds in highest concentrations in both oil samples are hydroxytyrosol, tyrosol and glycoside oleuropein.

Table 5: Phenolic Composition Concentrations (mg / Kg), Total Phenols Contents (mg/kg of oil) and Oxidative Stability (h) of Olive Oils from Olive Trees (Cv. Chemlali) Grown Under Fresh Water Irrigation (FW) and Saline Water Irrigation (SS) in 2004 and 2005.

	2004		2005	
	FW	SS	FW	SS
Tyrosol	42.5 ± 2.36^{a,w}	66.4±3.21^{b,w}	45±2.56^{a,w}	72.5±2.36^{b,x}
Hydroxytyrosol	84.5±3.56^{a,w}	96.7±2.06^{b,w}	88.5±2.47^{a,w}	105.4±3.56^{b,x}
Oleuropein	10.5±2.84^{a,w}	12.7±2.56^{a,w}	12.5±3.3^{a,w}	16.4±2.84^{b,x}
glycoside oleuropein	23.4±2.56^{a,w}	34.6±2.47^{b,w}	25.5±2.5^{a,w}	37.1±2.56^{b,w}
Vanillic	13.2±1.56^{a,w}	15.6±1.04^{a,w}	16.4±2.3^{a,x}	17.6±2.45^{a,w}
Caffeic	10.5±1.24^{a,w}	13.6±1.03^{b,w}	13.2±2.45^{a,x}	16.9±2.12^{b,x}
Syringic	8.6±1.2^{a,w}	10.7±1.24^{b,w}	10.7±1.24^{a,x}	12.4±1.24^{b,x}
p-coumaric	4.2±1.32^{a,w}	5.39±1.42^{a,w}	6.6±1.2^{a,w}	8.2±1.2^{a,x}
o-coumaric	3.2±1.02^{a,w}	4.6±1.25^{a,w}	5.3±1.32^{a,w}	7.1±1.32^{b,x}
Ferulic	2.96±1.43^{a,w}	4.5±1.65^{b,w}	4.25±1.02^{a,x}	6.8±1.22^{b,x}
Total phenols (mg/kg)	181.46±2.35$^{a,\ w}$	214.17±2.45$^{b,\ w}$	198.08±4.56$^{a,\ x}$	223.67±3.47$^{b,\ x}$
Oxidative stability (h)	16.02±1.02$^{a,\ w}$	18.84±1.45$^{b,\ w}$	16.43±2.03^{a,w}	21.73±2.14$^{b,\ x}$

ABBREVIATIONS USED: VOO, virgin olive oil; FW, fresh water treatment; SS, saline water treatment; Fw, fresh weight; ECe, electrical conductivity; FrW, fruit weight; FD, fruit diameter; FV, fruit volume; FWC, fruit water content; MI, maturation index; LSD, least significant differences; PAL, phenylalanine ammonia-lyase.

Il n'y a pas de glucoside dans l'huile donc pas d'oléuropéine et pas de glucoside oleuropéine. Il y a de l'oléuropéine aglycone et du ligstroside aglycone parmi les sécoiridoïdes principaux.

4. Conclusion

In conclusion, saline water (EC = 7,5 dSm^{-1}) used for olive tree (cv. Chemlali) irrigation appears to be beneficial not only for water resources management, but also have a direct effects on both quantitative and qualitative characteristics of virgin olive oil in the case of Chemlali olive cultivar tested in this experiment. The changes in VOO fatty acid and phenolic compositions induced perhaps by water deficit, resulting from saline water irrigation as has been confirmed. Recent researches confirmed the protective role of phenols, as natural antioxidants, against cardiovascular diseases and colon, breast and skin cancers. The higher levels of phenolic compounds found in Tunisian Chemlali olive oil would be with more benefits for people. Furthermore, severe studies report different pharmacological activities of olive oil phenols, other than antioxidant potential.

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S8-C2

Influence of water quality used in irrigation on the sensory properties of olive oil

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Abstract

This study was conducted to evaluate the influence of three water types applied to irrigate 'Nabali Muhasan' olive (*Olea europaea L*.) trees grown in an orchard in the north-east of Jordan on the sensory attributes of the produced olive oil.

The results of this study revealed the high salinity of the well water used.

On the other hand, the properties of rain water were significantly better than the standards for ground water whereas reclaimed wastewater (TWW) achieved tJordanian standard for TWW except for Na and HCO3.
Regarding the soil analysis of the olive orchard used in this study, no marked changes in the parameters analysed for the two years study (2006 &2007) were found.
Olive oils made from trees that received well and rain water either in 2006 or in 2007 crop seasons were free from any defect and were classified as extra virgin olive oil. On the other hand, the negative attributes fusty, musty, winey and muddy were detected in olive oils made from trees receiving reclaimed wastewater in 2006 crop year; whereas only two negative attributes i.e fusty and musty were detected in 2007 crop season.

The sensory attributes of olive oil samples obtained under the conditions of this study using experimental mill were better than those obtained by commercial press. Such results might be ascribed to the low quantities of olive fruits milled, low malaxation temperature used and the bitter cleanliness degree in case of experimental mill. As a result, it is difficult to conclude that the presence of negative attributes (defects) in the studied olive oils is due to the reclaimed wastewater treatment alone. However, more research is needed to clarify this poi

Keywords: Olive oil, treated waste water, rain water, well water, sensory properties, extra virgin

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S8-C4

L'importance de l'amélioration des techniques culturales pour assurer la durabilité et la productivité du secteur oléicole dans le sud tunisien

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Résumé

La filière oléicole dans le sud Tunisien constitue encore une spéculation importante par ses multiples implications sociale, culturelle, économiques et écologiques. Certes, l'aridité climatique et édaphique et la fragilité du milieu constituent une contrainte à la productivité du secteur, mais la région du sud dispose, en outre, de nombreux atouts, dont notamment la biodiversité, le savoir local, et pour cette raison, des efforts d'amélioration et de développement sont souhaitables et nécessaires.

Ce dans ce cadre que l'institut de l'olivier a mené des actions de recherches qui on pour objectifs d'améliorer la productivité et assure la durabilité du secteur tel que l'introduction d'autres cultures avec l'olivier (figuier, tomate, luzerne etc.….), l'amélioration des techniques culturales relatives aux travaux du sol pour réduire le phénomène d'érosion éolien et la perte en sol et l'épandage de la margine pour améliorer le statut organique des sols sableux initialement très faible.

Mots clés : Sud Tunisien, Durabilité, productivité, Techniques culturales.

Abstract

In the south of Tunisia, the olive trees orchards constitute even an important speculation by their multiple social, cultural, economic and ecological implications. Certainly, the aridity of climate and the fragility of this environment constitute a constraint to the productivity of the olive tree sector, but the southern Tunisian region disposes, in addition to numerous assets, of which notably the biodiversity, some local knowledge, and for this reason, many efforts of improvement and development are desirable and necessary.

In this setting, the olive tree institute led several research actions in order to improve the productivity and to assure the durability of the sector as the introduction of others culture with the olive tree (fig-tree, tomato, alfalfa etc.), the improvement of the technical culture relative to soil tilling in order to reduce the wind erosion phenomenon and the soil loss and the Mulching of the "margine", the Olive Mill Waste water, to improve the organic matter content of the sandy soils initially very weak.

1. Introduction et problématique

Dans le Sud Est tunisien, à bioclimat aride (100-200 mm de précipitation par an), on assiste à l'extension des cultures aux dépens de pâturage. Parmi ces cultures, celle de l'olivier qui tient une place prépondérante. Dans le gouvernorat de Médenine, on comptait déjà en 2007 un effectif d'environ 4,5 millions pieds (CRDA Médenine). Toutefois, dans cette région, l'oléiculture se heurte à des problèmes sérieux d'économie d'eau et d'érosion. Les techniques culturales relatives aux travaux du sol adoptées actuellement pour l'olivier, comme le labour conventionnel, se basent sur des passages fréquents au cours de toute l'année. Les agriculteurs considèrent la complète éradication de la végétation naturelle dans les interlignes nécessaire pour éliminer la concurrence en eau avec les arbres. En même temps, ils espèrent rompre la capillarité (effet mulch), augmenter l'infiltration des eaux de pluies et aérer le sol. Néanmoins, ces techniques sont à l'origine d'une exposition du sol aux agents érosifs du climat (vent et pluie) ce qui engendre une érosion accrue et un déchaussement des racines de la culture. Le travail fréquent par des outils non adéquat détruit la partie la plus superficielle du système racinaire des oliviers, la partie nécessaire à optimiser l'emploi de l'eau des précipitations

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peu abondantes. Le passage des outils de travail crée des semelles de labour qui réduisent sensiblement le taux d'infiltration de l'eau de pluie en profondeur entravant ainsi la recharge d'eau de profil. L'eau de pluie demeure ainsi prés de la surface, exposée à une évaporation intense et non exploitable par les racines qui ont été coupée par les façons culturales. En plus, le passage de tracteurs est à l'origine de la formation de zones de compaction superficielles.

Aussi la réduction de la couverture végétale, la perte de sa capacité de régénération et l'ameublissement des horizons de surface conduisent à la disparition de l'horizon sableux superficiel apte à stoker les eaux de pluie. Le sol est progressivement tronqué de ses horizons meubles de surface pouvant conduire à la mise à nu du substrat non altéré et quasiment imperméable. Dans les situations les plus fragiles, la mise en œuvre de ces pratiques entraîne un fort accroissement de l'érosion tant éolienne qu'hydrique. Dans les plaines sableuses, les vents entraînent le départ et le transport des éléments les plus fins du sol et peut mettre en suspension de très fines particules et les transporter sur des grandes distances. Les particules moyennes et fines peuvent être soulevées et redéposées alors que les grosses particules peuvent être soufflées en surface (saltation). L'abrasion qui en résulte peut réduire la dimension des particules de sol et augmente d'autant sa susceptibilité à l'érosion.

En outre l'activité oléicole est peu rentable, la faible productivité des plantations oléicoles, qui est liée à l'effet de plusieurs facteurs dont la sécheresse, la sénescence, la qualité des sols et le manque d'entretien des plantations affecte sensiblement la rentabilité économique de cette activité (B Karray ; A Mounir, 2007).

L'institut de l'olivier conscient de ces problèmes à développer un programme de gestion technique du sol et des plantations d'olivier dans les zones aride pour améliorer la productivité et assure la durabilité du secteur. Et a mené certaines actions de recherches (l'étude du flux d'érosion pour deux parcelles labouré par deux outils différents, l'étude de l'effet de l'épandage de la margine sur le sol sableux, l'introduction du figuier (Ficus carica L) Ces actions ont pour objectifs :

- Résoudre les problèmes liés à la mécanisation du travail du sol et cherché les outils adéquats pour l'ameublissement superficiel du sol toute en le protégeant contre l'érosion éolienne.

- L'amendement organique pour l'amélioration du statut du sol et l'amélioration des ses propriétés physiques structurales.

- Introduction du figuier (*Ficus carica L*.) dans le système oléicole des zones arides : un moyen de protection de l'environnement et d'amélioration de la productivité

2. Action 1: Etude de flux horizontal pour deux cas de labour

Le travail du sol jugé nécessaire pour la croissance et la production des cultures doit tenir compte des caractéristiques du sol et du climat afin d'éviter les risques d'érosion, le bon choix de l'outil adéquat présente beaucoup d'importance pour cette opération. C'est dans ce cadre que s'insère ce travail de recherche qui consiste à évaluer et étudier l'effet du travail du sol sur l'érosion éolien. Pour réaliser ce travail, on a calculé et mesurer le flux d'érosion pour deux parcelles labouré, une par les socs de scarifiage (queues d'hirondelle) habituellement très utilisés par les agriculteurs et l'autre par la charrue polysocs (jamoussi) dont l'objectif d'estimé les flux de saltation dans les zones arides tunisienne, où le phénomène de l'érosion éolienne est très actif, et par la suite comparer les flux horizontaux d'érosion pour les différents outils utilisés.

2.1. Mesure du flux horizontal d'érosion

En pratique, la mesure du flux d'érosion est effectuée moyennant un réseau de piège à sable de type BSNE (Big Spring Number Eight) installés sur des supports menus d'un système girouette. Chaque BSNE a une section rectangulaire bien déterminé mesurée moyennant un pied à coulisse. Pour suivre la variation du flux avec la hauteur, ces mini mâts ont été équipés de sept BSNE installés à des hauteurs comprises entre 5 cm et 100 cm par rapport au niveau du sol afin de couvrir la hauteur de la couche de saltation qui est de l'ordre de 80 cm dans la nature. En outre, le flux de saltation a été collecté après chaque événement érosif et la mesure a été réalisée sur deux parcelles soumises à deux techniques différentes du travail du sol afin de dégager les techniques les plus agressives.

2.2. Calcul du flux

Le flux horizontal q(z) (g.cm^{-2} .s^{-1}) à une hauteur donné z, exprime la concentration des particules en saltation à cette hauteur. Il est défini par la masse de matériel traversant par unité de temps une section verticale de surface unité, perpendiculaire à la direction du vent. Cette concentration est très forte au niveau des 10 premiers centimètres de la surface du sol Bagnold, (1941) ; Fryrear et al, (1991) et diminue avec la hauteur suivant une loi exponentielle ou en puissance. Fryrear et saleh, (1993) ont développé l'expression suivante pour décrire la variation du flux horizontal avec la hauteur :
q(z)=a exp(-bz)
Où a et b sont des coefficients d'ajustement exprimés respectivement en g^{-1} et g.cm^{-2}. L'intégration du flux sur la hauteur de la couche de saltation donne le flux horizontal d'érosion (Q).

2.3. Résultats

2.3.1. Charrue à dents

Tableau 1: flux mesuré et ajustement des paramètres du flux calculé pour la parelle traitée avec l'outil à dents (queues d'hirondelle).

	hauteur	poids Sable (g)	section des pièges	flux mesuré	hauteur bas piege	h section
piège 1bas	14	6,05	2,30	2,63	13,47	1,06
piège 1 haut	22,63	8,95	10,12	0,68	20,12	5,02
piège 2	35,085	5,18	9,96	0,52	32,62	4,93
piège 3	48,185	3,96	10,01	0,40	45,72	4,93
piège 4	61,155	2,43	10,31	0,24	58,68	4,97
piège 5	79,455	2,06	9,69	0,21	76,97	4,97
piège 6	119,195	1,32	9,38	0,14	116,72	4,95
		a	b			
q(z)=a exp(-bz)	paramètresrégression	9,223	0,092			

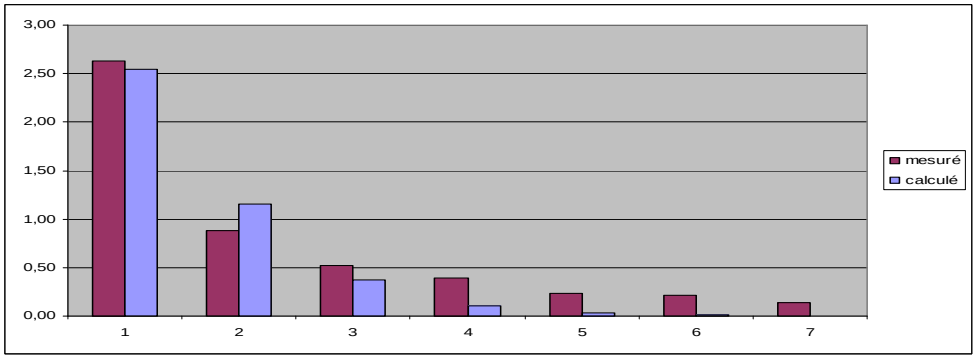

Figure 1: comparaison entre flux mesuré et calculé.

2.3.1. Charrue à socs

Tableau 2: flux mesuré et ajustement des paramètres du flux calculé pour la parelle traitée avec la charrue à soc (jamousi).

	hauteur	Poids Sable (g)	section des pièges	flux mesuré	hauteur bas piege	h section
piège 1bas	11,62	3,15	2,01	1,57	11,11	1,02
piège 1 haut	20,33	4,62	9,95	0,46	17,91	4,84
piège 2	31,69	2,78	11,21	0,25	29,01	5,36
piège 3	44,53	1,44	10,69	0,13	41,96	5,14
piège 4	60,35	1,11	10,60	0,10	57,61	5,07
piège 5	77,15	0,72	10,44	0,07	74,61	5,08
piège 6	117,10	0,49	10,02	0,05	114,61	4,97
		a	b			
q(z)=a exp(-bz)	paramètres régression	6,087	-0,118			

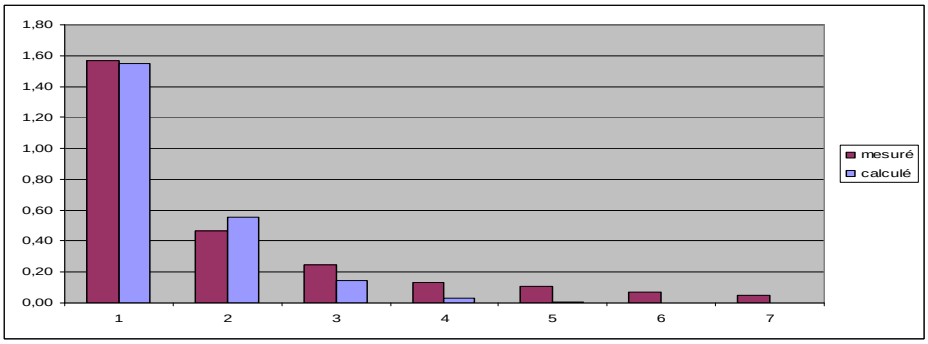

Figure 2: comparaison entre flux mesuré et calculé.

Dès lors que les paramètres de régression a et b sont connus, l'intégration du flux sur une hauteur comprise entre la surface du sol et 80 cm (50 cm) donne le flux horizontal d'érosion (Q) ($g.cm^{-1}.s^{-1}$) pour chaque parcelle.

Charrue à dents			**flux intégré sur 50 cm**
	a	b	
	9,22	-0,092	**99,34587482**

Charrue à socs			**flux intégré sur 50 cm**
	a	b	
	6,09	-0,118	**51,52351811**

La comparaison entre ces deux flux nous a permis d'avoir une idée sur l'ampleur qui prend le phénomène de l'érosion éolienne dans les zones arides tunisienne, puisque les flux ont été collecté événement par évènement, et puis de trancher entre deux outils de travail du sol afin de réduire au maximum la perte en sol dans la parcelle. Par ailleurs le soc semble être l'outil le plus approprié pour les sols sableux car il réduit à moitié les flux de saltation et permet le retournement du sol lors de la saison pluvieuse sur une profondeur allant de 15 à 25 cm. Elle crée par la suite, des conditions physiques favorables pour le développement et l'activité racinaire, bonne porosité et perméabilité suffisante. Alors que les queues d'hirondelle suite à des plusieurs passages cet outil conduit a la formation de semelle de labour.

3. Action 2: L'amendement organique pour l'amélioration des propriétés physiques structurales du sol

Le manque de la matière organique en zone aride en quantité suffisante contribue à la baisse de productivité des sols, compromettant ainsi les niveaux de rendement et la qualité des récoltes. Et pour le maintenir à un seuil optimal cela constitue une contrainte pour tout programme de conservation d'amélioration des sols. Aujourd'hui, des sources nouvelles de matières organiques peuvent être utilisées pour amender les sols tel que les margines qui s'accumulent d'une année à l'autre et constituent un véritable problème environnementale face au tonnage grandissant produit par les huileries qui dépassent les 700000 tonnes/an en Tunisie (source Direction Générale de Production Agricole) surtout après l'introduction du matériel d'extraction chaîne continue (3 phases) ce matériels nécessite des quantités importantes d'eau et génère des quantités importantes des margines (pour l'extraction d'une tonne d'olive il faut entre 1000 et 1200 litres d'eau). La valorisation de ces effluents par épandage dans les oliveraies du sud Tunisien caractérisé par un sol sableux est synonyme d'une meilleure gestion de l'environnement d'une part et d'amélioration de la cohésion du sol d'autre part. Ces constats ainsi réalisés, il nous a semblé nécessaire de faire le lien entre les différents doses des margines épandues apportés au sol (50,100, 200 m3/ha), et la stabilité de la structure d'un sol sableux connu comme instable. Cela nous a conduits à engager ce travail de recherche présenté ici et qui se propose :

* D'évaluer les effets des différents doses de margines sur l'évolution du taux de la matière organique, la stabilité structurale, la végétation naturelle et la perte en sol.

3.1. Evolution de teneur en matière organique dans le sol après 10 ans d'épandages:

La teneur en matière organique est déterminée selon la méthode de Walkley et Black qui consiste en une oxydation à froid par le bichromate de potassium ($K_2Cr_2O_7$) en milieu acide et titrage par le sulfate ferreux ($FeSO_4$ $7H_2O$). La matière organique est déterminé par l'équation **MO % = C % X 1.725**

Table 1: Evolution du taux de la matière organique après 10 ans d'épandages de la margine.

Doses	T0	T1	T2	T3
Moyenne	0.35 ± 0,12 a	0,49 ± 0,13 ab	0,60 ± 0,21 b	1,05 ± 0,28 c

L'analyse de la variance montre une nette amélioration du taux de la matière organique dans le sol de 0,35 % à 1,05 % (tableau 2). L'augmentation des taux de matière organique dans le sol est proportionnelle à la dose appliquée. Les différences observées pourraient être attribuées à la cinétique de minéralisation de la matière organique dépendant de l'importance des micro-organismes dans le sol traité (nombre et qualité) et les conditions climatiques. Ces résultats sont analogues à ceux de Cabrera et al, (1996) qui mentionnent que l'apport annuel sur un sol sableux contenant initialement 0,45 % de MO de 37 ou de 61 l/m2 de margines pendant trois années de suite, engendre un accroissement de la matière organique (1,62 % et 1,98% respectivement).

3.2. Effet de la margine sur l'agrégation du sol et la stabilité structurale

La stabilité des agrégats a été étudiée en se basant sur le concept du diamètre moyen pesé, exprimé dans la littérature anglo-saxonne comme « the mean weight diameter », qui a été introduit par Van Bavel, (1949), et son changement, 'the change in mean weight diameter », selon la méthode dite de tamisage sous eau de (De Leenheer et De Boodt, 1959).

Cette méthode consiste à comparer la distribution des différences fractions des agrégats du sol issue du tamisage à sec (état initial) à celle obtenue après un tamisage sous eau (état final). Le diamètre moyen pesé (DMP) à l'état initial et final peut être ainsi déterminé Sillanpää, M, (1958) ; Baver et al, (1972) par :

DMP = (II.1)

Avec : m_i = masse de la fraction i (g)
d_i = diamètre moyen de la fraction i (mm)
n = nombre total des fractions

le changement du diamètre moyen pesé qui correspond à l'indice d'instabilité (II) est alors obtenu par :

$II = (DMP)_s - (DMP)_h$ (II.2)

Où s et h désignant respectivement le tamisage à sec et sous eau.
L'inverse de cet indice n'est autre que l'indice de stabilité (IS) :

$$IS = \frac{1}{II} \qquad \text{(II.3)}$$

3.2.1. Résultats

Concernant le témoin sans margine les particules inférieure à 2mm est 100 %, La distribution granulométrique obtenue par 50m^3/ha caractérisée par une très forte proportion des particules les plus fines, avec 93% de la distribution inférieure à 2mm. Il s'agit bien d'un sol peu stable. Lorsque les agrégats ont été préalablement traités à la dose de 100m^3/ha, la distribution granulométrique se trouve décalé : le taux d'éléments fin ou particule inférieure à 2mm passe à 87%, alors que le taux d'éléments grossiers (ou agrégats) supérieure à 2mm passe de 7 à 13% avec une augmentation peu significative. Aux doses supérieures, ces taux d'agrégats supérieurs à 2 mm augmentent très fortement atteignant 34% avec le traitement de 200m^3/ha. Une réduction de toutes les classes inférieures à 2mm s'effectue simultanément. L'amélioration de liaison entre les particules a été obtenu avec la dose la plus élevée mais les agrégats de grande taille résiste moins à la désagrégation mécanique puisque l'indice de stabilité le plus bas est obtenu par la dose la plus élevée, les indices de stabilité sont respectivement 3,83, 2,94 et 2,31 pour les doses 50, 100, 200 m3/ha (tableau 1). Ces résultats peuvent être comparé à celle de Mellouli,(1996) qui a conclu qu'il existe une amélioration de la stabilité structurale pour les sols instable (les sables limoneux) suite à l'épandage de 50m^3/ha.

3.3. Effet de l'épandage de la margine sur la végétation naturelle

Il s'agit du nombre d'individus par unité de surface, dans notre cas nous avons utilisé un cerceau d'un mètre carré puis nous avons compté le nombre de plantes annuelles existantes dans la surface délimitée par le cerceau. La végétation spontanée stimulé par l'épandage de margine représente une autre source d'apport de matière organique après son incorporation dans le sol, aussi la présence de végétation est connue pour être le principal facteur limitant l'érosion éolienne.

3.3.1. Résultats

Tableau 2: Densité de végétation naturelle.

Doses	T0	T1	T2	T3
Moyen	2,66 ± 3,06 a	100,5 ± 21,67 ab	143,2 ± 21,31 b	1185,2 ±121,68 c

On a remarqué que la densité de l'herbement naturelle est très élevés (1185) au niveau de la parcelle qui a reçu la forte dose de la margine (200 m3/ha) cette végétation constitue le couvert végétal qui assure la protection de la surface par la limitation de l'érosion et l'opposition à l'écoulement de l'air. Cette végétation est très utile pendant la saison du printemps, période caractérisée par un vent très actif.

3.4. Effet de l'épandage de la margine sur la perte en sol

Pour un agrégat de sol, quand la force aérodynamique est supérieure à la somme de la force de gravité et la force de cohésion inter particulaire, l'agrégat commence à bouger. Deux vitesses sont importantes dans le processus de saltation. La vitesse seuil d'érosion U^*_t et la vitesse de friction U*. Pour chaque classe de taille de particule (D_p) il y a une vitesse seuil d'érosion. La vitesse de friction dépend de la vitesse de vent, de la densité moyenne des particules dans le sol ρ_p , de la présence d'éléments non érodables (végétaux, cailloux, roches) et l'humidité du sol et du taille des billons et sillions La quantification de la perte en sol déterminé par le flux mesuré dans la soufflerie et calculé en utilisant le modèle LISA élaboré par Gilles Bergametti.

$$Q = a.U_*(U_* - U_{*t}) \quad (g.cm^{-1}.s^{-1})$$

A = Coefficient d'érodabilité

U_* = vitesse de friction : $U_* = \frac{U(Z).K}{L_n = \frac{Z}{Z_0}}$

U_*t = vitesse de friction seuil
Z_0 = Rugosité de la surface
Z = Hauteur au dessus de la surface du sol.
K= Constante de Von Karman (~0.4)

3.4.1. Résultats

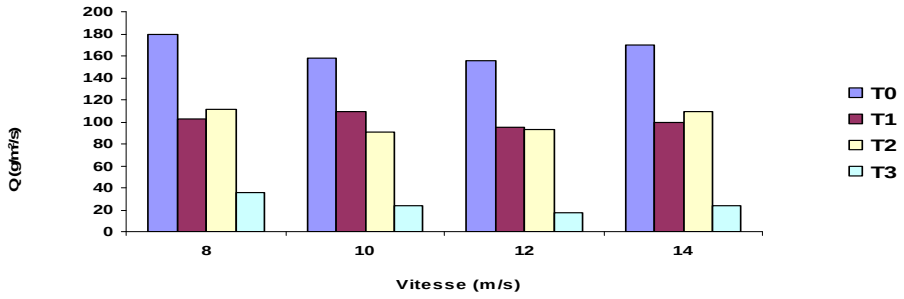

Figure 1: Flux mesuré dans la soufflerie a différentes vitesses.

Tableau 3: Flux calculé par le modèle LISA

Traitement	T0	T1	T2	T3
Flux (g/cm.s) en15 minutes	$9.92\ 10^{-2}$	$9.9\ 10^{-2}$	$9.65\ 10^{-2}$	$8.63\ 10^{-2}$

La perte en sol démunie avec l'épandage de la margine cette remarque est plus claire avec la dose la plus forte ????

4. Action 3: Introduction du figuier (*Ficus carica L.*) dans le système oléicole des zones arides : un moyen de protection de l'environnement et d'amélioration de la productivité (en cour de réalisation)

Une méthode possible de remédiation et d'amélioration des situations dégradées est la transformation de l'agro système oléicole actuel des zones arides par l'introduction du figuier (*ficus carica L*).

Pourquoi le figuier ?

- Facile à installer
- bien adapté à la région
- écoulement de production prometteuse
- conservation facile

4.1. Objectifs

L'objectif de ce programme de recherche est d'étudier les effets de l'introduction du figuier en intercalaire avec l'olivier sur :

L'état de surface du sol (éléments non érodables, croûtes, distribution granulométrique du sol, etc…), Flux d'érosion éolien, L'activité microbiologique du sol, L'ombrage et l'évapotranspiration, La productivité et rentabilité par hectare

4.2. Les effets attendus

- Rentabiliser les systèmes de productions oléicoles dans les zones arides

- Trouver un champ d'activité plus large pour la femme rurale à travers la création des unités de séchages de figuier.

- Ralentir l'effet de l'érosion éolien par l'implantation de figuier entre les lignes de l'olivier qui sont déjà très éloignés l'une à l'autre 24 m / 24 m.

- Amélioration de la biodiversité dans les zones arides et diversification des produits destinés à l'exportation.

- Passer d'un système de production monoculture à un système intégré (réhabilitation de l'écosystème).

4.3. Etudes préliminaires:

Concernant l'étude de l'effet de l'ombrage sur l'activité microbiologique du sol on a quantifié le nombre des bactéries pour le sol exposé totalement au soleil et pour le sol couvert par l'ombrage du figuier

4.3.1. Dénombrement de la microflore bactérienne totale

Il s'agit de la flore aérobie mésophile viable.

4.3.1.1. Préparation de la suspension du sol

* 10g de chaque échantillon nous ajoutons 90ml d'eau distillée stérile. Cette suspension est mise sous agitation à 250 tours/min pendant 30 minutes, Ensuite nous réalisons des dilutions jusqu'à 10^{-4}.

4.3.1.2. Ensemencement des boites de Pétri

* Le milieu utilisé est le nutriment Agar (28g de gélose nutritive et 5g d'agar) prêt à l'emploi. Pour cela, 0,1ml de chaque dilution est ensemencé en surface puis étalé dans trois boites de pétri. Ces boites sont incubées à 25℃ avec alternance d'obscurité et de lumière pendant 48h.

Le comptage est effectué par la méthode du nombre le plus probable (MPN), ceci permet de déterminer les micro-organismes viables. Elle consiste en une approche statistique basée sur une série de dilutions successives. Les résultats sont exprimés en unités formant des colonies par g de sol (UFC).

4.4. Résultats

Le nombre des bactéries dans un échantillon de sol exposé totalement au soleil : 52.66 10^4 CFU/g de sol. Le nombre des bactéries dans un échantillon de sol couvert par l'ombrage : 65.33 10^4 CFU/g de sol. Le décompte de la flore montre que l'ombrage a permis d'augmenter le nombre de micro-organismes dans le sol. D'une manière générale, la croissance de ces bactéries est contrôlée par plusieurs paramètres. En effets, la plupart des espèces ont une croissance optimale pour un pH entre 7,5 et 8, une température de l'ordre de 25 à 30 ℃ (dans la zone d'étude la température du sol à 20 cm dépasse 36℃ en été) et une concentration en oxy gène dissous entre 0,5 et 4mg/l Féray C., (2000)

5. Conclusion

L'amélioration des techniques culturales relatives aux travaux du sol améliore sa structure (bilan hydrique, aération et réduction du lessivage des éléments nutritifs) et ainsi garantir leur productivité. L'épandage de la margine Permet de limiter la déflation éolienne, phénomène très répandu dans les oliveraies du Sud Est suite au maintien de la couverture végétale, la création de mottes et l'apport d'amendement ce qui améliorer le statut organique des sols sableux initialement très faible et réduire le phénomène d'érosions éoliennes. Aussi Introduction du figuier (*Ficus carica L*.) dans le système oléicole des zones arides permet d'avoir un agro-système qui respecte la bonne santé des sols et leurs biodiversité, une meilleure résilience par rapport au changement climatique, réduction de l'érosion éolienne et les services en rapport avec la vie humaine (alimentation, nutrition, santé, revenus, identité culturelle, esthétique, zones créatives, et qualité de vie) par conséquence développement durable.

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S8-C5

Olive, Asparagus and animals: an agroforestry model for temperate climate in developed countries

Rosati A., Caporali S., Paoletti Q.

Abstract

Due to the high harvesting cost, traditional olive cultivation is rapidly becoming anti-economical in developed countries. This might result in abandonment of millions of hectares of agricultural landscape. Converting such a landscape to an olive-based agroforestry system, intercropping olives with other economically viable crops, can provide economic sustainability, allowing maintenance of a traditional agricultural landscape, which is also functional to tourism activities. Here, the cultivation of a wild asparagus species (*Asparagus acutifolius*) as an understory crop in the olive orchard is proposed. The possible inclusion of animal raising in the olive-asparagus policolture are discussed. Olives, wild asparagus and animals could be managed together as a productive agroforestry system, where each crop benefits the others, while producing more income on the same land.

Key words: olive, asparagus, animal, agroforestry, policulture.

Olivier, Asperge et animaux: un modèle de l'Agroforêsterie pour les pays développés avec climat tempéré

Résumé

À cause des coûts élevés de la récolte, la culture traditionnelle de l'olivier dans les pays développés est en train de devenir rapidement antiéconomique. Il est probable que ceci porte à l'abandon de millions d'hectares du paysage agricole. Convertir tel paysage en un système "agroforestier" basé sur l'olivier, en associant l'olivier avec d'autres cultures économiquement viables, il peut offrir durabilité économique, en permettant de conserver un paysage agricole traditionnel qui est ouvert aussi aux activités touristiques. Ce travail propose la culture d'une espèce d'asperge sauvage (*Asparagus acutifolius*) comme une culture de sous-bois dans l'oliveraie. Il est également proposé de faire du pastoralisme avec des animaux d'élevage dans la polyculture olivier-asperge. Oliviers, asperges et animaux pourraient être gérés comme un système "agroforestier", où chaque culture apporte bénéfice aux autres et en même temps en produisant plus des revenus dans le même terrain et en particulier autour des villes.

Mots clés: olivier, asperge, animal, agroforêsterie, polyculture.

1. Introduction

Due to the high harvesting cost and the cost of other field practices that require much labor, traditional olive cultivation is rapidly becoming anti-economical in developed countries where hand labor is very expensive. Decoupling of agricultural funding from the production (i.e. 100% decoupling for olive in Italy) and the probable end of agricultural subsidies after 2013, might result in the abandonment of millions of hectares of agricultural landscape in Europe. This might negatively affect not only the agricultural activities and income, but also the landscape of several European countries where olive has been historically important and very much part of the landscape. In some of these countries, olive trees are protected and cannot be removed, even if the orchard is abandoned or unproductive. In this context, new ways of deriving an income from olive orchards would be highly desirable.

One way to do this, is to grow other valuable crops under the olive orchard canopy, creating an olive policulture; that is, and agroforestry system where the olive is the tree component. The additional income deriving from such crops would justify maintenance of the olive landscape, while growing more

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crops on the same land would generate scale economy, lowering the cost of olive production, hence making it more competitive. Maintenance of traditional landscapes, like the olive landscape, is important also from an economical point of view in that situation where the beauty of the landscape is functional to tourism activity. Italy, for instance, has over 20 thousand “agri-turism” enterprises (on-farm hospitality): if each of them benefits from 50 hectares of surrounding landscape, this results in one million hectares that contribute to the tourism business.

Old agriculture was based on policulture or agroforestry, but in modern times the agricultural design in western countries has been simplified in an attempt to transform farming in a process more similar to industry, where scale economy can be achieved and labor reduced. In this process the environmental costs associated with modern farming have not been accounted for. The recently increasing interest for more sustainable farming provides an opportunity and a challenge to design new agricultural systems, which are ecologically sustainable but also economically viable (i.e. high yields and low labor). To design such systems, a better understanding of the needs and functions of plants and animals is necessary so that different species can be integrated in a single system where they give some yield while providing ecological functions that help reducing outside inputs and labor.

The theoretical basis of such an approach has been amply discussed, and there are many successful practical examples. However, most of these examples regard farming in developing countries where labor is still relatively inexpensive and usually provided within the family, farm size is small, and expected income is low. In developed countries successful examples are less frequent because agriculture has come further away from been ecologically designed: hand labor is expensive and replaced by machines which usually require large farm size and monocultures, which in turn, lead to heavy use of chemicals. Redesigning such systems with an agroecological approach might imply radical changes, which are difficult to implement, but also hard to accept culturally. However, some recent example exist. Within an European agroforestry research project, the “SAFE” (Silvo-Arable Forestry for Europe) project, cereals have been grown among large rows of trees, both crops being completely mechanized. This system has shown increased yield compared to the monocultures, while providing environmental benefits. Other positive examples of successful agroforestry in western countries are related to the economical advantages associated with the environmental benefits of the presence of trees, as in windbreaks, protection of riparian vegetation, prevention of erosion and consequent economic losses, like the reduction of water holding capacities of filled-up water reservoirs.

In the present paper, the cultivation of a wild asparagus species (*Asparagus acutifolius*) as an understory crop in the olive orchard is proposed as a possible way to increase both income and sustainability of olive growing. The potential yield and the market of such asparagus is discussed as well as the ecological design that justifies its inclusion in the olive orchard and possible strategies for the field management of the proposed agroforestry system are suggested.

2. Cultivation and market of *Asparagus acutifolius*

Wild asparagus (*Asparagus acutifolius*), was known and probably cultivated in ancient times (Aliotta et al., 2004), but not cultivated in modern times until very recently (Rosati, 2001). This species is one of the eight wild asparagus species present in Italy and the Mediterranean area (Bozzini, 1959) where it has always been enjoyed in traditional cooking (Venezia et al., 1993; Rosati, 2001; Fiori et al., 2001; Adam, 2004; Pieroni, 2005; Della et al., 2006). The spears are gathered from plants grown in the wild and sold in local markets. Given the high price of these spears in the existing market, recently some farmers have attempted to cultivate this species (Rosati 2001; Adam, 2004). Cultivation may supply this market which, up to now, has been limited by the availability of produce from the wild, and allow its expansion. However, little information on suitable field techniques is available to support farmers in the cultivation of this potential new crop. Such techniques differ from those of the cultivated asparagus (*Asparagus officinalis*) since the two species have quite different ecological requirements and yields (Aliotta et al, 2004; Rosati et al., 2005). Unlike *Asparagus officinalis*, the wild *A. acutifolius* is evergreen and prostrate, requiring different strategies for weed management and needing less light, water and nutrients, in view of the lower biomass produced, which results in lower yields (i.e. about 10-15% of the yields of cultivated asparagus). The lower yield is mostly due to the small size and weight of spears: about 5g per spear of 0.3m (Rosati et al, 2005; Benincasa et al., 2007). The low productivity makes this new crop suitable for marginal rural areas where high quality and price, rather than high yields, is the strategy for market crops.

The frugality of the wild asparagus, currently cultivated using seeds from local, non-selected wild plants, allows for a crop virtually free of pests and diseases, perfectly suited for organic or any other natural farming techniques.
The two species differ not only botanically and in ecophysiological requirements but also in their use: *A. officinalis* is used as a vegetable and therefore consumed in large quantities, while *A. acutifolius*, having a much stronger flavour, is used as a condiment in small quantities. This allows for a higher price of the spears, which ranges usually between 7 and 25 € per kg (i.e. two to four times the price of cultivated asparagus).

The most important problem that has limited success in growing *A. acutifolius*, has been that its seeds have a strong dormancy and do not germinate easily (Venezia et al, 1993). A suitable technique for production of transplants has been proposed (Rosati and Falavigna, 2000). More recent information has been published on field practices, yield potential, and labor requirements for cultivation and harvest (Rosati et al., 2005; Benincasa et al., 2007). In the following section some of the main results are summarized.

Wild asparagus (*A. acutifolius*) bare root transplants, obtained as described by Rosati and Falavigna (2000) have been transplanted in rows 1m apart and 0.33m within the row (Rosati et al., 2005), or rows 0.8m apart and either 0.4 or 0.25 within the row (Benincasa et al., 2007). In this wild asparagus the crown, although large, does not rise with time as with the cultivated asparagus, so planting must not be as deep. Harvest usually begins two or three years after transplanting, depending on transplant size, nutrient and water availability, soil type and climate. The spears are harvested in the spring when they first emerge, as soon as they reach sufficient height (0.3-0.5m). Harvest is protracted for 4 to 8 weeks or until spear diameter starts decreasing. The prickly vegetation can be removed before harvest, allowing for easier and faster harvest, but this might affect negatively plant vigor and yield in the following years (Rosati et al., 2005).
Harvest efficiency (i.e. kg of spears harvested per hour of labor) ranges between 7.2 kg/h and 3.0 with vegetation removal and between 3.8 kg/h and 1.2 without vegetation removal (Rosati et al., 2005; Benincasa et al., 2007).

Yield ranges between 1 and 1.5 tons per hectare but can easily reach 2 tons per hectare with good field practices and good quality transplants (Rosati et al., 2005; Benincasa et al., 2007). Given the high prices of the spears, which may range between 10 and 30 euro per kg, the wild asparagus appears to be an economically interesting crop, even though harvesting is a very expensive practice. In fact, hypothesizing 500 hours per hectare (i.e. cutting the vegetation before harvest), a sale price of 10 € per kg of spears and a cost of 10 € per hour of labour, harvest cost would represent one third of the crop's gross income. Even with more conservative numbers, the crop should easily pay for the labour, which might represent an opportunity of self-employment in small farms and/or in rural marginal areas. Alternatively, the spears could be harvested directly by the consumers in pick-your-own operations associated with tourism. Market potentials must be carefully assessed before planting.

3. The olive-asparagus agroforestry

Despite the fact that the literature hitherto available reports data for the cultivation of wild asparagus as a monoculture, the use of *Asparagus acutifolius* as an understory crop in policultures, including an olive-based agroforestry, has long been suggested (Rosati et al., 2001). Even though no data are available, there are several reasons to believe that such policulture could be ecologically and economically beneficial. Below I will discuss some such reasons.

The wild asparagus here proposed grows spontaneously in the Mediterranean area, sharing similar ecological needs as the olive, though the asparagus range is wider than that of the olive. In fact, both species are drought and heat tolerant, can grow on deep as well as shallow soils as long as there is no water logging, and tolerate well rocky soils. In addition, the asparagus species proposed, is also shade tolerant, unlike the olive, thus it can easily grow in the thin shade of the olive tree. In a recent study, Villalobos and co-workers (2006) have demonstrated that the olive maximum yield is obtained when the olive trees intercept 55% of the incoming light. With greater canopy densities the trees produce less oil, having to invest in vegetative growth to reach light. Therefore, about 45% of the available light cannot be used by the olive orchard. This radiation, however, can be used by the asparagus here studied which, as mentioned, is shade adapted. The productivity of the olive orchard can therefore be increased by producing not more oil, but a second crop.

The fact that this asparagus cannot produce large biomasses and its productivity is only about 10-15% of that of the cultivated asparagus, already suggests that this species needs not only little light, but also less nutrients to achieve its maximum potential yield, making its monoculture an ecological non-sense (i.e. a waste if light and nutrients that cannot be exploited by this crop with limited biomass and yield potential). Instead, the amount of light that is transmitted by the olive orchard is probably sufficient for the little biomass produced by the asparagus, while the light intercepted by its canopy, below the olive orchards, does not affect the light absorption by the olive canopy above. Similarly, the nutrients available in a marginal soil should be sufficient and there is no need for a very fertile soil as there is for the cultivated *Asparagus officinalis* with much greater yields. In conclusion, the low yield potential and the frugality of the wild asparagus, together with its shade and drought tolerance, suggest that its cultivation under the canopy of the olive orchard represents an optimal ecological niche, allowing two crops to be obtained on the same land with no detriment on the yield of either crop.

The overlapping of the natural distribution of olives and wild asparagus represents a guarantee that the asparagus will grow well and with little pest and disease problems in the olive orchard. The species is rather tolerant to the few pests and diseases that affect it, like the asparagus beetle (*Crioceris asparagi*) and the spotted asparagus beetle (*Crioceris duodecimpunctata*) among the pests and the asparagus rust (*Puccinia asparagi*) and purple spot, otherwise called fern spot (*Stemphylium vesicarium*) among the diseases.

Another good reason to grow wild asparagus under the olive canopy is that the asparagus is perennial and does not require tilling. Most olive orchards are now managed with green mulch and no tilling, in order to increase soil organic matter and water storage and to prevent soil erosion. An understory crop that requires soil cultivation would interfere with such practices and lead to reduced benefits. The wild asparagus is a long lasting perennial and, once established, does not require tilling, but only mowing of the grass, which is also needed by the olive orchard.

Figure 1: This is how a modern olive orchard could look like.

Mowing the weeds represents both a cost and en ecological toll, like any use of machinery. In olive orchards, animals have been used recently to obtain weed control without machinery, while obtaining a further animal crop. A selective weed control by the animals, leaving the olive trees intact, is obtained using animals that do not eat olive leaves or animals that cannot reach them. As for the asparagus, given that the vegetation is prickly, most animals, like sheep, tend not to eat it. Other animals like geese could be used as well, as they are used for weeding many crops in the USA (Geiger et al., 2006). The animals must be kept out of the orchards during the harvest period, both to avoid damage to the emerging spears as well as for sanitary reasons. Similarly, organic fertilization or phytosanitary compounds should not be administered before and during spear harvest.

One possible problem of the proposed policulture could be the interference of the prickly vegetation during olive harvest. This vegetation could easily get caught in the screens used for olive harvest if these are laid directly to the ground. Removing the asparagus vegetation during olive harvest in the fall is not advisable as this would probably affect asparagus plant vigor by removing the source of photosynthesis (i.e. the evergreen vegetation) for the whole winter and the following spring during harvest, thus depleting plant reserves too much. However, with mechanical harvesting as trunk shakers equipped with an umbrella or with over-the-row harvesters used for the super high density orchards, this should not be a problem. Other systems to prevent such problems could be probably developed.

4. Conclusions

The wild asparagus (*Asparagus acutifolius*), for its ecological needs and its frugality appears suitable as an understory crop in the olive orchards, with the potential to provide additional income on the same land, while increasing diversity in the field and with no negative effects on the olive yield. Although management of the asparagus-olive policulture, as well as the assessment of the market

potential, must be carefully evaluated, this agroforestry system could allow an increase of income and sustainability of olive orchards, especially in marginal areas. This agricultural model, together with others that could be developed, could contribute to maintaining the olive landscape, which is both historically and economically important in many areas of Europe and the world.

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Thème 5

Bonnes pratiques de fabrication de l'huile d'olive et d'élaboration des olives de table

S9-C1

Essais de désamérisation biologique des olives à l'échelle industrielle

I. Janati Idrissi & M. Rahmani

Résumé

Des essais de fermentation des olives vertes désamérisées par trois souches sélectionnées de *Lactobacillus plantarum,* résistantes à l'oleuropéine ($F_{1,}$ H_{24} et L_{15}), et par voie chimique ont été menés au sein d'une unité industrielle de production des olives de table à Fès/ Maroc. Les analyses physico-chimiques et microbiologiques des saumures montrent que les olives désamérisées chimiquement, fermentent (pH=4,0) au bout de 60 jours. Cependant, pour les olives désamérisées par voie biologique, le pH atteint des valeurs de 4,0 au bout de 40 jours de saumurage, mais l'amertume n'a disparu qu'au bout de 120 jours. Par ailleurs, l'évaluation organoleptique des olives fermentées a montré une meilleure qualité sensorielle des olives désamérisées par voie biologique.

Mots clés : Olives vertes, fermentation lactique, oleuropéine, *Lactobacillus plantarum, désamérisation.*

Trials on biological debittering of olives at an industrial scale

Abstract

Trials on debittering of green olives were conducted by three strains of *Lactobacillus plantarum,* resistant to oleuropein ($F_{1,}$ H_{24} & L_{15}), and by chemical means in an industrial unit specializing in table olives, at Fes/ Morocco. Physico-chemical and microbiological analyses carried on the olive brines showed that olives debittered chemically completed fermentation (to pH 4.0) after 60 days. However, for the biological debittering process, fermentation was shortened to 40 days but the bitterness disappeared only after 120 days. Organoleptic evaluation of fermented olives showed better sensorial characteristics in olives debittered with the biological process.

Key words: Green olives, lactic fermentation, oleuropein, *Lactobacillus plantarum, debittering.*

1. Introduction

Les olives de table vertes « style espagnol » constituent une part importante dans la production mondiale en olives de table. Ce procédé consiste en un traitement alcalin des olives vertes, afin de dégrader l'oleuropéine qui est un composé phénolique responsable de leur amertume (Shasha et Leibowitz, 1961), pour donner de nouveaux composés phénoliques non amers (le dihydroxy-phényl éthanol et l'acide élénolique) (Figue 1) Cette opération est suivie de phases de rinçage et e saumurage (Fernandez Diez, 1971). Ensuite, les olives sont fermentées par les bactéries lactiques, en particulier les *Lactobacillus plantarum*. La désamérisation chimique des olives engendre des rejets alcalins très polluants et contribue à l'altération de la qualité nutritionnelle et sensorielle du produit. Ainsi, l'introduction d'une désamérisation biologique des olives de table, comme alternative au procédé classique, s'avère nécessaire en vue de produire des olives « Bio » tout en respectant l'environnement.

En effet, de nombreux travaux (Ciafardini et al., 1994 ; Marsilio et *al*.,1996, 1999 ; Marsilio et Lanza, 1998) ont été réalisés dans le but d'étudier la possibilité d'hydrolyser l'oleuropéine par voie biololgique par des souches de *Lactobacillus plantarum*, isolées partir des saumures en pleine fermentation. Les résultats de ces études ont montré que les souches testées hydrolysent l'oleuropéine *in vitro* par l'action de la β-glucodidase dans une première étape pour donner l'oleuropéine aglycone, puis par une estérase pour donner l'hydroxytyrosol et l'acide élénolique, composés non amers (Fig. 1).

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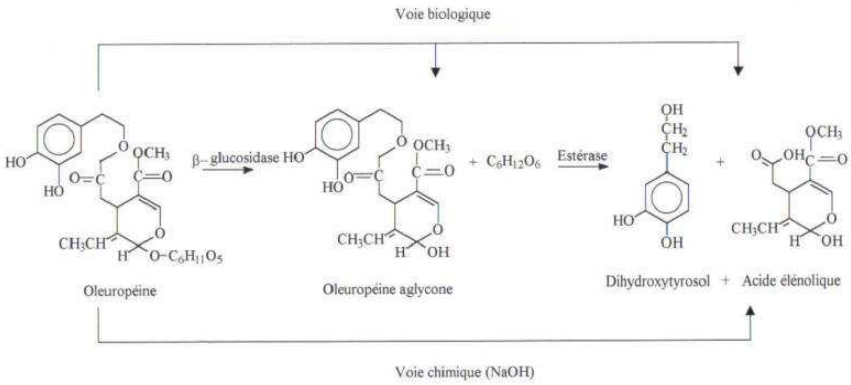

Figure 1: Voies de dégradation de l'oleuropéine, d'après Marsilio et Lanza (1989) .

La désamérisation biologique des olives a été testée à un stade expérimental et n'a pas été entamée à un échelon industriel. Aussi, le présent travail a pour but de sélectionner des souches de *Lactobacillus plantarum* ayant une forte activité oleuropéinolytique et d'expérimenter, à échelle industrielle, la désamérisation biologique des olives.

2. Matériel et méthodes

2.1. Sélection des souches de bactéries lactiques résistantes à l'oleuropéine

Des souches pures de bactéries lactiques ont été isolées sur un milieu MRS Agar à partir de saumures industrielles, du laboratoire, des feuilles d'olivier *« Picholine marocaine »* des margines et des grignons. Ces souches ont été identifiées comme étant des bacilles Gram + non sporulés.

Les souches isolées ont été inoculées sur un milieu MRS Agar contenant 1% (m/v) d'oleuropéine (Extrasynthèse, Genay, France). Les boites de Pétri inoculées ont été incubées pendant trois jours à 30℃. Le milieu MRS Agar à composition normale (san s oleuropéine) a été ensemencé et incubé dans les mêmes conditions pour servir comme témoin.

2.2. Sélection des souches à forte activité oleuropéinolytique

Les souches résistantes à l'oleuropéine ont été inoculées dans les tubes contenant 9 ml de MRS liquide additionné d'un ml d'une solution d'oleuropéine à 1% (m/v). Ensuite, les tubes ont été incubés à 30℃ pendant 96 heures. Après 48 heures d'incuba tion et à la fin de celle-ci, 2 ml d'échantillon de chaque tube on été prélevés et centrifugés à 12.000 x g pendant 10 minutes. Le surnageant a été extrait avec 3 ml d'un mélange chloroforme-méthanol (2/l, v/v). Les extraits ont été agités à l'aide d'un vortex pendant 1 minute et centrifugés à 12.000 x g pendant 10 minutes. Le surnageant a été extrait avec 3 ml d'un mélange chloroforme-méthanol (2/1, v/v). Les extraits ont été agités à l'aide d'un vortex pendant 1 minute et centrifugés à 3.500 x g pendant 5 minutes. Le surnageant a été évaporé à sec et le résidu a été récupéré dans l'acétonitrile (Ciafardini et *al*. 1994). Le même substrat a été utilisé pour l'étude de l'activité estérasique.

L'oleuropéine résiduelle a été dosée par CLHP suivant la méthode de Brenes et *al*. (1998), en utilisant une colonne sphérisorb 5 ODS (250 x 4,6 mm) et un détecteur UV, réglé à 280 nm avec un gradient eau-acétonitrile (ajusté à pH 2,3 avec l'acide phosphorique). Ce gradient est composé de deux stades ; le premier allant de 95% à 75% en eau pendant 20 minutes et le deuxième allant de 75% à 50% en eau pendant 10 minutes. Le débit a été réglé à1 ml/min.

2.3. Identification des bactéries oleuropéinolytiques

Les bactéries oleuropéinolytiques ont été identifiées par un ensemble de tests physiologiques et biochimiques (catalase, acidification, tests d'incubation à 30℃ et 45℃, production de gaz ; la fermentation des sucres a été évaluée par les galeries API-50CHL).

2.4. Etude de l'hydrolyse de l'oleuropéine

2.4.1. Activité β-glucosidase

L'activité β-glucosidase a été étudiée en utilisant comme substrat le 5-bromo-4-chloro-3-indoly-β-D-glucopyranoside (X-gluc) (Sigma Chemical Co., Saint-Louis, Mo.). Dans les boites de Pétri contenant le MRS solide modifié, on a ajouté 0,2 ml d'une solution N,N diméthyl formamide contenant 0,3 % de X-Gluc (m/v). Les boites de Pétri ont été ensuite séchées pendant 1 heure à l'obscurité. Ces boites ont été inoculées par les colonies bactériennes et incubées à 30℃ pendant 48 heures. Les colonies produisant la β-glucosidase se colorent en bleu (Ciafardini et *al*., 1994).

2.4.2. Activité estérase

Le substrat est le même que celui utilisé pour la sélection des souches oleuropéinolytiques.

Le surnageant a été évaporé à sec sous un courant d'azote puis converti en dérivés triméthylsilyls après un chauffage à reflux à 40℃ pendant 30 minut es en présence de l'agent de silylation composé du mélange pyridine-hexaméthyldisilazane-tri-méthylchlorosilane (2 :1 :1, v/v) (Marsilio et *al*., 1996).

Le CG-SM a été réalisée avec un chromatographe Carlo Erba GC 8000, équipé d'un détecteur sélectif de masse tisons Trio 1000. La colonne utilisée est une colonne capillaire DB5 de 30 m de longueur et de 0,32 mm de diamètre. Le gaz vecteur est l'hélium (20 Kpa). La programmation de la température et la suivante : de 50℃ à 150℃ à 5℃/min et de 150℃ à 300℃ à 10℃/min. La température de l'injecteur est de 200℃ ; la quantité injectée est de 1µl.

Le spectre de masse de l'hydroxytyrosol a montré un pic moléculaire [M+] 370 (36) et un pic de base 267 (100).

2.4.3. Conduite de l'essai de fermentation

L'essai de fermentation a été mené au sein d'une unité industrielle de production d'olives de table à Fès. Les olives utilisées pour cet essai proviennent d'un même lot, de la variété «*Picholine marocaine*». Six fûts en plastique d'une capacité de 140 kg d'olive, lavés et désinfectés, ont été remplis d'olives de calibre 260/290 puis de saumure fraîche à 10° Bé. Ces fûts ont été ensuite inoculés par la biomasse déjà préparée à partir des trois souches sélectionnées. Pour chaque souche, deux fûts ont été inoculés, à raison de 10^7 UFC/ml de saumure. Pour l'essai témoin, deux fûts lavés et désinfectés ont été remplis d'olives du même lot ayant subi une désamérisation à la soude à 3 Bé pendant 8 heures, suivie de deux lavages, le premier ayant duré 3 heures et le deuxième 6 heures, et un saumurage à 10° Bé.

2.4.4. Analyses physico-chimiques

Il s'agit des déterminations de pH, de l'acidité libre, et des teneurs en NaCl et en sucres réducteurs.

2.4.5. Analyses microbiologiques:

Il s'agit de dénombrements de bactéries lactiques, de levures et de la flore mésophile aérobie totale (FMAT).

2.4.6. Evaluation organoleptique des olives de table

Une note globale variant de 1 à 9 a été attribuée pour la description des différents attributs sensoriels (odeur, saveur, texture, apparence externe).

3. Résultats et discussion

Les souches F_1, H_{24} et L_{15} ont été sélectionnées pour leur activité oleuropéinolytique prononcée (>90%) et ont été isolées respectivement à partir des feuilles d'olivier, de saumures d'olives tournantes et de saumures d'olives vertes. Les résultats des tests d'identification ont monté qu'il s'agit de souches de *Lactobacillus plantarum*.

3.1. Hydrolyse de l'oleuropéine

Le test d'incubation en présence de X-Gluc a montré que les souches étudiées ont une activité β-glucosidasique. En effet, selon Marsilio et *al.* (1988), le premier composé intermédiaire de la dégradation enzymatique de l'oleuropéine est l'oleuropéine aglycone, grâce à l'action de la β-*glucosidase.*

L'analyse chromatographique des dérivés triméthylsilyls des extraits du MRS liquide de l'oleuropéine, inoculé par les souches de bactéries lactiques sélectionnées, montre que le produit d'hydrolyse de l'oleuropéine est l'hydroxytryrosol, identifié par son spectre de masse, tandis que l'acide élénolique n'a pas été identifié. Ces résultats sont en accord avec ceux trouvés par Marsilio et *al*. (1996) qui suggèrent que ces produits d'hydrolyse de l'oleuropéine sont obtenus sous l'action d'une activité estérasique.

3.2. Suivi des paramètres physico-chimiques au cours de la fermentation:

Le suivi des paramètres physico-chimiques tout au long de la fermentation nous a permis de conclure au bon déroulement de ce processus pour les essais non désamérisés, saumurés à 10° Bé, puis inoculés avec les souches F_1, L_{15} et H_{24} ainsi que pour l'essai témoin désamérisé par une solution de soude à 3° Bé, pouis saumué à 10° Bé.

En effet, le pH a montré une diminution remarquable (de 7,0 à 5,0) pour les essais inoculés et ce, dès le troisième jour après saumurage. Les valeurs de pH atteintes à la fin de la fermentation étaient de l'ordre de 4, au bout de 41 jours de fermentation (Fig. 2).

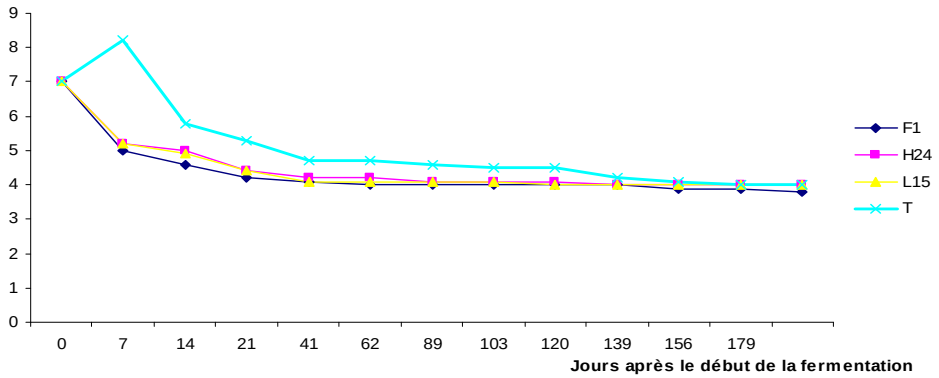

Figure 2: Evolution du pH dans les saumures de fermentation.

Concernant l'acidité libre (exprimée en grammes d'acide lactique pour 100 ml de saumure), les valeurs obtenues à la fin de la fermentation sont de l'ordre de 0,8 % pour tous les essais. Ces valeurs témoignent des bonnes conditions de fermentation, en particulier la température et l'anaérobiose. Concernant l'essai témoin, les valeurs de l'acidité au cours de la première semaine restent faibles par rapport à celle dans les olives non désamérisées. Ces valeurs sont corrélées avec celles du pH lors de la même période.

La teneur en NaCl a baissé sous l'effet des échanges diffusifs. En effet, par gradient de concentration, le sel passe de la saumure vers la pulpe des olives jusqu'à l'établissement de l'équilibre à des valeurs de 7° Bé pour les essais inoculés et 6° Bé pour l'essai témoin. Cette différence est due probablement au passage plus facile du sel de la saumure vers les olives à travers la paroi qui est attendrie par le traitement alcalin.

Les concentrations des sucres réducteurs dans les saumures ont atteint leur maximum à 0,7% ; 0,8% et 0,4%, respectivement pour les essais inoculés et l'essai témoin. Tout au long de la fermentation, ces concentrations étaient plus élevées dans les saumures correspondant aux essais inoculés, ce qui peut être expliqué par la perte des sucres réducteurs causée par le traitement alcalin et le lavage dans l'essai témoin. Cette perte peut atteindre 50% environ de la teneur initiale de ces composés dans l'olive (Marsilio et *al,.* 1998).

3.3. Analyses microbiologiques

Les résultats du dénombrement des micro-organismes ont mis en évidence le développement d'une flore importante, surtout pendant les premières semaines de fermentation. Pendant cette période, on a noté un nombre important de bactéries lactiques ($10{,}5.10^6$ UFC/ml pour les essais biologiques et 10^6 UFC/ml pour l'essai inoculé par la souche L15 ; contre $2{,}6.10^6$ UFC/ml pour l'essai témoin).

On dénote ensuite une diminution des bactéries dont la population se maintient à un niveau plus au moins stable jusqu'à la fin de al fermentation ($3{,}2.10^5$ UFC/ml pour les essais biologiques et $6{,}5.10^5$ UFC/ml pour l'essai témoin).

On remarque aussi une diminution de la FMAT (de l'ordre 5.10^2UFC/ml pour tous les essais) et des levures (de l'ordre de 5.10^3 UFC/ml pour les essais, qui est due à l'acidification du milieu.

3.4. Evaluation organoleptique des olives biologiques

Les résultats de l'évaluation organoleptique des olives biologiques nous ont permis de classer le produit dans la catégorie "très bonne".

3.5. Conclusion

A la lumière des résultats obtenus au terme de la présente étude, on peut confirmer que les olives fermentées par voie biologique présentent toutes les qualités pour qu'elles soient commercialisées. Dans le but de faciliter l'introduction de ce nouveau procédé à une échelle industrielle. On se propose de développer les cultures « starter » et d'optimiser les conditions de fermentation (teneur en NaCl de la saumure, température, teneur des substances fermentescibles, anaérobiose et la concentration de la charge bactérienne du "starter").

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S9-C2

Infection par des bactériophages au cours de la fermentation de l'olive de table

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Résumé

Une infection de phages pendant la transformation des olives de table fermentées pourrait se traduire dans une substantielle diminution et, dans certains cas, même dans une cessation totale de la production d'acide lactique par les ferments lactiques. Donc la présence éventuelle de phages a été recherchée pendant la fermentation des olives. Les olives ont été traitées comme des olives de table verte selon le style traditionnel grec, et divisé en neuf lots. À quatre saumures on a ajouté, comme cultures de demurrage, souches sélectionnées de *Lactobacillus plantarum*; les cinq autres saumures ont été laissés à fermenter spontanément. Après une semaine on a observé l'inhibition de croissance des souches inoculées, nous amenant à soupçonner une éventuelle attaque de phages. Pour vérifier cette hypothèse on a effectué un test de turbidité. La présence de phage a été démontrée par l'inhibition de la croissance de souches testées cultivées en présence d'une aliquote de la saumure privée de cellules microbiennes par filtration stérilisante. Les phages ont également été isolés à partir de saumures fermentées naturellement. La diminution de la turbidité du milieu a indiqué la présence présumée de phages capables de lyser les cellules bactériennes sensibles. La présence de bactériophages a été confirmée par microscopie électronique (SEM). La présence démontrée de phages lytiques active contre *Lactobacillus plantarum* pourrait représenter un sérieux obstacle à l'obtention d'un régulier processus d'acidification. Rechercher des souches résistantes aux phages pourrait aider à surmonter ce problème

Phage infection during fermentation of table olives

Abstract

A phage infection in fermented table olive processing results in a serious decrease and, in some cases, even a total failure in the production of lactic acid by the starter cultures Olives were processed as green table olives according to the traditional Greek-style, with the addition of *Lactobacillus plantarum* B1 as starter culture. After one week, growth inhibition of inoculated strain was observed, leading us to suspect a possible phage attack. To verify this hypothesis, a turbidity test was applied. Phage presence was demonstrated by the growth inhibition of tested strains grown in the presence of an aliquot of the brine, deprived of microbial cells by sterilizing filtration. The decrease in the turbidity of the medium indicated the presumptive presence of phage particles able to lyse sensitive bacterial cells. The presence of bacteriophages was confirmed by scanning electron microscopy. The demonstrated presence of lytic phages active against *L. plantarum* could represent a serious obstacle on the regular acidification process. Search for phage resistant strains could help to overcome this problem.

Key-words: table olives, *Lactobacillus plantarum*, phage infection, fermentation, lactic acid bacteria.

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S9-C3

Extraction de l'huile d'olive vierge par le système de centrifugation continu à deux phases : influence de différentes variables du processus, sur certains paramètres de qualité de l'huile

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Résumé

Dans le but de préserver à l'huile d'olive ses meilleures caractéristiques physico-chimiques et organoleptiques, de nouveaux procédés sont apparus, dont notamment la chaîne continue à deux phases.
L'extraction de l'huile par la chaîne continue intervient par effet de la force centrifuge qui accentue la différence entre les poids spécifiques des liquides non miscibles et des matières solides et permet donc la séparation continue et simultanée de l'huile, de l'eau (margines) et des grignons.

L'évolution et le progrès technologique, qu'ont connu les procédés d'extraction de l'huile d'olive, a fait que les systèmes utilisant la centrifugation de pâte ont pris de la place au dépends des autres. Ce pendant, leur utilisation est loin maîtrisée.

Dans le cadre de rationalisation du système continu (chaîne continue à 2 phases) nous avons voulu connaître l'effet de la température, du temps de malaxage, et du débit de la pâte injecté au décanteur sur les caractéristiques physico-chimiques de l'huile.

Le contenu en polyphénols, chlorophylle, caroténe et la stabilité étaient en relation avec les variations de température, le temps de malaxage et le débit de la pâte.

Mots clés : parameters de qualité

Assessment of a two-phase extraction system on olive oil quality parameters

Abstract

The new methods for olive oil extraction use industrial decanters to separate all the phases by centrifugation. The efficiency of centrifugation is achieved thanks to the differences between specific weights of non miscible liquids and solid materials. Thus, it permits simultaneous and continuous separation of oil, water (olive mill wastewater) and husk. Several technological advances were realized in order to obtain better olive oil characteristics such as physical, chemical and organoleptical traits. Among these novel progresses, appeared the two-phase oil decanter. Yet, satisfactory industrial and technical management of this apparatus is not reached. The aim of this work is the assessment of the effects of the temperature and the duration of mixing and paste injection flow in the decanter on olive oil quality parameters. Several correlations were found between the polyphenol, chlorophyll and carotene contents as well as the oil stability and the studied parameters of the extraction process.

Key words: quality parameters

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S9-C4

Phenolic and volatile compounds from oils of three olive cultivars introduced in the north of Tunisia

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Abstract

The behaviour of three European olive varieties, AscolanaTenera, Koroneiki and Picholine, cultivated in the north of Tunisia, was studied of the quality indices and the minor compounds of their oils. α-Tocopherol showed high values varied from 337 for Ascolana to 424 mg/Kg for Koroneiki whereas total phenols showed the highest value for Picholine oils. Significant differences were observed for the aroma composition between the oils from the foreign cultivars. The major volatile component was the C-6 aldehyde fraction whose content varied greatly between the different varieties studied: The *E*-2-hexenal content ranged from 1.6 mg/kg of oil in the AscolanaTenera variety to >5 mg/kg for the Picholine and Koroneiki cultivars. Therefore, results showed good adaptation for Koroneiki and Picholine to the Tunisian cultivation conditions.

Keywords: Aroma profile / Introduced European cultivars / Olive oil / Phenolic compounds.

1. Introduction

Oleiculture has great economic and social importance in the countries of the Mediterranean region. While Tunisia is a relatively major producer country after the European Union and it is ranked forth. However, the predominated Tunisian cultivar, *Chemlali* variety, is characterized by high levels of palmitic and linoleic acids and sometimes by a low level of oleic acid. A major effort has been made recently for improving the quality of olive oil produced in Tunisia through intervarietal controlled crossings (Rjiba *et al.*, 2009; Dabbou *et al.*, 2006) and the introduction of new cultivars (Dabbou *et al.*, 2009) constitutes means for providing cultivars having a better oil quality. The aim of this work was to study the behavior of three European cultivars introduced in the north of Tunisia.

2. Materials and methods

Plant material and growing areas selected. Olive fruits (*Olea europaea* L.) of the *Ascolana Tenera* (Italy), *Koroneiki* (Greece) and *Picholine* (France) varieties were collected in November 2006 at a known ripeness index from trees all located in the same orchard (a National Collection, 20 km away from Tunis, in North-East Tunisia) and which benefited from the same cultural practices. The experimental field in Béjaoua is characterised by a rainfall of 500 mm/year and a temperature varying from a minimum of 18℃ to a maximum of 32℃. Olives were transformed into oil within 24h. Only undamaged and uninfected fruits were processed at laboratory scale system (Abencor analyser). Finally, the oils were decanted and immediately stored in dark bottles until analysis. *Analytical methods. Quality parameters* (Free Fatty acids, peroxide value and UV absorption characteristics) were carried out following the analytical methods described in the EUC (1991). *Phenolic fractions* were extracted by liquid-liquid extraction (Montedoro *et al.*, 1992) and analysed by HPLC as reported by Selvaggini *et al*. (2006), using an Agilent Technologies system model 1100 (Agilent Technologies, Palo Alto, CA, USA). *α-tocopherol* was measured by the method reported by Psomiadou *et al*. (1998). *Volatile compounds* were determined by headspace solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC/MS), as previously reported by Servili *et al.* (2001) using the 65-mm Carbowax/divinylbenzene fibre (Supelco, Bellefonte, PA, USA) and a GC Varian 4000 equipped with a 1078 split/splitless injector coupled with a Varian Saturn 3 mass spectrometer. The volatile compounds were identified as reported by Dabbou *et al.* (2009). *Statistical analysis.* Significant differences among varieties studied were determined by an analysis of variance which applied a Duncan test with a 95% significant level ($P<0.05$), using the SPSS programme, release 11.0 for windows (SPSS, Chicago, IL, USA). All analyses were carried out in triplicate and the results were presented as means of three repetitions.

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3. Results and discussion

Analytical parameters of olive oils. Physico-chemical characteristics of virgin olive oils obtained from olives of three varieties were presented in Table 1. All the oils produced and analyzed were completely within the limits established by IOOC (2008) as required for the 'extra virgin' category., except for the *Ascolana Tenera* variety having a *K*232 value of 2.7 (Table 1). This value can be attributed to the oxidation of the sample during the processing technology, because the raw material was carefully selected, picked and processed. In fact, cultivar or origin area had no significant influence on these analytical parameters, which were basically affected by factors causing damage to the fruits, e.g., olive fly attacks or improper systems of harvesting, transport and storage of olives (Kiritsakis, 1998; Ranalli & Angerosa, 1996; Ben Temine *et al.*, 2006).

Table 1: Physico-chemical characteristics of the virgin olive oils from the different European olive cultivars grown in the studied area.

Characteristics	Ascolana Tenera	Koroneiki	Picholine
Free fatty acids	0.36 ± 0.06^{a}	0.27 ± 0.06^{a}	0.26 ± 0.03^{a}
Peroxide value	16.78 ± 1.07^{a}	7.48 ± 0.7^{b}	3.42 ± 0.12^{c}
K232	2.78 ± 0.09^{a}	2.01 ± 0.17^{b}	1.59 ± 0.08^{c}
K270	$0.15 \pm 0.01^{a,b}$	0.18 ± 0.03^{a}	0.12 ± 0.03^{b}

Values are the means of the three different VOO samples (n=3) ± standard deviations. Different letters indicate significant differences (P<0.05) between cultivars.

Phenolic compounds (Table 2) are of fundamental importance in virgin olive oil for their nutritional properties, sensory characteristics, and the shelf life of the product (Morello *et al.*, 2004; Servili *et al.*, 2008). In particular, the phenolic compounds are potent antioxidants, and can confer a marked bitter taste or a sweet taste typical of some virgin olive oil (Artajo *et al.*, 2006; Esti *et al.*, 2009). In addition, they are thought to play an important role in human diets as preventative agents against several diseases (Owen *et al.*, 2000, 2004) and are divided in two groups, hydrophilic (phenols) and lipophylic compounds (tocopherols).

Table 2: Phenolic composition (mg kg^{-1}) evaluated in the virgin olive oil samples from the different European olive cultivars grown in the studied area.

Ithanol	Ascolana Tenera	Koroneiki	Picholine
-Tocopherol	337.4 ± 1.3^{c}	424 ± 1.0^{a}	392 ± 0.9^{b}
3,4-DHPEA	1.60 ± 0.9^{b}	5.50 ± 0.0^{a}	1.10 ± 0.0^{b}
p-HPEA	34.03 ± 0.45^{b}	19.93 ± 0.55^{c}	43.57 ± 0.06^{a}
3,4-DHPEA-EDA	54.53 ± 0.15^{c}	58.20 ± 0.6^{b}	116.20 ± 0.1^{a}
p-HPEA-EDA	22.90 ± 0.0^{c}	37.23 ± 0.85^{b}	43.80 ± 0.1^{a}
(+)-1-Acetoxypinoresinol	3.00 ± 0.0^{c}	6.57 ± 0.06^{b}	9.40 ± 0.0^{a}
(+)-1-Pinoresinol	25.70 ± 0.4^{a}	9.53 ± 0.06^{b}	6.27 ± 0.06^{c}
3,4-DHPEA-EA	33.90 ± 0.1^{c}	73.83 ± 1.35^{a}	54.13 ± 0.06^{b}
Total Phenols (HPLC)	174.63 ± 0.15^{c}	210.80 ± 3.5^{b}	274.50 ± 0.0^{a}

Values are the means of the three different VOO samples (n=3) ± standard deviations. Different letters indicate significant differences (P<0.05) between cultivars.

Phenols: The total phenol concentrations (Table 2), established by HPLC, showed significant differences (p<0.05) between different studied olive oils where the ranges were from 174.63 to 274.5 mg kg^{-1}. However, the highest amount of the total phenols was present in *Picholine* olive oil followed by *Koroneiki* oil. These differences were approximately the same for the chromatographic profiles of the different oils. Furthermore, the chromatographic profile showed that the most important phenolic compounds that have been identified in the studied olive oils were phenolic alcohols (hydroxytyrosol, 3,4-DHPEA and tyrosol, *p*-HPEA), secoiridoid derivatives (the dialdehydic form of elenolic acid linked to tyrosol (*p*-HPEA-EDA), the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) and oleuropein aglycone (3,4-DHPEA-EA)) and lignans (pinoresinol and acetoxypinoresinol). These results proved previous works (Bendini *et al.*, 2007; Servili *et al.*, 2004; Hrncirik & Fritsche, 2004).

Table 2 showed that the concentrations of lignans varied inversely (Table 2). In fact, concentration of (+)-1-acetoxypinoresinol ranged from a minimum for *Ascolana Tenera* (3 mg kg^{-1}) to a maximum for *Picholine* olive oil (9 mg kg^{-1}). However, the concentration of (+)-1-pinoresinol was the highest for *Picholine* olive oil (25 mg kg^{-1}) and the lowest for *Ascolana Tenera* (6 mg kg^{-1}). For the simple phenols, the level of tyrosol ranged from 20 to 44 mg kg^{-1}, and was generally higher than that of hydroxytyrosol which ranged from 1 to 6 mg kg^{-1} and was found in all samples analyzed. These results were in accordance with those of several authors for olive oil varieties (Hrncirik & Fritsche, 2004). Moreover, a clear variation can also be observed on the basis of the oils' phenol content (Table 2). In particular, extra virgin olive oil having a low degradation level, estimated by quality parameters, had a high content of the secoiridoid derivative 3,4-DHPEA-EDA and low contents of the simple phenolic compounds 3,4-DHPEA and *p*-HPEA, which was the case of *Picholine* olive oil the most abundant in 3,4-DHPEA-EDA and *p*-HPEA-EDA (116.2 and 43.8 mg kg^{-1}, respectively). On the other hand, oils having an intermediate or an advanced degradation level showed a lower content of 3,4-DHPEA-EDA and higher contents of 3,4-DHPEA and *p*-HPEA. These data are in agreement with the results of Lavelli (2002). Consequently, the concentration and composition of phenolic compounds were strongly affected by olive cultivar (Tura *et al.*, 2007) since agronomical and technological factors were the same for the varieties studied.

α-tocopherol, the main tocopherol species of virgin olive oil (Table 2) seem to be higher in the studied varieties where contents ranged from 337.4 mg kg^{-1} for *Ascolana tenera* to 424 mg kg^{-1} for *Koroneiki* olive oils. *Picholine* oil showed a moderate concentration (392 mg kg^{-1}) but higher than the amount found by Sakouhi *et al.* (2008). In addition, the three olive cultivars, which grown in the same area, differed significantly in α-tocopherol content. This difference probably linked to genotype characteristic and metabolic behavior of each cultivar (Sakouhi *et al.,* 2008).

Volatile compounds, being responsible for most sensory properties of virgin olive oils, play a significant role in the evaluation of the overall oil quality and in the generation of preferences among consumers (Angerosa, 2002) since they are the only parameters that consumers can appraise directly, while other quality features (e.g. chemical composition) are not always labelled on the bottle (Tura *et al.*, 2008). For a better understanding of the aromatic profile of each cultivar, the total content of volatile compounds with chemical affinity or similar biosynthetic pathway was taken in account (Table 3). The total volatile compounds ranged from 17.2 mg kg^{-1} (*Ascolana tenera*) to 23.9 mg kg^{-1} (*Koroneiki*).

Results of SPME–GC analysis of the oil headspace volatile composition (Table 3) showed a large number of substances (23 compounds) consisting mainly of alcohols, esters, as well as flavour active aldehydes and ketones. In fact, the identified compounds were mainly alcohols (such as 1-hexanol, (*Z*)-2- hexen-1-ol and (*E*)-3-hexen-1-ol) which ranged from 0.1 mg kg^{-1} to 12.1 mg kg^{-1}. Furthermore, the quantities of aldehydes differed according to the cultivar with a wide variability from 0.1 to 5.5 mg kg^{-1}, including (*E*)-2-Heptenal and (*E*,*E*)-2,4-Hexadienal that are not originated from lipoxygenase pathway (LOX). However, ketones and esters were also present in small amounts. In addition, (*Z)*-Hexenyl acetate was present in the aroma of *Picholine* and *Ascolana Tenera* oils in low amounts, thus indicating a low presence of alcohol acyl transferase (AAT) whereas it was completely absent in *Koroneiki* oil. Comparing volatile compounds of the three virgin olive oils collected in the same period and planted in the same area, significant differences were observed indicating that olive oil aroma compounds accumulate differently according to the cultivar (Angerosa *et al.*, 1999). Then, *Koroneiki* oil seems to be the richest in alcohols, followed by *Picholine* and *Ascolana Tenera* (17.1, 16.3 and 13.2 mg kg^{-1}; respectively), whereas *Picholine* was the richest in aldehydes (6.3 mg kg^{-1}).

The LOX pathway is a biochemical reaction scheme that accounts for most of the aroma fraction of olive oil containing C6 aldehydes, alcohols and esters (Angerosa, 2002). In fact, the amount of compounds produced from the oxidation of linolenic acid (73.7 and 85.7% of the total of C6 compounds for *Ascolana Tenera* and *Koroneiki* varieties, respectively) was greater than the amount of those biogenerated from the oxidation of linoleic acid (Table 3). Thus, hexanal, the main volatile oxidation product derived from the 13-hydroperoxide, which is one of the major hydroperoxides formed by autoxidation of linoleic acid (García-Martínez *et al.*, 2009), was approximately absent in the studied olive oils. In addition to LOX pathway compounds, the aroma of the studied VOO contained reasonable amounts of pentene dimers and C5 compounds.

Table 3: Volatile composition (mg kg^{-1}) of virgin olive oil, identified by HS-SPME-GC/MS, of the different European olive cultivars grown in the studied area.

Compounds	**Ascolana Tenera**	**Koroneiki**	**Picholine**
6-Methyl-5-hepten-2-one	tr^{b}	tr^{b}	tr^{a}
(E)-2-Heptenal	tr^{a}	nd^{b}	0.1 ± 0.1^{a}
(E,E)-2,4-Hexadienal	0.1 ± 0.1^{c}	0.1 ± 0.1^{b}	0.2 ± 0.1^{a}
(E,E)-2,4-Hexadienal	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}	0.4 ± 0.1^{a}
2-Methyl-1-propanol	0.5 ± 0.1^{a}	0.1 ± 0.1^{c}	0.3 ± 0.1^{b}
2-Methyl-1-butanol/3-Methyl-1-butanol	0.1 ± 0.1^{a}	0.1 ± 0.1^{b}	0.1 ± 0.1^{c}
Benzyl alcohol	0.1 ± 0.1^{b}	0.1 ± 0.1^{a}	0.1 ± 0.1^{b}
Phenylethyl alcohol	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}
Phenol	tr^{a}	$tr^{a,b}$	tr^{b}
3-Pentanone	0.6 ± 0.1^{c}	1.2 ± 0.1^{a}	0.7 ± 0.1^{b}
1-Penten-3-one	nd^{b}	nd^{b}	nd^{a}
(E)-2-Pentenal	nd^{b}	nd^{a}	0.1 ± 0.1^{a}
1-Penten-3-ol	0.2 ± 0.1^{a}	0.3 ± 0.1^{b}	0.3 ± 0.1^{b}
1-Pentanol	0.1 ± 0.1^{c}	0.1 ± 0.1^{a}	0.1 ± 0.1^{b}
ΣC5 compounds	1.0 ± 0.1^{a}	1.6 ± 0.1^{c}	1.2 ± 0.1^{b}
Ethyl acetate	0.8 ± 0.1^{b}	nd^{c}	0.1 ± 0.1^{a}
Hexanal	tr^{b}	tr^{c}	tr^{a}
1-Hexanol	3.4 ± 0.1^{a}	3.0 ± 0.1^{b}	4.4 ± 0.1^{b}
Hexyl acetate	0.2 ± 0.1^{b}	nd^{c}	nd^{a}
ΣC6 compounds from LA	4.4 ± 0.1^{b}	3.0 ± 0.1^{c}	4.6 ± 0.1^{a}
(E)-2-Hexenal	1.6 ± 0.1^{c}	5.3 ± 0.1^{b}	5.5 ± 0.1^{a}
(E)-2-Hexen-1-ol	3.7 ± 0.1^{c}	12.1 ± 0.1^{a}	9.3 ± 0.1^{b}
(E)-3-Hexen-1-ol	0.2 ± 0.1^{a}	0.1 ± 0.1^{c}	0.1 ± 0.1^{b}
(Z)-3-Hexen-1-ol	4.5 ± 0.1^{a}	0.8 ± 0.1^{c}	1.2 ± 0.1^{b}
(Z)-3-Hexenyl-acetate	0.3 ± 0.1^{a}	nd^{c}	tr^{b}
ΣC6 compounds from LnA	10.3 ± 0.1^{c}	18.2 ± 0.2^{a}	16.2 ± 0.1^{b}
Total LOX products	14.7 ± 0.2^{c}	21.2 ± 0.2^{a}	20.8 ± 0.1^{b}
Total esters	1.3 ± 0.1^{a}	-	0.1 ± 0.1^{b}
Total Ketones	0.6 ± 0.1^{c}	1.2 ± 0.1^{a}	0.7 ± 0.1^{b}
Total aldehydes	1.9 ± 0.1^{c}	5.6 ± 0.1^{b}	6.3 ± 0.1^{a}
Total alcohols	13.2 ± 0.1^{c}	17.1 ± 0.1^{a}	16.3 ± 0.1^{b}
Total volatile compounds	17.2 ± 0.1^{c}	23.9 ± 0.2^{a}	23.6 ± 0.1^{b}

Values are the means of the three different VOO samples (n=3) ± standard deviations. Different letters indicate significant differences ($P<0.05$) between cultivars.

The detection of these compounds indicated the existence of an additional branch of the LOX pathway leading to the production of C5 compounds through the alkoxy radical. Consequently, the production of C6 and C5 compounds through the enzymatic oxidation of linolenic and linoleic acids is affected by the cultivar since the degree of ripeness of fruits and their processing conditions were the same (Angerosa *et al.*, 1999).

4. Conclusion

According to results of volatile compounds, phenols and □-tocopherol, we can conclude that virgin olive oils produced from three European cultivars introduced in Tunisia contain a higher number of volatiles. Also, the total phenol content was higher. Furthermore, from the nutritional aspect based on measurements of total antioxidant and volatiles, virgin olive oils produced are to be preferred due to their abundance in aldehydes. In contrast to volatiles and phenols, tocopherol content of these oils appeared to be very consistent between the different olive varieties. These introduced European olive cultivars could be used for blending their oils with autochthonous olive oils, or used for crossings. Finally, the geographic area appears to have a significant effect on the qualitative characteristics and the chemical composition of olive oil.

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Chemical composition of Arbequina, Chemlali, Chetoui virgin olive oil in relation to extraction system; storage conditions and preferens of consumers

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Abstract

Virgin olive oil is characterized by particular aroma, taste and color that distinguish it from other vegetable oils. Virgin olive oil consumption is currently increasing thanks to its excellent organoleptic and nutritive qualities. Our work was carried out to assess the effect of cultivar, extraction method and the packaging on olive oil. Three olive varieties cultivated in different regions on Tunisia have been used: Chetoui (south), Chemlali (centre), and Arbequina the Spanish variety introduced in the south.

Each variety was processed by three extraction systems: two-phase centrifugation, three-phase centrifugation and press system and stored in four types of packaging (opaque glass, transparent glass, metal and polyethylene terephtalate PET) during six months. Analysis revealed significant statistical differences in some parameters mainly in phenol contents, oxidative stability, chlorophyll and carotenoid compound. Most of the quality indices showed significant variations among olive varieties. The study showed that the best packaging which preserves the quality of olive oil is opaque glass. Finally, the sensorial analysis demonstrates that oils extracted by centrifugation system are more preferred by consumers than oils extracted by pressure system.

Key words: olive oil, packaging, sensorial analysis.

Composition chimique d'huile d'olive vierge d'Arbequina, Chemlali, Chetoui en retation avec le système d'extraction, les conditions de stockage et les préférences des consommateurs

Résumé

L'huile d'olive est très appréciée pour sa flaveur caractéristique et sa valeur biologique et nutritionnelle. Ces caractéristiques sont fortement liées à la qualité qui, elle-même, est influencée par plusieurs paramètres tel que la maturité des olives, la variété, les techniques culturales, les modes d'extraction…

Parmi les facteurs, nous nous sommes intéressés dans le présent travail à l'étude de l'impact de la variété, du système d'extraction et du matériau d'emballage utilisé pour la conservation sur la qualité finale de l'huile d'olive. Les huiles utilisées pour cette étude appartiennent à trois variétés cultivées dans différentes régions : chétoui du Nord, chemlali du Centre et arbequina du Nord ; chaque variété a été triturée par deux systèmes d'extraction : le système par centrifugation (deux phases, trois phase) le système par presse. Chaque huile à été conditionnée dans quatre types d'emballage différents : le PET, le verre opaque, le verre transparent et le métal et conservée pendant deux mois.

Les résultats trouvés ont permis de mettre en évidence que les principaux critères de qualité de l'huile d'olive tels que l'acidité, la teneur en carotène et en chlorophylle, la teneur en polyphénols totaux ainsi que la stabilité oxydative sont fortement influencés par le système d'extraction et la variété.

D'autres paramètres comme l'extinction spécifique à 232 nm et 270 nm ainsi que l'indice de peroxyde ne présentent de différence significative que pour l'effet variétal.

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L'influence des interactions entre ces deux paramètres se trouve significative pour l'acidité, l'IP et les phénols totaux.

Concernant la nature de l'emballage, elle n'est pas significative sur l'acidité et l'IP, par contre elle présente une différence significative pour le K232 et le K270.

Ainsi, l'interaction entre système d'extraction-contenant, variété-contenant et terme-contenant n'ont aucun effet significatif sur les critères de qualité.

De même, l'analyse sensorielle a montré qu'il y a une différence significative entre les systèmes d'extraction et les attributs positifs de l'huile ainsi qu'entre le système d'extraction et les notes de préférence des consommateurs.

Mots clés : huile d'olive, emballage, stabilité oxydative, évaluation sensorielle.

1. Introduction

Olive Oil Extra Virgin (HOVE) is - undoubtedly - the more natural it is a good protector and controller of the balance of our health. Its properties beneficial to health and nutrition than justify its price. It represents one of the fat the most popular Mediterranean populations. It is well known for its balanced composition of fatty acids and especially for its high oleic acid composition, in natural antioxidants such as polyphenols and tocopherols as well as for its delicious flavor. The quality of olive oil varies not only depending on the variety, soil and climatic conditions but also many factors in the production cycle, the extraction process and conservation and the material of packaging used. However, like other vegetable oils, virgin olive oils undergo oxidation during storage and therefore it is necessary to limit degradation by the good preservation and good choice of container packaging (Grati Kamoun, 2006). Thus, the objective of this work is to study, initially, the influence of the variety of retrieval system (centrifuge and pressure) and certain storage conditions, specifically the type of packaging (transparent glass, opaque glass, metal and polyethylene tetraphthalate (PET)) on the final quality of olive oil. In a second step, we propose to study the preferences of the oil through a consumer panel.

2. Materials and methods

2.1. Oil extraction and storage conditions

The study focused on different oils from three varieties which were obtained by two extraction systems: system by centrifugation (two and three phases) and pressure system. Oils were packaged in transparent glass of 90 ml, opaque glass bottles of 90 ml and metal cans of 160 ml and polyethylene tetraphtalate (PET) bottles of 90 ml. Then the bottles were completely filled to avoid the air space and were stored at room temperature for two months. Monitoring of quality criteria has been performed on these samples every 15 days.

2.2. Oil analyses

2.2.1. Analytical parameters

Free fatty acid (FFA), peroxide value (PV) and Ultra Violet absorption were determined according to the European Communities official methods (EEC Reg. 2568, 1991).

2.2.2. Fatty acid composition

Fatty acid composition was carried out according to the COI norms (COI/T.20/Document: 24 (2001)). Fatty acid methyl esters (FAMES) were prepared by dissolving 0.1 g of olive oil in 2 mL of heptanes and a methanolic solution of KOH (0.2 N). FAMES were separated and quantified by gas chromatography using a model 5890 series II instrument (Hew-lett-Packard Ca, Palo Alto, CA, USA) equipped with a flame ionisation detector, and a fused silica capillary column HP – INNOWAX (30 m length· 0.25 mm i.d. and 0.25 lm of film thickness). Iodine values were calculated from FA percentages using the formula elaborated by Torres & Maestri, (2006). Oxidative susceptibility was calculated from FA composition according to the formula established by Cert et al. (1999).

2.2.3. Total phenol content

Total phenols were extracted following the method proposed by Gutfinger (1981). Olive oil (2.5 g) was put into a test tube with 5 mL hexane and 5 mL methanol/water (60:40 / v / v) added. Aliquot from the methanol/water layer were added to Folin Ciocalteu reagent and 1 mL sodium carbonate. The phenol content was determined spectrophotometrically at 725 nm and the concentration was expressed as mg of gallic acid per kg of virgin oil.

2.2.4. Pigments content

Beta-carotenes and chlorophylls (mg/ kg) were determined at 470 and 670 nm, respectively, in cyclo-hexane using the specific extinction values, according to the method of Minguez-Mosquera et al. (1991).

2.2.5. Evaluation of oxidative stability

The oxidative index was determined by the Rancimat Model 743 (Metrohmn, Switzerland). Three grams of the sample were introduced in the test tube and heated at 120 ℃, and air was bubbled through the oil at a flow rate of 20 L h^{-1}.

2.2.6. Sensory analysis

The sensory evaluation was performed by two different tests: the analytical test which determine the sensory characteristics of the oil and ranking it according to its sensory profile; this analysis is performed by an expert jury corresponding a group of subjects selected according to the guidelines of international standards. Panel members are trained to recognize the different flavor characteristics and measuring their intensities using the standard COI/T.20/Doc No 15/Rév.2 (2007). The hedonic tests are conducted by a panel of 200 consumers through a questionnaire. The tasters choose, for each sample, the category that corresponds to their degree of appreciation. The different samples were coded and presented to consumers by randomization and repetition. All varieties were tasted by the same panel of consumers, not trained in order to know the olive oil most appreciated by consumers.

2.3. Statistical analyses

The values of the parameters studied were expressed in average, the number of repeat is 3; the comparison between samples was performed by analysis of variance (ANOVA) followed by test "t" of Student for averages inappropriate. These tests were made through the software Statgraphics version 5.0, the significance level α is 0.05.

3. Results and discussion

3.1. Analytical parameters

Analysis of the acidity function of the retrieval system shows acidity values between 0.2 and 1.3%. All acidity results from the centrifuge system (two and three phases) to meet the standard COI (2009) (acidity <0.8 for the extra virgin), while the pressure system generates an oil more acidic (0.5 to 1,3%). These results are confirmed by the work of Torres and Maestri (2006) and Chimi (2006). The largest change of acidity was observed in oils from the pressure system. From the box diagram of the acidity we can see that the average acidity of different samples obtained by the SP system is shifted to the median which highlights the tendency of these oils with an acidity important than the oils obtained by centrifugation system with two and three phases. These results are consistent with results found by Torres and Maestri (2006) and Chimi (2006).

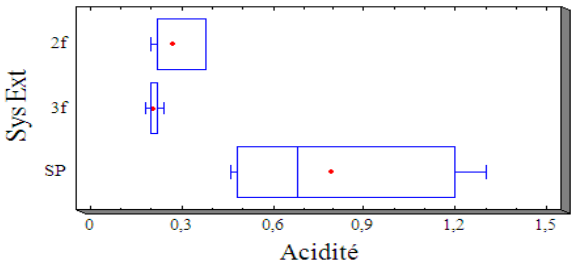

Figure 1: Diagram box acidity.

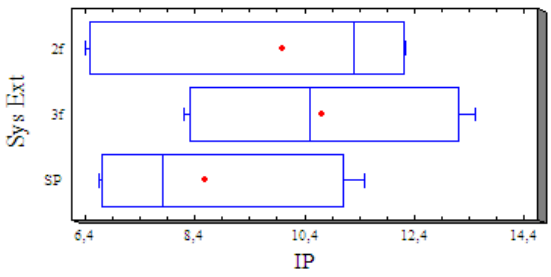

Figure 2: Diagram box of peroxide index.

The results for the specific extinction show that all values are in the K_{232} standard (COI, 2008) which sets a limit of K_{232} < 2.5. The lowest value was recorded in oil from the system three phases, it is of the order of 2.136. The highest is observed in oil from the system by pressure. Regarding the K270, all samples meet the standards of the COI (K270 <0.22). The lowest value is observed in oil from the system in three phases, it is of the order of 0.142. The highest value (0.162) was observed in oil from the system by pressure.

All mean values of the peroxide index (PI) of different samples meet the standard (COI, 2009) which sets a value of PI < 20 méq d'O2 / Kg. Statistical analysis showed that the influence of the extraction system on the peroxide value is not significant. This result is confirmed by the work of Gimeno et al. (2002). The lowest index is recorded in the oil obtained by pressure system (8,594 meq O2 / kg) while the highest (10.704 meq O2 / kg) was observed in oil from the system three phases.

3.2. Evaluation of oxidative stability

The statistical study showed that the influence of the extraction system on the oxidative stability was significant (p <0.05). The highest average is observed in the oil from the system in two phases (10.96 h) while the lowest average value is observed in the oil from the system by pressure (8.56778 h). This allows us to conclude that oil from the two phase system is more resistant to oxidation and extraction centrifugal systems generate oil more stable. These results are consistent with those found by Salvador et al. (2003).

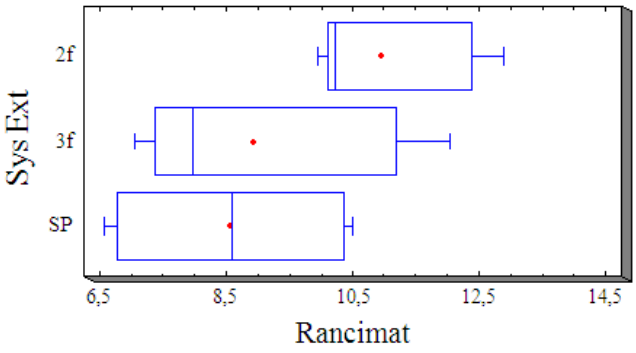

Figure3: Diagram box oxidative stability.

3.3. Chlorophylls and B-Carotenes content

The study of the influence of the extraction system on the chlorophyll content of different samples showed a significant difference that mainly depends on the extraction system (P <0.05). This result is in compliance with the work of Torres and Maestri (2006) who reported that there was no significant difference between the extraction system and the chlorophyll content. The oils from the two-phase system have the highest values (8.16 ppm). However, the lowest value is observed in oils from the pressure system (6.09 ppm).

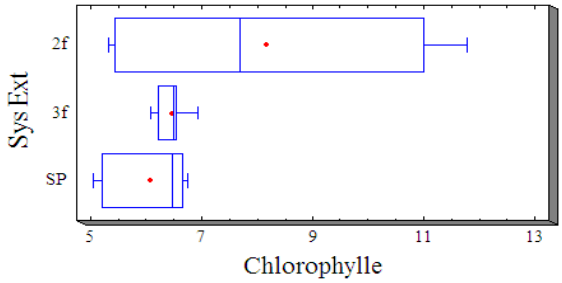

Figure 4: Diagram box of chlorophylls.

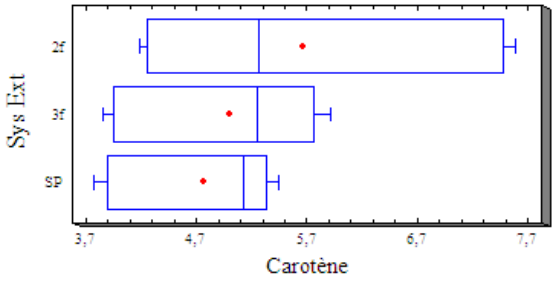

Figure 5: Diagram box of Carotenes.

The highest content (5.66) is observed in oils extracted from the two-phase system while the lowest (4.77) is recorded in oils crushed by pressure. Taking into account that the carotenoids, especially the β-carotene are potent antioxidants thanks to their ability to trap oxygen species (Morello et al., 2004). Therefore, it is possible to say that the oils with the highest values β-carotene (oils obtained from the two-phase system) are more resistant to oxidation.

3.4. Total phenol content

The highest content is observed in the oil obtained from the two-phase system; the lowest level is observed in the sample from the three-phase extraction system.

In fact, the phenols of the olive paste are soluble in water and oil; the addition of water during the mixing operation reduces the concentration of phenols in the oil phase. So the two-phase centrifugation system better preserves the amount of polyphenols, as the addition of water is very low during the mixing. This result is consistent with the work of Gimeno et al. (2002).

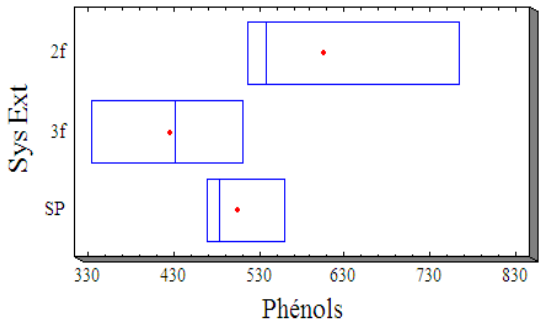

Figure 6: Diagram box of chlorophylls.

3.5. Sensory analysis

3.5.1. Sensory analysis by the expert panel

3.5.1.1. Effect of the extraction system on the attribute notes

The statistical study shows that there is a significant effect of the extraction system on the bitter attribute. The highest score was observed in the two-phase system. The lowest grade was observed in the pressure system. Statistical analysis shows that there is a significant difference between the extraction systems and fruitiness. The two-phase system gives a fruitier oil than the three-phase and pressure systems.

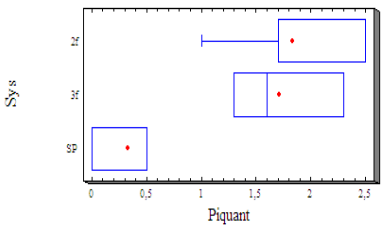

Figure 7: Diagram box of the astringency attribute.

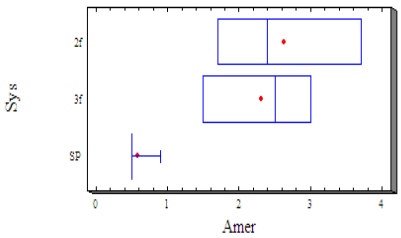

Figure 8: Diagram box of the bitter attribute.

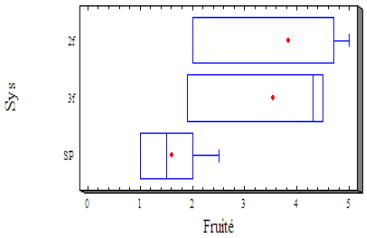

Figure 9: Diagram box of the fruity attribute.

3.5.2. Sensory analysis by consumers

Statistical analysis reveals a significant effect of the extraction system on the grades given by consumers. Those under the age of 30 preferred oils from the three-phase system than the oil obtained from the two-phase and the pressure systems. Yet, consumers whose age is above 30 preferred oils from the two-phase system rather than the three-phase and the pressure systems.

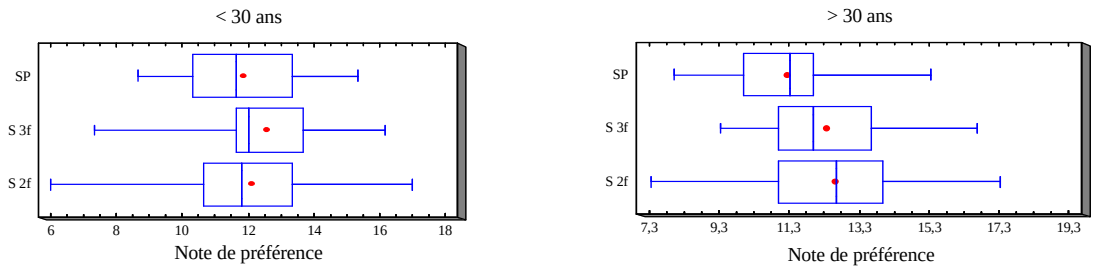

Figure 10: Diagram box of the grades of consumer preferences.

3.6. Effect of packaging on the quality of the oil acidity

By analyzing the diagram box of acidity, we note that the values of acidity meet all the standards (COI, 2009). The oil stored in a glass container, either opaque or transparent, has the lowest acidity content, while the oil stored in a PET packaging has the highest content. So the glass is the best type of packaging material for the preservation of oil followed by the metal packaging in the second rank and eventually the PET in the third rank.

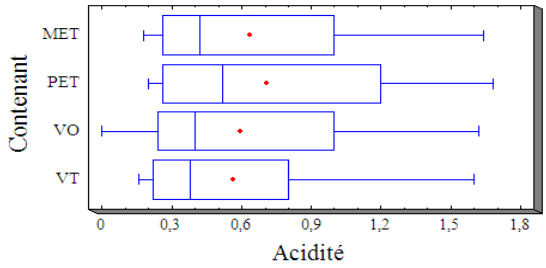

Figure 11: Diagram box of Acidity.

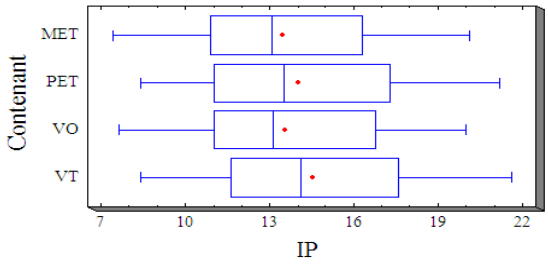

Figure 12: Diagram box of peroxide index.

VT: clear glass; VO: opaque glass, PET: polyethylene terephthalate; MET: metal; PI: peroxide index

The analysis diagram of the PI box shows that all values are classified according to the standard (COI, 2009). The oil stored in opaque glass shows the lowest value of PI, the one stored in PET has the highest value. But statistical analysis by ANOVA doesn't show a significant effect of container on the PI.

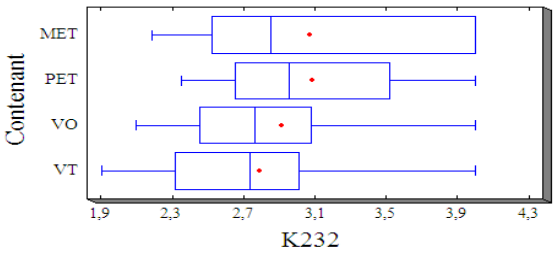

Figure 13: Diagram box of K232.

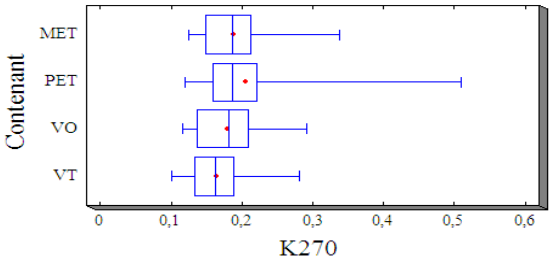

Figure 14: Diagram box of K270.

VT: clear glass; VO: opaque glass, PET: polyethylene terephthalate; MET: metal

Statistical analysis shows that there is a significant influence of packaging on the K232 ($P < 0.05$). Oils packaged in PET at room temperature have values significantly higher than those packaged in glass. So PET promotes primary oxidation, due to its permeability to oxygen. These results are identical to those found by Ben Tekaya (2007a). Similarly for K232, statistical analysis shows that there is a significant effect of packaging on the K270. The highest value is observed in oils packaged in PET. The lowest is observed in oils packaged in glass; result consistent with that of Ben Tekaya *et al.,(*2007b).

4. Conclusion

Upon completion of this work, we have noted that the main quality index essentially acidity, peroxide value, the K232, K270 and the oxidative stability are influenced by variety and retrieval system type of packaging used. This study has therefore concluded that: Variety "Chetoui" is the most stable point of view resistance to oxidation than the varieties 'Chemlali' and 'Arbequina' due to its richness in antioxidants (polyphenols, chlorophyll and carotene) and C18: 1 and C18:2 . The extraction system by two-phase centrifugation allows for better oil quality (physico-chemical and sensory) that the system by centrifugation three phases and pressure, which can give the best oils to improve the condition grinding conditions: splitting the amount of water added during mixing, low temperature of 25 °C and mixing time between 45 and 60 min. The opaque glass is the most appropriate packaging for packaging olive oil because it preserves more nutritional quality and taste and do not let light through.

Consumers with an age <30 years preferred oils of the variety "Chemlali 'from the extraction system by centrifugation three phases. By cons consumers aged over 30 prefer the variety of oils "Chetoui 'from the extraction system by centrifugation two phases.

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S9-C7

Composition and Antioxidant Activity of Olive Leaf Extracts from Greek Olive Cultivars

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Abstract

The phenolic composition of olive leaves of the Greek cultivars *koroneiki, megaritiki* and *kalamon* grown in North Greece was determined using LC/MS. Furthermore, the antioxidant activity of olive leaf extracts from the above three cultivars, obtained using solvents of increasing polarity (petroleum ether, dichloromethane, methanol and methanol/water 60%) was studied. The antioxidant activity of the olive leaf extracts was determined using the stable free radical diphenylpicryl-hydrazyl (DPPH) test, as well as the oxidative stability index (OSI) in comparison to the synthetic antioxidant TBHQ and commercial oleoresin (rosemary extract). The ability of phenolic compounds to inhibit the lipoxygenese (LOX) activity was also investigated. The ten main components determined in the olive tree leaf extracts for the cultivars koroneiki and kalamon were: secologanoside, dimethyloleuropein, oleuropein diglucoside, luteolin-7-O-glucoside, rutin, oleuropein, oleuroside, quercetrin, ligstroside and verbascoside.

Respective compounds for the cultivar megaritiki were: secologanoside, dimethyloleuropein, oleuropein diglucoside, luteolin7-O-glucoside, oleuropein, oleuroside, quercetrin and ligstroside. In all three cultivars, oleuropein represented the main phenolic component.. When methanol/water (60%) was used, as solvent, more phenolic compounds were determined. The total phenols determined in the extracts, obtained by successive extractions using the above solvents, were 6094, 5579 and 6196 mg/Kg (mg gallic acid/kg dried olive leaves) for the cultivars megaritiki, kalamon and koroneiki, respectively. Among all extracts, methanol/water extracts exhibited the highest antioxidant activity applying the DPPH and OSI methods. The antioxidant activity of olive oil samples containing the above additives during the OSI method followed the sequence: synthetic antioxidant TBHQ **>** commercial oleoresin **>** olive tree leaf extracts **>** control. Likewise, methanol/water extracts significantly inhibited soybean lipoxygenase, although some small differences in the activity among the extracts of the different cultivars of olive leaves were observed. A positive correlation was shown between the antioxidant activity of leaf extracts and the total phenol content.

Keywords: olive leaves, antioxidant activity, phenolic components, lipoxygenase activity, DPPH

1. Introduction

Lipid oxidation has been one of the main interests of the scientific community for centuries. Researchers are continuously seeking those natural antioxidants that will sufficiently protect fats and oils from oxidation. Synthetic antioxidants are very effective and stable under usual processing and storage conditions of oils (Kiritsakis et al. 2003). They have however certain disadvantages, such as relatively high cost. Moreover, there are scientific reports on the possible toxicological effects of synthetic antioxidants, such as BHA, BHT and TBHQ (Gharavi et al. 2007). Although there are no conclusive results on the safety of these substances, world-wide interest has arisen for the recovery and utilization of antioxidants from natural sources (Loliger et al. 1996). Research has focused on antioxidant compounds derived from leaves and fruit of olive trees, numerous fruits and vegetables, as well as aromatic plants and spices (Gamel and Kiritsakis 1999; Papagrigoriou et al. 2007; Gkanatsiou 2007)**.**

Recently, research has focused on the antioxidant properties of olive tree leaf extracts (Kähkönen 2000). Salta et al. (2007) enriched commercially available oils (olive oil, sunflower oil, palm oil and a vegetable shortening) in polyphenols by adding olive leaf extract. Results showed that both antioxidant capacity and oxidative stability were substantially improved for all oils studied. A level at 400 ppm of free phenolics, extracted from olive leaves exhibited high antioxidant activity and was superior to that of butylated hydroxy toluene (BHT) in retarding sunflower oil oxidative rancidity (Farag et al. 2003).

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Aliquots of concentrated crude olive leaf juice were added to sunflower oil and heated to 180℃. The samples exhibited remarkable antioxidant activity and at a concentration of 800 ppm were superior to that of BHT in increasing sunflower oil stability (Farag et al. 2007). A phenol extract of high hydroxytyrosol content obtained from olive leaves (*Olea europaea* L.) increased the oxidative stability of different food lipids (butter, lard and cod liver oil) (. De Leonardis, 2008). The enrichment of refined olive and husk oils with olive leaf extract and its hydrolysate extract by Bouaziz et al. (2008) resulted in an increased resistance to oxidative deterioration due to its phenolic antioxidants content. The authors suggested that both hydrolysate and leaf extracts are excellent antioxidants and may serve as substitutes for synthetic antioxidants.

Due to the increasing interest in the use of natural antioxidants, the present study was carried out to evaluate the phenolic composition of selected olive leaves of three Greek cultivars and to determine the antioxidant activity of olive leaf extracts obtained by different solvents of increasing polarity.

2. Materials and Methods

2.1. Materials

Olive leaves were collected from an olive orchard located in the area Trilofos of Thessaloniki in the fall of 2007. Four trees of each of the cultivar *koroneiki, megaritiki* and *kalamon* were selected. Samples were taken from each tree including different parts of the tree and were mixed. Leaves were left to dry at room temperature for one week before use. 1,1.Diphenyl 2-picryl hydrazyl (DPPH), Folin-Ciocalteu reagent, lipoxygenase (Kiritsakis 1983; Bouaziz et al. 2008; De Leonardis et al. 2008; Zheng and Wang 2001) type I-B (soybean) and linoleic acid (sodium salt), 99% purity, were purchased from Sigma (St Louis. MO, USA). TBHQ was obtained from Eastman Co, while the commercial oleoresin was purchased from (Kalsec. Co., MI, USA). All chemical reagents were of analytical grade.
Extra virgin olive oil from the island of Crete, with acidity of 0.5% and peroxide value of 7 meqO_2/kg oil, was used.

2.2. Phenol extraction from the olive leaves

Phenols were extracted from the olive leaves by successive extractions, using solvents of increasing polarity (*petroleum ether, dichloromethane, methanol and methanol/water 60%*). 10 g of dried leaves from each cultivar were mechanically milled and placed in Erlynmeyer flasks. 200ml of petroleum ether were added into each flask and left for 24 h at room temperature followed by filtration using Whatman 47mm x 0.45 Micron filters. The filtrates were evaporated in a rotary evaporator. The dry residues of the olive leaves were returned in the flasks, where 200 mL dichloromethane were added and contents were kept for another 24 h. The procedure was repeated as shown in Fig. 1, with methanol and methanol/water 60%. All extracts were evaporated under mild conditions of temperature (40-45℃), in order to avoid the decomposition of phenolic compounds.

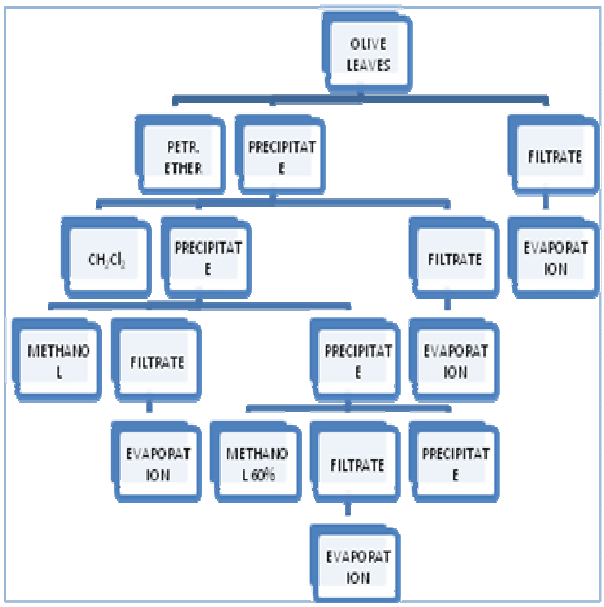

Figure 1: Flow diagram for the preparation of olive leaf extracts with successive extractions.

Dry residues obtained from each cultivar and solvent treatment were analyzed for their phenol content.

2.3. Determination of Total Phenol Content

The total phenol content of the obtained fractions was determined using the method of Zheng and Wang (2001) with few modifications: 5 mg of dry residue from each solvent extraction was dissolved in 1 ml DMSO. 100 μl of this solution were transferred to a volumetric flask to which 500 μL of Folin-

Ciocalteu phenol reagent and 400 μL of 7.5% sodium carbonate solution were added. The mixture was shaken thoroughly and kept for 1.5 h at 30^{o}C, in the absence of light. The absorbance of the blue colour formed was measured at 765 nm. The concentration of total phenol compounds for each extract was calculated on the basis of a standard curve obtained using gallic acid as the standard (twelve serial-2 fold dilutions to give a range of 0.01-0.001 mg/ml in triplicate). Results were expressed as mg of gallic acid per 100 g of dried weight.

2.4. Evaluation of the antioxidant activity with OSI apparatus

Given amounts of the dry residues from each cultivar, the synthetic antioxidant TBHQ and the commercial oleoresin (rosemary extract) were dissolved in DMSO. The stock samples were of such concentration that by adding 1 mL of each solution to 4 g of olive oil, mixtures of oil containing 100 ppm phenols were prepared. A control sample, containing 4 g of olive oil and 1 ml DMSO, was also prepared. The flow rate of air in the OSI apparatus was set at 16L/h and the temperature at 110℃. The OSI values, which correspond to the beginning of the propagation period or the end of the initiation period, were automatically recorded.

2.5. Interaction with DPPH stable free radical

The DPPH method (Kontogiorgis and Hadjipavlou-Litina, 2005) was used to determine antioxidant activity of olive leaf extracts. 20 μL from the stock solution of the sample (approximately 2.5 mg in 1 mL DMSO) were dissolved in absolute ethanol to a final volume of 1 mL and then added to 1 mL DPPH (0.1 mM, in absolute ethanol). The reaction mixture was kept at room temperature. The optical density (OD) of the solution was measured at 517 nm, after 20 and 60 min. The optical densities of the samples in the absence of DPPH were subtracted from the corresponding OD with DPPH. The % reduction values were determined and compared to appropriate standards.

$$\%\text{Reduction} = \frac{\text{control OD (mean)} - \text{sample OD (mean)}}{\text{control OD (mean)}} \times 100$$

2.6. Soybean lipoxygenase inhibition

All extract samples were initially dissolved in DMSO (approximately 2.5 mg in 1 mL DMSO). 10 μL or 1 μL of the solution were mixed with 100 μL of sodium linoleate (0.1 mM) and 0.2 mL of the enzyme solution (1/9x10^{-2}% w/v salt solution pH=9). Samples were incubated at room temperature for 3 min. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid was recorded at 234 nm and compared to an appropriate standard inhibitor (caffeic acid IC_{50}=600 μM) (Zheng and Wang, 2001; Kontogiorgis and Hadjipavlou-Litina, 2005).

2.7. Analysis of the phenolic compounds in olive leaf extracts

LC-MS analysis was performed in a Finningan LCQ Deca ion-trap mass spectrometer (Finningan MAT, San Jose, CA, USA) coupled with a Thermo Separation series liquid chromatographic system (Thermo products, San Jose, CA, USA) consisting of UV3000, AS3000, P4000, SCM1000, a membrane degasser and an injection valve (100μl loop). An XTerraR RP 18, 3.5μm, 4.6x150mm (Waters, Ireland), with an XTerraR RP 18, 3.5μm, 4.6x10mm guard column. Elution was performed at a flow rate of 1.0 ml/min, using as mobile phase a mixture of water/acetic acid (99.9:0.1 v/v) (solvent A) and acetonitrile/acetic acid (99.9:0.1 v/v) (solvent B). Both eluents were of HPLC grade and filtered through a 0.20μm filter disk (for solvent A) and a 0.45μm filter disk (for solvent B). The injection volume was 20μl. The solvent gradient changed according to the following conditions: from 96% (A)-4% (B) to 76% (A)-24% (B) in 35min, 53% (A)-47% (B) in 15min, 100% (B) in 10min and 96% (A)-4%(B) in 10min followed by 10min of conditioning the column with the initial conditions. Chromatograms were acquired at 232nm, 280nm and 370nm. The HPLC data were collected and processed by Chromeleon data system.

The MS analyses were carried out using an electrospray (ESI) interface operating in both positive and negative mode using the following conditions: Helium gas was used as a stealth gas at a flow rate of 28 arb. The electrospray voltage was 3.70kV and the heated capillary temperature and voltage were maintained at 270^{o}C and -37.00V respectively for negative mode. The tube lens offset voltage was set at -55 V. For positive mode scanning, the electrospray voltage was 4.70kV, the heated capillary

temperature and voltage were maintained at 270^{o}C and 15.00V respectively, while the tube lens offset was set to 45V. The molecular ions were scanned from 100.0 to 2000.0 (m/z) in such a scanning order that full-scan mass spectrum was followed by a tandem mass spectrum (MS/MS). The MS data were collected and processed by Xcalibur data system. The system was optimized for oleuropein on the m/z ratios of 539 and 541 corresponding to the negative and positive ion of oleuropein respectively.

3. Results and Discussion

3.1. Analysis of phenolic compounds

Figure 2 shows the phenolic compounds isolated from the olive cultivars megaritiki, koroneiki and kalamon. Nine compounds namely: demethyloleuropein, oleuropein diglucoside, luteolin-7-O-glucoside, rutin, oleuropein, oleuroside, quercetrin, ligstroside, verbascoside were identified in the cultivar megaritiki, while ten compounds (secologanoside, demethyloleuropein, oleuropein diglucoside, luteolin-7-O-glucoside, rutin, oleuropein, oleuroside, quercetrin, ligstroside, verbascoside) were identified in kalamon cultivar and the same apart from secologanoside and ligstroside were identified in koroneiki cultivar. Oleuropein was by far the main constituent in all three cultivars. The compounds identified were in accordance with the related literature (Savourin, 2001).

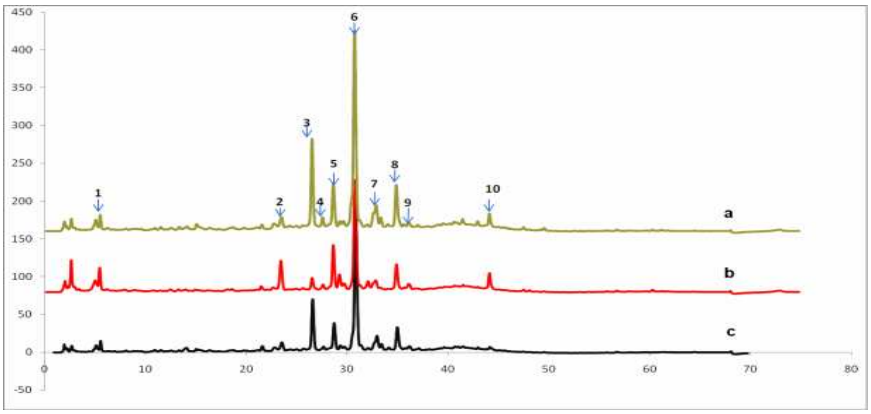

Figure 2: RP-HPLC chromatograms of the three cultivars extract 1t 280nm UV absorbance (1: Secologanoside, 2: Demethyloleuropein, 3: Oleuropein diglucoside, 4: Luteolin-7-O-glucoside, 5: Rutin, 6: Oleuropein, 7: Oleuroside, 8: Quercetrin, 9: Ligstroside, 10: Verbascoside). (a: Kalamon, b: Koroneiki, c: Megaritiki).

Two oleuropein derivatives (Fig. 3) were identified during MS analysis. The two peaks represent oleuropein (Fig. 4, retention time 31.96 min) and oleuroside (Fig. 4, retention time 34.01 min) which have the same mass. The only difference in structure between the two compounds is the position of the olefinic double bond in the elenolic acid moiety so the identification based on MS was not possible.

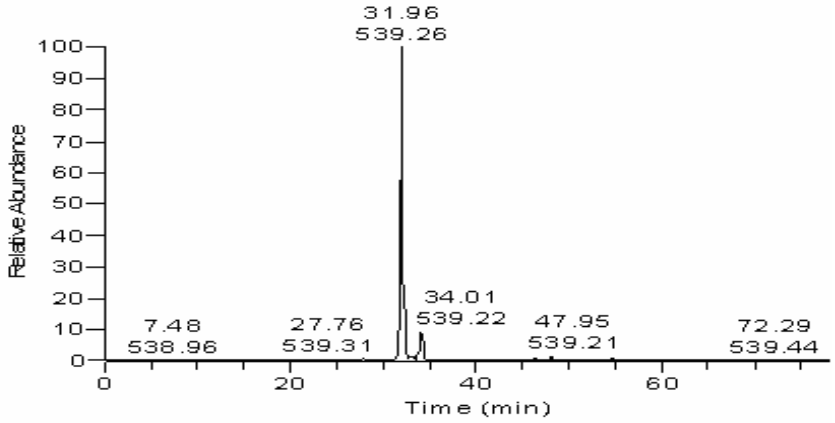

Figure 3: Oleuropein derivatives identified during MS ion scanning (Oleuropein, retention time 31.96 and Oleuroside (retention time 34.01).

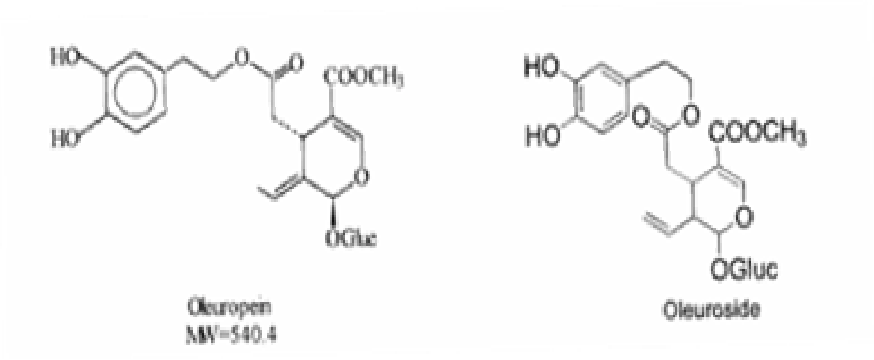

Figure 4: Oleuropein (retention time 31.96) and oleuroside (retention time 34.01).

3.2. Determination of Total Phenols

Table 1 shows the concentration of the main phenolic compounds found in the olive leaves of the three tested cultivars. Table 2 on the other hand, shows the amount of the dry residues collected from the three cultivars and the four kinds of solvents tested to obtain the phenolic extracts. The highest amount was isolated when methanol/water was used. Among the cultivars, koroneiki gave the highest amount of extract followed by megaritiki and kalamon. The weight extract was for the cultivars koroneiki, megaritiki and kalamon 1.48, 1.44 and 1.43 g respectively. When petroleum ether was used as the solvent, the lowest amount of phenols was obtained from all the three cultivars.

Table 1: Concentration of the main phenolic compounds determined in the olive leaves of the three cultivars (mg of phenols in 100 g olive leaves).

Compound	Kalamon	Koroneiki	Megaritiki
Oleuropein diglucoside	4.13	1.44	0.96
Rutin	1.59	1.66	1.11
Oleuropein	8.48	4.89	3.26
Quercetin	1.74	1.07	0.71

Table 2: Weights (g) of dry residues of phenolic extracts.

Megaritiki	0.227+0.305+25.925+34.469= 60.926 mg
Kalamon	0.238+0.137+21.052+34.358= 55.785 mg
Koroneiki	0.137+0.267+27.533+34.024= 61.961 mg

Combining the results of the Tables 3-6, the total mg of phenols in each cultivar were:

Solvent	Cultivar		
	Kalamon	Koroneiki	Megaritiki
Petroleum Ether	0.0542	0.049	0.0484
Dichloromethane	0.291	0.3036	0.3543
Methanol	0.9483	1.1971	1.1522
Methanol 60%	1.4316	1.4793	1.4362

Table 3: Phenol concentration when petroleum ether was used as solvent.

Cultivar	mg gallic acid in 100 g sample	mg phenols in dry residues
Megaritiki	470	0.227
Kalamon	440	0.238
Koroneiki	280	0.137

Table 4: Phenol concentration when dichloromethane was used as solvent

Cultivar	mg gallic acid in 100 g sample	mg phenols in dry residues
Megaritiki	86	0.305
Kalamon	47	0.137
Koroneiki	88	0.267

Table 5: Phenol concentration when methanol was used as solvent

Cultivar	mg gallic acid in 100 g sample	mg phenols in dry residues
Megaritiki	2250	25.925
Kalamon	2220	21.052
Koroneiki	2300	27.533

Table 6: Phenol concentration when methanol/water (60%) was used as solvent

Cultivar	mg gallic acid in 100 g sample	mg phenols in dry residues
Megaritiki	2400	34.469
Kalamon	2400	34.358
Koroneiki	2300	34.024

The amount of olive leaves used was 10g. Thus, the phenol concentration in mg/Kg for the cultivars, megaritiki, kalamon and koroneiki was 6093, 5579 and 6196 respectively. Amounts of phenols determined in the present work are substantially lower than those reported by Skerget et al. (2004). Differences observed may be related to the use of different cultivars and different procedures of extraction.

3.3. Evaluation of the antioxidant activity with OSI apparatus

As Table 7 shows, all olive leaf phenolic extracts extended the initiation period of oxidation compared to the control. They enhanced the oxidative stability by 5.5 to 6.5 hrs or 30% compared to the control. TBHQ showed the strongest effect, increasing the oxidative stability by 10 hrs or 47%. Commercial oleoresin also increased antioxidant activity, extending the initiation period by 9,2 hrs or 42%. TBHQ seems to be the strongest antioxidant in other studies as well (Wanasundara and Shahidi, 1994).

3.4. Interaction of olive leaf extracts with the stable free radical of DPPH (1,1-diphenyl-2-picryhydrazyl)

Figure 5 shows the results of the interaction of the olive leaf extracts from three different cultivars with the stable free radical of DPPH. The % interaction was determined after 20 and 60 min.

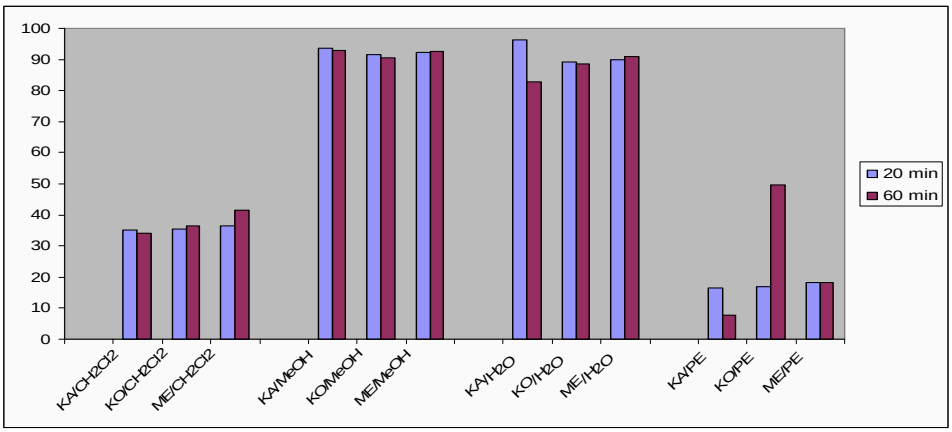

Figure 5: Interaction of olive leaves extracts with DPPH for 20 and 60 min. (Cultivars: ME: Megaritiki, KO: Koroneiki, KA: Kalamon, Solvents: CH_2Cl_2: Dichloromethane, MeOH: Methanol, H_2O: MeOH 60%, PE: Petroleum ether).

Almost all petroleum ether and dichoromethane extracts were found to be less active against DPPH. Between the two, dichloromethane extracts presented higher reducing activity than petroleum ether extracts, except in the case of cultivar koroneiki after 60 min. The interaction with DPPH does not increase with time. Only in the case of koroneiki cultivar/petroleum ether, there was a significant increase in reducing activity with time. On the contrary, the reducing activity of the kalamon cultivar/petroleum ether seemed to decrease with time, whereas for megaritiki it remained constant. Almost all methanol and methanol/water (60:40) extracts presented the same antioxidant activity. Their activity did not seem to be time dependent. Only in kalamon cultivar/methanol-water extract, the reducing activity decreased after 60 min.
All of the olive leaf extracts presented antioxidant activity. However there was no statistically significant difference ($p<0.05$) among the antioxidant activities of the cultivars. The petroleum ether extracts of koroneiki and kalamon cultivars were the least potent, due to their low polyphenol content.

3.5. Determination of soybean lipoxygenase inhibitory activity induced by the extracts from the olive tree leaves of four greek cultivars

The soybean lipoxygenase assay was used as an indication of the antioxidant activity of the extracts with the higher DPPH interaction values. Linoleic acid was used as substrate for soybean lipoxygenase. Linoleic acid is enzymatically converted to a conjugated diene, which results to a continuous increase in absorbance at 234 nm. A mixture of DMSO and buffer served as control (no enzyme inhibition), while the reported value for caffeic acid was used as a positive control.
The inhibitory activity of the extracts of olive tree leaves from the three different Greek cultivars was tested at two different concentrations (10 μL and 100 μL). Almost all the extracts presented the same inhibitory activity at the higher concentration (Fig. 6). Significant differences were observed at the lower concentration (10 μL) of the extracts. The inhibitory activity was concentration dependent.

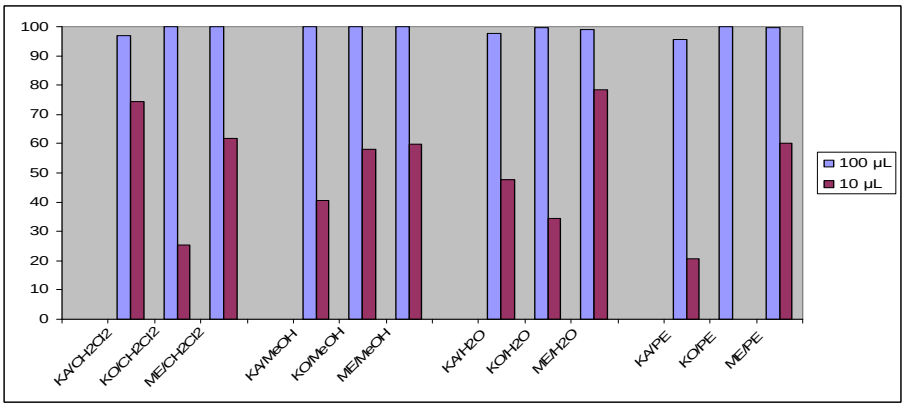

Figure 6: Soybean lipoxygenase inhibition by 10 and 100 μL olive leaf extracts (Cultivars: ME: Megaritiki, KO: Koroneiki, KA: Kalamon, Solvents: CH_2Cl_2: Dichloromethane, MeOH: Methanol, H_2O: MeOH 60%, PE: Petroleum ether).

There was a statistically significant difference ($p>0.05$) among the extracts and the cultivars respectively. Almost in all cases the methanol and methanol/water (60:40) extracts were the most potent against lipoxygenase. The dichloromethane extracts of kalamon and megaritiki cultivars presented a higher inhibitory activity against LOX as compared to the koroneiki cultivar. Most of the LOX inhibitors are antioxidants or free radical scavengers (Skerget et al. 2004), since lipoxygenation occurs via a carbon-centered radical. Based on present results it is obvious that the % DPPH scavenging activity is correlated to the LOX % inhibition (Fig. 6). Certain studies (Muller 1994) suggest a relationship between LOX inhibition and the ability of the inhibitors to reduce Fe^{+3} at the active site to the catalytically inactive Fe^{+2}. Many flavonoids and other phenolic derivatives inhibit soybean lipoxygenase through the above proposed mechanism (Muller 1994). Thus the presence of compounds with a free -OH group could account for the inhibition effect shown in tested samples. It has been suggested that olive leaves are a source of antioxidants acting as radical scavengers (Papoti and Tsimidou, 2009.

4. Conclusion

The present study showed that due to the diversity and complexity of the natural mixtures of phenolic compounds in the olive leaf extracts of various cultivars it is rather difficult to compare their antioxidant activities. There were also some antioxidant activities in olive leaves that may be attributed to other unidentified compounds or to synergistic interactions.

The concentration of phenols obtained from the three different Greek cultivars (koroneiki, megaritiki and kalamon) varied with type and polarity of solvent used for the extraction. The highest amount of phenols was extracted when methanol/water 60% was used. Among the culivars, koroneiki seemed to have the highest concentration of phenols. Many different phenolic compounds were determined in the leaves of the three olive cultivars. However, oleuropein was found in the highest concentration in all three cultivars.
When the OSI method was applied, using virgin olive oil as the substrate, the antioxidant activity of additives followed the order: Synthetic antioxidant TBHQ **>** Commercial oleoresin **>** Olive tree leave extracts **>** Control.

The LOX inhibitory activity was affected by the solvent polarity used. Results obtained for this enzyme, using petroleum ether as solvent, confirmed the results of the DPPH method and gave an indication for the higher antioxidant activity of the phenolic compounds present in the cultivar koroneiki.
The olive leaf extracts present significant antioxidant activity which renders such products useful for the enhancement of the oxidative stability of edible oils.

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S10-C1

Études organoleptique, chimique et spectrale d'huiles d'olives vierges d'AOC françaises

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Résumé

Les caractéristiques sensorielles, chimiques (acides gras et triglycérides) et spectrales (Moyen infrarouge) d'huiles d'olive vierges de six AOC françaises (Aix-en-Provence, Haute-Provence, Nice, Nîmes, Nyons and Vallée des Baux de Provence) (n= 600) ont été déterminées sur des huiles provenant de six années de récolte consécutives. L'évaluation du fruité, de l'amertune et du piquant n'est pas suffisante pour décrite les AOC. Aussi, il est nécessaire de décrire les huiles par des descripteurs analogiques qui sont estimés par les dégustateurs selon des critères définis. Une Analyse en Composante Principale utilisant 34 paramètres (acides gras, triglycérides et squalène) permet de différencier les six AOC. Toutefois les AOC Aix-en-Provence et Vallée des Baux de Provence ne sont pas complètement résolues car elles possèdent toutes les deux, deux variétés principales en commun (*Salonenque* et *Aglandau)*. L'utilisation conjointe de la spectrométrie infrarouge et de l'analyse multivariée (PLS-DA) constitue une approche originale pour étudier les relations entre les AOC, leur composition et leur origine géographique. L'interprétation spectroscopique des premiers vecteurs de régression indique que chaque AOC est caractérisée par un acide gras ou un triglycéride spécifique. De bons résultats ont été obtenus malgré la similitude de composition des deux AOC Aix-en-Provence et Vallée des Baux de Provence. La spectroscopie infrarouge constitue une méthode alternative à l'authentification des huiles d'olive vierges de six AOC françaises.

Mots clés : Huile d'olive vierge, AOC, analyse sensorielle, analyse chimique, analyse infrarouge, chimiométrie, authentification.

Abstract

The sensory and chemical (fatty acid and triacylglycerol compositions) characteristics of six registered designations of origin (RDOs) of French virgin olive oils (Aix-en-Provence, Haute-Provence, Nice, Nîmes, Nyons and Vallée des Baux de Provence) (n= 600) were determined over five consecutive year harvest periods. The evaluation of the fruity, bitter and pungent attributes was insufficient for describing the RDOs, so it was necessary to complete the oil descriptions with descriptive attributes (analogical describers) estimated by the tasters. Principal Component Analysis, using 34 parameters, has ensured to differentiate the six RDOs. The Aix-en-Provence and Vallée des Baux de Provence RDOs are not completely differentiated because the two RDOs have two principal varieties in common : *Salonenque* and *Aglandau*. The morphograms of fatty acid and triacylglycerol compositions are genuine fingerprints of the six RDOs. The combination of mid infrared spectroscopy with multivariate analysis (PLS-DA) provides an original approach to study profile of virgin olive oils (VOOs) in relation to composition and geographical origin. Spectroscopic interpretation of regression vectors has shown that each RDO is correlated to one specific component of VOO according to their cultivar compositions. The results are satisfactory, in spite of the similarity of cultivar compositions between two denominations of origin (Aix-en-Provence and Vallée des Baux de Provence). Chemometric treatment of MIR spectra makes it possible to obtain similar results to those obtained by time-consuming analytical techniques such as GC and HPLC, and constitute a fast and robust tool for authentication of these French VOOs.

Keywords: Virgin olive oil, RDO, sensory, chemical analysis, infrared analysis, chemometry, authenticity

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1. Introduction

La détermination de la traçabilité des produits de l'industrie agroalimentaire est devenue essentielle en raison des problèmes de santé publique qu'ils peuvent présenter ainsi que pour assurer la loyauté des échanges commerciaux. La traçabilité nécessite de disposer d'outils non subjectifs pour connaître l'ensemble des éléments permettant de suivre le cheminement d'un produit de son origine jusqu'à son utilisation par les consommateurs. La filière oléicole n'échappe pas à cette nécessité de traçabilité.

À ce jour, environ 200 variétés différentes d'oliviers ont été dénombrées en France parmi lesquelles une quinzaine sont des variétés principales pour la production d'huiles d'olive vierges (HOV) (Moutier et al., 2004)**.** 30% environ de la production française sont commercialisés et valorisés soit sous forme d'huiles monovariétales soit sous forme d'assemblages avec le label Appellation d'Origine Contrôlée (AOC) (Aix-en-Provence, Corse, Haute-Provence, Nice, Nîmes, Nyons, Provence et Vallée des Baux de Provence). Diverses approches ont été proposées pour répondre à la demande de traçabilité des HOV en utilisant des caractéristiques chimique ou organoleptique. Toutefois, la détermination de la traçabilité constitue un véritable défi analytique qui nécessite la mise en œuvre de méthodes permettant la détermination de paramètres multiples pour répondre à cette demande émergente.

Cette étude porte sur les huiles de 6 AOC françaises (Aix-en-Provence, Haute-Provence, Nice, Nîmes, Nyons et Vallée des Baux de Provence), pour lesquelles les caractéristiques organoleptique, chimique (composition en acides gras et en triglycérides) et spectrales seront déterminées et utilisées pour la reconnaissance de leur origine.

2. Matériels et méthodes

2.1. Analyse sensorielle

Les intensités de fruité, d'amer et de piquant ont été évaluées selon la méthode d'analyse organoleptique décrite dans le Règlement Européen n°2568/91. Les analyses ont été réalisées sur des huiles des campagnes oléicoles 1996/1997 à 2005/2006 (Aix-en-Provence, n= 27 ; Haute-Provence, n=31 ; Nice, n=38 ; Nîmes, n=18 ; Nyons, n= 19 ; Vallée des Baux de Provence, n=48). Les dégustations ont eu lieu deux mois et quatorze mois après la date de production. Pendant cette période, les échantillons sont conservés à 12°C à l 'abri de la lumière. Ces échantillons d'huiles ont fait parallèlement l'objet d'une dégustation analogique selon la méthode présentée par Pinatel et al (2004) qui fournit une classification des descripteurs analogiques selon leur importance, à la fois en intensité et en reconnaissance par les divers membres du jury. Le nombre maximal de descripteurs pris en compte est de 14.

2.2. Analyses chromatographiques

2.2.1. Echantillons

600 échantillons provenant du Centre Technique de l'Olivier, (CTO), Aix-en-Provence, France et sont représentatifs de lots commerciaux produits dans les moulins français. Ils sont issus de cinq campagnes oléicoles successives (2000/2001-2004/2005. Les AOC Haute-Provence (n=92), Nice (n=102), Nyons (n=104) sont constituées d'une seule variété principale. Les AOC Aix-en-Provence (n=130) et Vallée des Baux de Provence (n=131) peuvent être constituées de trois ou quatre variétés principales dont deux sont obligatoires sans que leurs proportions ne soient imposées. L'AOC Nîmes (n=41) doit être constituée d'au moins 60% de la variété Picholine.

2.2.2. Préparation et analyse des esters méthyliques d'acides gras

La préparation et l'analyse des esters méthyliques ont été précédemment décrites par Ollivier et al. (2006).

2.2.3. Analyse des triglycérides

L'analyse des triglycérides a été précédemment décrite par Ollivier et al. (2006).

2.3. Analyses spectrales

Les spectres (100 accumulations) sont enregistrés entre 4000 et 600 cm^{-1} avec une résolution spectrale de 4 cm^{-1} sur un spectromètre Thermo Nicolet Avatar à transformée de Fourier (source Ever-Glo, détecteur DTGS). Tous les échantillons sont enregistrés en utilisant un accessoire de réflexion totale atténuée (ATR) munie d'un cristal de diamant. Chaque spectre est la moyenne de 3 spectres indépendants.

2.4. Chimiométrie

Cette étude utilise une méthode de prédiction, la régression PLS appliquée conjointement aux données spectrales et aux données d'origine codée en binaire. Cette méthode est une alternative à la régression linéaire multiple classique. La régression PLS est une méthode d'analyse de données initiée par Wold (1966) qui a connu un fort développement par la suite (Geladi & Kowalski, 1986 ; Tenenhaus, 1998). Ses premières applications à l'analyse quantitative remontent aux années 1980 (Mardia et al, 1981). Les applications chimiométriques sont réalisées à l'aide du logiciel Unscrambler 9.6, distribué par la société CAMO (Computer Aided Modelling, Trondheim, Norway).

2.5. Morphogramme

Une représentation graphique radiale (morphogramme), réalisée à partir d'un tableur est fondée sur l'écart des variables (acides gras et triglycérides) par rapport à une moyenne déterminant l'origine (0%) des mêmes variables issues d'une base de données (FATG-BD02). Cette base de données a été établie à partir des compositions en acides gras et en triglycérides provenant d'HOV de 46 variétés françaises, de cinq AOC françaises et d'HOV provenant de six pays méditerranéens (Espagne, Italie, Grèce, Tunisie, Maroc, Turquie) totalisant 1400 échantillons d'huiles. Sur chaque morphogramme, qui correspond à une AOC donnée, chaque axe donne pour une variable donnée, la position de la moyenne centrée à 50 % des valeurs centrales pour cette variable. Cette moyenne est établie à partir des valeurs comprises entre le premier et le troisième quartile, bornes qui sont aussi représentées sur le graphique par le tracé en pointillés. Ces trois points sont positionnés sur chaque axe en pourcentage par rapport à la variation maximale observée pour la variable correspondante sur l'ensemble des huiles de la base de données.

3. Résultats et discussions

3.1. Analyse sensorielle

La description des six AOC françaises, selon le Règlement Européen n°2568/91, avec les trois descripteurs positifs : fruité, amer et piquant obtenus ne permettent pas de les différentier. On ne peut en effet distinguer certaines appellations que par l'amertume qui permet de séparer les huiles de Nîmes, des huiles de trois autres appellations, Nice, Nyons et Vallée des Baux de Provence. Ce sont les descripteurs analogiques qui peuvent permettre à l'analyse sensorielle d'obtenir une discrimination plus efficace. Le tableau 1 donne l'ordre d'importance des différents descripteurs relevés pour chacune des appellations obtenus à partir des profils analogiques des mêmes huiles.

Tableau 1: Principaux descripteurs analogiques des huiles d'olive vierges de six AOC françaises.

AOC	Premier descripteur	Deuxième descripteur
Aix-en-Provence	Artichaut cru (2,0)[a]	Feuille/herbe (3,0)
Haute-Provence	Artichaut cru (1,0)	Banane verte (2,0)
Nice	Amande fraîche (1,0)	Artichaut cru (3,0)
Nîmes	Prune (1,0)	Foin frais (2,5)
Nyons	Pomme (2,0)	Noisette fraîche (4,0)
Vallée des Baux de Provence	Artichaut cru (1,0)	Amande fraîche (2,0)

[a] La valeur entre parenthèse correspond à la médiane des positions du descripteur au classement par ordre d'importance.

Ces résultats constituent une première étape pour la recherche de descripteurs en vue de construire des moyens d'identification et de contrôle des huiles d'olives en appellation. Les résultats les plus intéressants, de ce point de vue, sont ceux qui fournissent, pour une appellation donnée, un

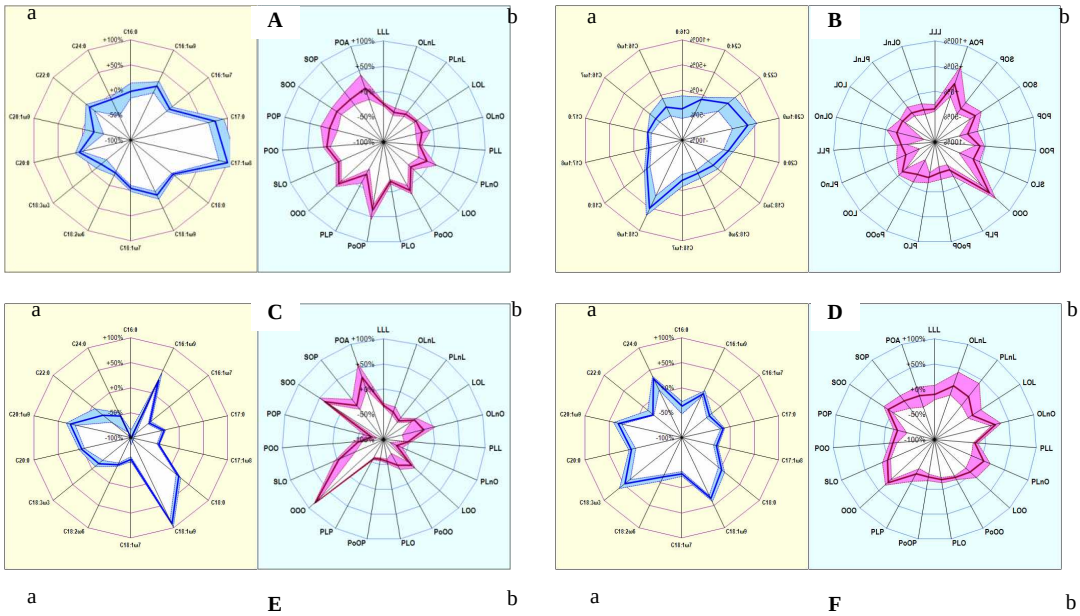
descripteur très spécifique en premier. C'est le cas pour le descripteur "prune" sur l'AOC Nîmes. La présence d'un descripteur spécifique en deuxième position comme le descripteur "banane verte" sur l'AOC "Haute-Provence", est aussi très informatif. Par contre, le descripteur "fleur de genêt" de l'AOC Nice, bien que très spécifique, est trop souvent minoritaire ou non perçu pour être utilisé dans une grille de contrôle de profil.

3.2. Analyses chimiques

Tous les échantillons étudiés possèdent les mêmes 14 acides gras et les mêmes 19 triglycérides mais à des taux différents suivant les AOC (Ollivier et al., 2006 ; Ollivier et al., 2007). Les compositions en acides gras et en triglycérides des six AOC sont présentées sous forme de morphogrammes basés sur la banque de données (FATG-BD02). Les AOC Haute-Provence, Nice, Nîmes et Nyons qui sont constitués d'une seule variété principale, ont des morphogrammes différents et caractéristiques pour les acides gras (a) et les triglycérides (b) (Figure 1). L'AOC Haute-Provence (cv *Aglandau*) (Figure 1A) est caractérisée par des valeurs voisines de 100% pour les acides margarique (17 :0) et margaroléique (17:1n-8). Les autres acides sont proches de la moyenne (0%) à l'exception des acides linoléique (18:2n-6) et linolénique (18:3n-3) qui y sont légèrement inférieurs. Les triglycérides sont voisins de la moyenne sauf PoOP qui avoisine le niveau de 50% tandis que LLL, OLnL, PLnL, LOL et PLL sont inférieurs à 0%.

L'AOC Nice (cv *Cailletier*) (Figure 1B) possède des valeurs comprises entre 0 et 50% pour les acides oléique (18 :1n-9), gondoïque (20:1n-9) et béhénique (22:0). Les autres acides se situent à des valeurs proches ou inférieures à 0% notamment les acides palmitique (16:0), palmitoléique (16:1n-7), margarique (17:0) et margaroléique (17:1n-8). L'acide stéarique (18:0) dont le taux est le plus bas de toutes les AOC caractérise l'AOC Nice. Les triglycérides sont en accord avec les acides gras avec OOO proche de 50% et des valeurs proches de 0% ou inférieures pour les autres triglycérides.

L'AOC Nyons (cv *Tanche*) (Figure 1C) est caractérisée par des valeurs voisines de 50% pour les acides hypogéique (16:1n-9) et stéarique (18:0) et une valeur supérieure à 50% pour l'acide oléique (18:1n-9). En revanche, l'acide palmitique (16:0) est proche de 100% et les acides palmitoléique (16:1n-7), margarique (17:0), margaroléique (17:1n-8) et Z-vaccénique (18:1n-7) ont des pourcentages de l'ordre de 50%. Cette AOC est la plus riche en acide oléique (18:1n-9) et trioléine (OOO) des six AOC. L'AOC Nîmes (*cv Picholine, Négrette*) (**Figure 1D**) présente des morphogrammes spécifiques. Nîmes est particulièrement riche en acides linolénique (18:3n-3) (~50%) et gondoïque (20:1n-9) (~50%) et a des valeurs de l'ordre de 50% pour les acides palmitique (16:0), palmitoléique (16:1n-7), margaroléique (17:1n-8) et Z-vaccénique (18:1n-7). Les triglycérides OLnL et OLnO sont voisins de 50% tandis que PoOO, PoOP et POP et SOP sont proches de 50%.



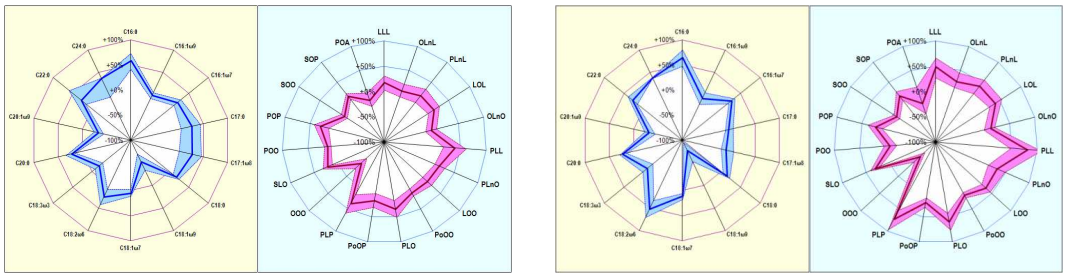


Figure 1: morphogrammes des acides gras (a) et des triglycérides (b) des AOC Haute-Provence (A), Nice (B), Nyons(C), Nîmes(D), Aix-en-Provence (E) et Vallée des Baux de Provence (F).

Les Figures 1E et 1F montrent les acides gras et les triglycérides des AOC Aix-en-Provence (cv : *Aglandau, Salonenque, Cayanne*) et Vallée des Baux de Provence (cv : *Salonenque, Aglandau, Grossane, Verdale des Bouches du Rhône*) qui résultent des variétés qui les constituent. Les formes des représentations des deux AOC présentent de fortes similitudes. Ce résultat était prévisible puisque les deux AOC ont en commun deux variétés principales *Aglandau* et *Salonenque*. Une analyse fine du morphogramme des acides gras de l'AOC Aix-en-Provence montre que cette AOC possède le cultivar *Aglandau* comme variété majoritaire en raison des taux élevé en acides margarique (17:0) et margaroléique (17:1n-8). Une analyse similaire pour l'AOC Baux de Provence permet de conclure que c'est le cultivar *Salonenque* qui est majoritaire en raison des taux élevés des acides palmitique (16:0) et linoléique (18:2n-6) présents dans cette huile. Ces résultats sont en accord avec les populations des ces cultivars (*Aglandau* et *Salonenque*) sur les aires de production de ces AOC.

3.3. Analyse spectroscopique

Les spectres moyens infrarouges sont organisés en matrice et les différentes AOC sont repérées par un codage binaire. Pour une origine considérée, un échantillon est codé 1 lorsqu'il appartient à cette origine et 0 lorsqu'il n'en fait pas parti. Une analyse discriminante est réalisée en utilisant la méthode PLS-DA (Galtier et al., 2008)**.** Une étape préliminaire est réalisée afin de déterminer si les huiles proviennent d'une AOC française ou d'une huile étrangère. Dans ce cas, un modèle d'étalonnage est réalisé avec un total de 100 HOV dont 50 proviennent de France et 50 proviennent des principaux producteurs d'huile d'olive (Espagne, Grèce, Italie, Turquie). Le but étant de savoir si l'HOV est française, un seul modèle PLS-DA est réalisé considérant l'origine "France" par rapport à toutes les autres. On considère, dans ce cas, les quatre origines étrangères comme une seule pour la construction du modèle. Le modèle est ensuite validé sur 100 HOV françaises et sur 45 HOV étrangères n'ayant pas servi au calcul du modèle Si pour un échantillon d'HOV, l'origine prédite est "France", les modèles correspondant aux six AOC françaises lui sont alors appliqués. Les HOV françaises sont toutes prédites avec une valeur supérieure 0,75, celles des autres origines sont prédites avec une valeur inférieure à 0,25. Tous les échantillons sont donc bien prédits, et aucun ne se trouve proche de la valeur seuil à 0,5. La distinction de l'origine géographique "France" ou "étrangère" ne pose donc aucun problème.

Pour la reconnaissance des différentes AOC françaises, l'étude est menée sur 342 échantillons (250 échantillons sont utilisés pour la calibration et 92 pour la prédiction). Les résultats en prédiction sont rassemblés dans le Tableau 2.

Tableau 2: Prédiction de l'appartenance d'huiles d'olive vierges à une AOC française.

	Aix-en-Provence	Haute-Provence	Nice	Nîmes	Nyons	Baux
Nombre d'échantillons prédits	21	13	15	9	13	21
Nombre de coefficients de régression	6	6	6	5	4	6
Aix-en-Provence	**20** (95%)	0	0	0	0	1
Haute-Provence	0	**13** (100%)	0	0	0	0
Nice	0	0	**15** (100%)	0	0	0
Nîmes	0	0	0	**8** (89%)	0	0
Nyons	0	0	0	0	**13** (100%)	0
Vallée des Baux de Provence	1	0	0	0	0	**21** (100%)

Régression PLS-DA obtenue après traitement SNV des spectres dans la gamme 4000-650 cm^{-1}.

Cette bonne classification des échantillons en fonction de leur AOC peut s'expliquer par l'interprétation des vecteurs de régression, et en particulier du premier de chaque modèle. Il est connu que le premier coefficient de régression est une bonne approximation du composé pur discriminant (Haaland & Thomas, 1988) dans le cas de régression PLS. Donc, les coefficients de régression obtenus pour chaque modèle AOC sont des approximations du composé discriminant des AOC françaises. L'interprétation de ces coefficients est très utile pour permettre la compréhension des modèles AOC en fonction de leur composition chimique.

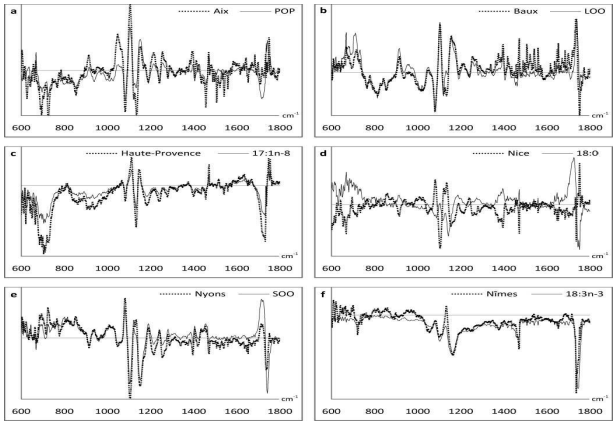


Figure **2:** Comparaison des coefficients de régression des modèles AOC et des modèles acides gras et triglycérides obtenus à partir des spectres MIR .

Les coefficients de régression obtenus pour les modèles de prédiction des AOC ont été comparés à ceux obtenus pour l'analyse quantitative des acides gras et des triglycérides des HOV (Galtier et al., 2008). Chaque coefficient de régression de chaque modèle AOC présente une grande similitude avec un coefficient de régression des modèles acides gras et triglycérides (**figure 2)** : l'AOC "Aix-en-Provence" et le triglycéride POP ; l'AOC "Haute-Provence" et l'acide gras 17:1n-8 ; l'AOC "Nice" et l'inverse de l'acide gras 18:0 ; l'AOC "Nîmes" et l'acide gras 18:3n-3 ; l'AOC "Nyons" et le triglycéride SOO ; l'AOC "Vallée des Baux" et le triglycéride LOO.

Le Tableau 3 présente le taux de ces six composés dans les six AOC françaises déterminés par chromatographie. Ce tableau montre que ces acides gras ou ces triglycérides sont effectivement caractéristiques pour chacune des AOC. En ce qui concerne les AOC "Aix-en-Provence", "Haute-Provence", "Nyons" et "Vallée des Baux de Provence", le pourcentage des différents composés associés (POP, LOO, SOO et 17:1n-8) est discriminatoire car il est le plus élevé dans chaque AOC associée. Au contraire, pour l'AOC "Nice", le pourcentage de 18:0 est le plus faible par rapport aux autres AOC ; cela explique que les coefficients de régression soient inversés.

Tableau 3: Proportions des six composés discriminatoires pour chaque AOC française (résultats chromatographiques).

AOC	18 :0 (%)	17 :1n8 (%)	18 :3n3 (%)	POP (%)	SOO (%)	LOO (%)
Aix-en-Provence	2,45	0,20	0,59	3,77	3,35	14,71
Haute-Provence	2,32	0,31	0,54	3,38	3,66	12,04
Vallée des Baux de Provence	2,36	0,13	0,59	3,68	3,10	15,81
Nice	2,04	0,09	0,57	2,78	3,30	12,64
Nîmes	2,34	0,14	0,85	3,10	3,62	13,62
Nyons	2,58	0,08	0,56	2,02	4,47	12,29

4. Conclusion

L'analyse sensorielle et les compositions en acides gras et en triglycérides permettent de caractériser les huiles d'olive vierges des six AOC françaises étudiées. La corrélation existant entre la composition chimique d'une HOV et son origine variétale ou géographique a rendu possible à partir des spectres MIR, d'une part la distinction d'huiles d'AOC françaises d'huiles étrangères, et d'autre part l'identification de l'appartenance à une AOC des HOV françaises.

La comparaison des coefficients de régression des modèles de prédiction des AOC déterminés par spectroscopie infrarouge à ceux déterminés à partir des analyses chromatographiques montre des similitudes importantes. Chaque AOC possède un composé susceptible de la différencier des cinq autres.

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S10-C2

Authentification de l'huile d'olive dans l'alimentation animale par la technique de RT-PCR

George Perrakis, Emmanuel Politis & **George Siragakis**

Résumé

Pendant les deux ans derniers, plusieurs fermiers utilisent de l'huile d'olive dans le feedstuffs pour avoir la meilleure qualité de viande. Le but de cette étude était d'établir un essai analytique à base d'ADN sensible pour la détection d'huile d'olive dans la nourriture d'animal. Pour l'isolement d'ADN 3 échantillons de fourrage ont été utilisés leur contenu de pétrole après soxhlet l'extraction. En plus, l'huile d'olive pure dans de différentes quantités (conforme à 100 %, 20 %, 10 %, 5 % et 1 % d'huile d'olive dans la nourriture d'animal) a été appliquée pour déterminer la limite de cette technique de détection. L'essai a été davantage évalué sur la nourriture d'animal après l'enrichissement avec 20 % et 10 % d'huile d'olive. Une étude comparative a été faite de deux différents kits commerciaux (Quiagen et r-Biopharm) pour la récupération d'ADN d'arbre vert olive de haute qualité. L'effet boule de neige polymerase ultérieur (PCR) a été conçu en utilisant des abécédaires d'ADN universels amplifiant le chloroplaste trnL (UAA) intron. La traçabilité de l'huile d'olive pourrait être fondée sur le polymorphisme de cet intron. Pour *Olea europaea* cela a une grandeur de 548 bp. La limite de détection a été déterminée comme 20 %.

Mots clés : Huile d'olive, détection dans les aliments, polymorphisme, DNA, RT-PCR.

Olive oil identification in feed by RT-PCR

Abstract

During the last two years, several farmers use olive oil in the feedstuffs in order to have better quality of meat. The aim of this study was to establish a sensitive DNA-based analytical assay for the detection of olive oil in animal feed. For the isolation of DNA 3 fodder samples were used their oil content after soxhlet extraction. In addition, pure olive oil in different quantities (corresponding to 100%, 20%, 10%, 5% and 1% of olive oil in animal feed) was applied in order to determine this technique's limit of detection. The assay was further tested on animal feed after enrichment with 20% and 10% of olive oil. A comparative study was made of two different commercial kits (Quiagen and r-Biopharm) for the recovery of high quality olive tree DNA. Subsequent polymerase chain reaction (PCR) was designed by using universal DNA primers amplifying the chloroplast trnL (UAA) intron. Olive oil traceability could be based on this intron's polymorphism. For *Olea europaea* it has a size of 548 bp. The limit of detection was determined as 20%.

Key words: olive oil, identification, feed, DNA, RT-PCR.

1. Introduction

Over the past few years olive oil nutritional value has been highly appreciated by consumers from all over the world regardless their own national diet habits. Olive oil is considered to be a bioactive food not only in the Mediterranean basin where it is mainly produced and consumed but throughout the globe, even in distant countries from olive tree's natural habitat such as China and Japan. This was also noticed during the recent commercial exhibition "Elaiotechnia" (olive oil art) where participating exhibitors were even from Asia and visitors coming to learn about olive oil and promote it to their countries even from far-away Malaysia.

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2. Material and Methods

2.1. New products with olive oil

Meat and products originating from it are considered to be responsible for causing a series of contemporary health syndromes such as cardiovascular disease, obesity etc. The new trend in food industry is to use the positive mind-set of the consumers regarding olive oil in a way that other products, considered up to now as less nutritional and healthy, to be promoted. It is worth mentioning some examples: the biggest Fast Food brand in Greece decided to use olive oil for frying, the two major greek sausage and cold meat product companies use olive paste and olive oil in their products, one of the biggest dairy industry launches Mediterranean yogurt with olive oil and much more similar cases.

Except from the above mentioned, several products can be found in the market based on olives such as olive sweets prepared the same way as greek traditional syrup fruit sweets, olive liquor e.t.c. that are currently promoted excessively because of olive's oil positive and dynamic impact on consumers. Olive oil is even used in animal feed in order to improve meat quality. At least three poultry production units in Greece are following this approach.

Food Allergens Lab with a specialized scientific group in all its three laboratories (Crete-Athens-Cyprus) counts many years of experience in olive oil research. On September 2009, a proposal was submitted from our laboratory to the Institution of Research Promotion of the Cyprus Republic and it is expected to be approved in the "New Products and Services" framework. This is a proposal about a research program concerning the production of ice-cream with cyprus olive oil.
This kind of actions assist to further develop and expand olive tree cultivation and olive related culture globally thus giving hope to olive tree cultivators who watch their products getting constantly a lower price.

2.2. New identification method

Is it possible to confirm a manufacturer's claim that its product contains olive oil? The main difficulty is that olive oil is present in foodstuffs in a mixture of other oils and fat ingredients. This means that it can not be detected by classical analytical methods such as fatty acid or sterol profiling by the use of gas chromatography because olive oil's lipid ingredients exist in other food materials as well and no distinguishing of their source is possible. Hence a more accurate, sensitive and selective way is necessary to be found.

Food Allergens Lab has developed a method based on molecular biology techniques in order to detect olive oils' presence in complex food matrices so that its addition in different products can be validated. The innovation in this new analytical method is the use of Polymerase Chain Reaction (PCR) which identifies olive oil by means of olive tree DNA presence and is characterized by high sensitivity and selectivity.

An overview of the experimental procedure can be seen in Figure 1.

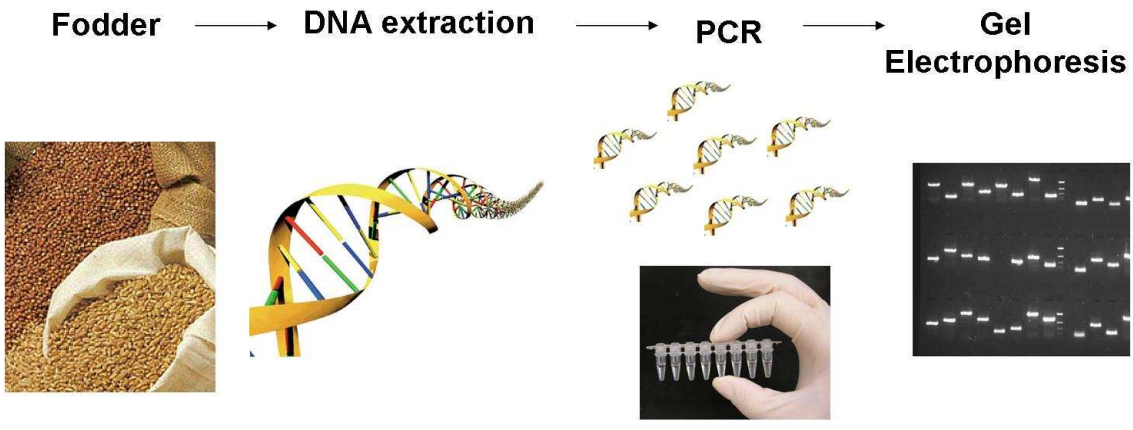

Figure 1: Overview of the experimental set up.

Samples:

Firstly it was necessary to test different samples from where olive DNA could be extracted. Fodder with olive oil enrichment claim and animal feed with olive oil added by us were used.as starting materials. Fodder samples were mixtures of wheat soy, corn, plant oils and other ingredients of plant origin. In addition, pure olive oil in different quantities (corresponding to 100%, 20%, 10%, 5% and 1% of olive oil in animal feed) was applied in order to determine this technique's limit of detection. Except from the above mentioned, DNA from *Olea europaea* leafs was also isolated so that it could be used as a positive control. Leafs were chosen as a DNA source because of their higher DNA extraction yields and easier DNA isolation.

DNA extraction:

For the recovery of high quality olive tree DNA, extraction took place by using two different commercial extraction kits (Quiagen and r-Biopharm) according to their manufacturers' protocols and a comparative study was made. In order to achieve better extraction performance, samples were manipulated not only per se, but also after soxhlet extraction for a more concentrated and clean oil content. In this case (fat content after soxhlet extraction) or when pure olive oil was used for the isolation of DNA, successive centrifugation steps took place in order to acquire an adequate amount of pellet (plant cells and other debris of plant origin) that then went through the DNA extraction process.

Polymerase Chain Reaction:

Subsequent PCR was designed by using universal DNA primers amplifying a noncoding region, the chloroplast trnL (UAA) intron which varies in size depending on the plant. Its sequences have been widely used for reconstructing phylogenies between closely related species or for identifying plant species [1,2]. Olive oil traceability could be based on this intron's polymorphism. For *Olea europaea* it has a size of 548 bp. Other plant species result in PCR products of different DNA size. For instance, soy has an intron size of 585 bp. The primers' sequences (Spaniolas et al. 2008) are:

A1:CGAAATCGGTAGACGCTACG
A2: GGGGATAGAGGGACTTGAAC

In every PCR reaction the following reagents and the applied PCR program are presented in the Table 1.

Table 1: PCR experimental conditions used and The PCR program applied was:

REAGENT	VOLUME (μl)
Buffer	5
MgCl2	5
dNTPs	1
Primer Plant A1	1,5
Primer Plant A2	1,5
DNA Polymerase	0,25 (AmpliTaq) , [or 0,50 (Taq)]
DNA	5
H2O	30,75

95 °C for 10 min
95 °C for 30 sec
50 °C for 30 sec — 40 cycles
72 °C for 1 min
72 °C for 10 min
4 °C constantly

Apart from the samples, some controls were also applied in order to ensure that PCR has worked properly. Except from the positive control (mentioned previously) an extraction blank and amplification blank were necessary in order to ensure that at any stage of the process no contamination took place (Taberlet et al. 2007) . If the extraction blank or the amplification blank give birth to a DNA band at the end, then during DNA extraction or during the preparation of the PCR reactions something went wrong and plant DNA from the environment or from other samples was present. In addition, controls were added that check for inhibition of the PCR by substances present in the DNA extract, by spiking samples with olive tree DNA.

Gel Electrophoresis:

Visualization of the results was performed by gel electrophoresis of the PCR products. Each plant species' was separated on a DNA size basis and olive tree DNA could be identified after comparison of the amplicon size with a suitable ladder. For best separation results, agarose gel was made very dense and long. Electrophoresis was applied at 120 Volt for approximately 100 min and DNA bands were visible when exposed to UV light.

3. Results and Discussion

DNA extraction was complicated and sometimes problematical due to the oily texture of the samples. The 2 commercial DNA extraction kits did not differentiate regarding DNA yield and DNA purity. The amount of isolated DNA was increased after consecutive centrifugations of the olive oil. This can be explained by the fact that plant debris and cell residues were condensing into pellets that were then all resuspended in the same extraction buffer and treated as one sample.

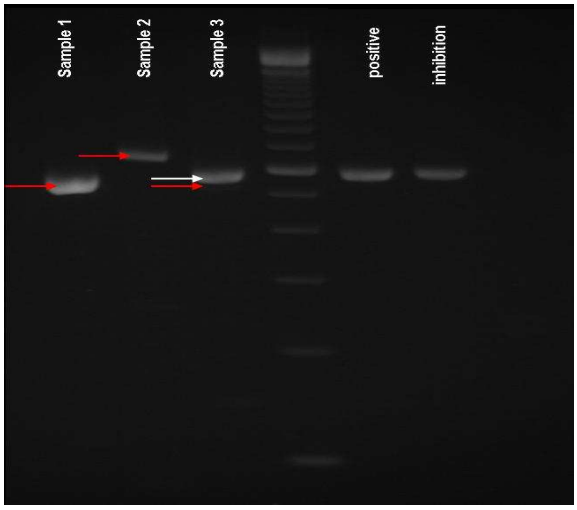

Figure 2: DNA bands after PCR and agarose gel electrophoresis. From the right to the left: Inhibition control, Positive control (*Olea europaea* DNA obtained from olive tree leafs), DNA ladder, and 3 fodder samples. White arrows indicate DNA from olive oil and red arrows DNA from other plants.

PCR did not always give the expected results because of impurities inhibiting the reaction. Hence many repetitions were made in order to achieve having no contamination, no inhibition and the production of amplicons corresponding to olive tree. DNA gel electrophoresis revealed that, except from *Olea europaea*, other plant species such as soy (amplicon size 585 bp) gave DNA bands (see Figure 2, bands indicated by red arrows). Occurrence of multiple bands was something normal since several plant materials took part for the production of animal feed. Olive oil was detected at a percentage as low as 20% (v/w). Smaller amounts could not be recovered and amplified by PCR. Therefore the limit of detection was determined as 20% (Figure 3). Variations and modifications on DNA extraction and PCR protocols did not result in higher sensitivity.

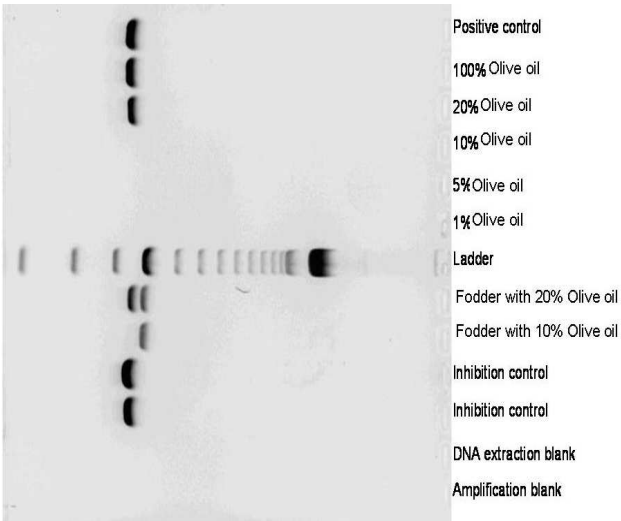

Figure 3: Visualization of results after gel electrophoresis. Above the ladder: 100%, 20%, 10%, 5% and 1% of olive oil in animal feed (for the determination of this technique's limit of detection). Olive oil was not detected when it was less than 20% (v/w). Below the ladder: samples compared to PCR controls.

A limit of detection equal to or lower than 0.5% (v/w) is necessary in order for this method to be used for quality control applications. Consequently improvements, optimization and standardization of this method need to be done. Nonetheless, this study indicates that it is a first step towards establishing a sensitive DNA-based analytical assay for the detection of olive oil in food.

4. Conclusion

There is an international trend towards the enrichment of novel and functional foods with olives and olive oil. The identification of olive oil presence in complex foodstuff could be accomplished by the use of molecular techniques. The detection limit of the method developed by Food Allergens Lab has been determined at 20% (v/w). This new technique is currently used more extensively in many food matrices and the results are indicative that it could be exploited for quality control applications provided that it can be further optimized. It has been already presented excessively in two recent international conferences: at the 3rd Greek Lipid Forum on June in Athens and at the 10th Chemistry Conference of Greece and Cyprus on July in Heraclion.

Acknowledgment

We thank the Department of Horticultural Genetics and Biotechnology of the Mediterranean Agricultural Institute of Chania (Greece) for providing us their expertise on this subject.

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S10-C3

L'huile d'olive aromatisée : Elaboration et évolution des caractéristiques chimiques en fonction du stockage

Grati Kamoun Naziha, Amani Rebai, M.T Hamdi et M.N Arous

Résumé

Devant le manque de diversification de la gamme commerciale de l'huile d'olive tunisienne mise sur le marché, nous nous sommes proposé d'élaborer une nouvelle gamme d'huile d'olive de qualité, ayant des vertus nutritionnelles et thérapeutiques, « huiles d'olive aromatisées ». Ces huiles sont obtenues par l'addition de plantes aromatiques et des arômes naturels .Elles sont gardées dans un endroit frais à une température moyenne de 20 ℃ et leur stabili té oxydative en fonction du temps de stockage a été suivi.
Les résultats obtenus montrent que l'addition des plantes aromatiques entraîne une augmentation des pigments (chlorophylles et carotènes) et des antioxydants (polyphénols et tocophérols). L'huile aromatisée au romarin est la plus riche en ces pigments et en tocophérols. Pour les teneurs en polyphénols, les huiles aromatisées à la lavande et au basilic possèdent les teneurs les plus élevées. Par contre, l'aromatisation n'a pas influencé significativement la composition acidique étant donné que ce paramètre dépend essentiellement du génotype.

Des tests de préférence réalisés auprès de 300 consommateurs représentant différentes catégories d'âge et de niveau social ont mis en évidence que l'huile aromatisée au citron est la plus appréciée au niveau du goût et de l'odeur alors que l'huile aromatisée au romarin et au thym sont les plus appréciées au niveau de la couleur. Par ailleurs, l'étude de la stabilité oxydative des huiles d'olive aromatisées montre que les critères de qualité (acidité, K270 et indice de peroxyde) augmentent au cours du stockage alors que la teneur des composés mineurs (polyphénols, tocophérols, chlorophylles et carotènes diminuent. Ce sont les huiles aromatisées au romarin qui présentent une stabilité oxydative plus élevée par rapport aux huiles témoins et les huiles additionnées par d'autres arômes.

Mots clés: Huile d'olive, aromatisation, stabilité oxydative, stockage.

Flavoured Tunisian Olive Oils: Production and Study of the physicochemical characteristics evolution during the storage

Abstract

The aims of the present work were to produce several flavoured olive oils and to evaluate the physico-chemical characteristics and oxidative stability change of these oils during the storage
The aromatized olive oils that present the dietetic and therapeutic virtues were prepared by maceration for 10 days of fresh selected Mediterranean aromatic plant (3% w/w of rosemary, lavender, lemon, basil and thyme) and addition of certain natural extract aromas plant. Oils samples were kept in glass bottles and stored at fresh place at 20℃.
The resistance to oxidation of these selected flavoured oils was compared to a control samples by measuring PV, K232 and K270 values and change in chlorophyll, carotenes and polyphenols contents during storage. Obtained results confirmed the previous ones and demonstrated that addition of aromatic plants causes a slight increase in free acidity and viscosity, no significant change in the acid composition but a significant enhances pigment and antioxidants content.
Moreover, the results show that rosemary olive oil is the richest aromatized olive oil in the pigment and tocopherols. Concerning the polyphenols, the flavoured olive oils prepared with the addition of lavender and basil have the highest content.
A flavoured olive oil should not only satisfy the sensory requirements of consumers but also should present some other qualities appreciated in the food market, e.g. improved keeping quality compared to that of the plain oil. Furthermore, the investigation beside 300 consumers demonstrated that aromatized olive oil with the citron was the most appreciated.
The following of the change of oxidative stability and quality criteria's (acidity, PV, K232 and K270) of flavored olive oils show the increase of these parameters during the storage. However, we observed a significant reduce of pigment and antioxidants content. A rosemary flavoured olive oil was most stable against oxidation during the storage.
Key words: olive oil, aromatization, oxidative stability, storage

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Thème 6

Nouveaux usages et alternatives de valorisation des produits de l'olivier (nutrition, santé, cosmétique, énergie, environnement)

S11-C1

Présentation du projet RESOLIVE pour la production d'énergie renouvelable par digestion anaérobie des margines et des margions de l'industrie oléicole en Méditerranée

Bárbara De Mena Pardo, Dr. Gerhard Schories

Résumé

L'UE comprend les pays principaux des producteurs d'huile d'olive, un secteur qui a un potentiel énorme pour l'implémentation des énergies renouvelables. Les activités prévues pour le RESOLIVE projet, subventionné sous FP7 dans le cadre du thème <<Recherche au profit de SMEs>> s'adressent directement à l'adaptation de solution d'énergies renouvelables pour l'industrie d'huile d'olive. Un objectif principal de ce projet sera la définition des conditions spécifiques pour l'implémentation des solutions des énergies renouvelables dans l'industrie d'huile d'olives. Dans RESOLIVE, des tests laboratoires sur la digestion anaérobique seront exécutés pour optimiser les techniques actuelles pour la production de biogaz. Ce papier présentera le progrès obtenu, y compris une analyse des résultats.

Anaerobic digestion of olive mill wastewater and 2-phase olive mill residues in the framework of the FP7 project RESOLIVE (Adaptation of renewable energy solutions for the olive oil industry)

Abstract

The EU comprises leading producer countries of olive oil, a sector that has a huge potential for the implementation of renewable energies. The activities carried out in the project RESOLIVE, funded under FP7 in the "Research for the benefit of SMEs" scheme, directly address the adaptation of renewable energy solutions for the olive oil industry. The one of the main objectives of this project is defining specific conditions for the implementation of renewable energy solutions in the olive oil industry. Within RESOLIVE a full program of laboratory scale tests on anaerobic digestion is being carried out in order to optimize the existing techniques for biogas production. This paper will present the progress obtained, including an analysis of the results.

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S11-C2

Medicinal properties of olive leaves phenolic compounds in cholesterol fed rats

Jemai Hedya, Ines Feki, Mohamed Bouaziz, Abdelfatteh El Feki, Sami Sayadi

Abstract

The positive correlation existing between phenolic compounds consumption and cardiovascular diseases decreased prevalence is well established. This fact is related to the polyphenols capacity in reducing some cardiovascular risk indicators mainly hypercholesterolemia and LDL- C oxidation. Indeed, the medicinal properties of phenolic compounds are due to their antioxidant effects. The present study was designed to test the lipid- lowering and antioxidant activities of olive leaves polyphenols in hypercholesterolemic diet fed rats. Our results show that olive leaves polyphenols have significantly restored the lipid metabolism, the hepatic antioxidant enzymes activities and the histological organisation of the heart, the liver and the aorta. These findings highlight the olive leaves and confirm that olive by- products polyphenols beneficially affect the risk factor of cardiovascular diseases.

Key words: Antioxidant, Cardiovascular diseases, Hypercholesterolemia, Olive leaves, Phenolic compounds

Propriétés médicinales des composants phénoliques contre le cholesterol pour l'alimentation des rats nourris avec des feuilles d'olivier

Résumé

La corrélation positive qui existe entre la consommation des composés phénoliques et la diminution de la prévalence des maladies cardiovasculaires est bien établie. Cet effet est lié principalement au pouvoir des polyphénols à modérer certains indicateurs de risque cardiovasculaire, principalement l'hypercholestérolémie et l'oxydation des LDL-C. En effet, les propriétés médicinales des composés phénoliques sont dues à leurs effets antioxydants. La présente étude a été conçue pour tester les activités antioxydantes et hypo- lipidique des polyphénols issus des feuilles de l'olivier chez des rats hypocholestérolémiques. Nos résultats montrent que ces polyphénols ont significativement rétablie le métabolisme lipidique et les activités des enzymes antioxydantes hépatiques. Ces résultats mettent en valeur les feuilles de l'olivier et confirment leur implication dans la diminution de l'incidence des facteurs de risque des maladies cardio-vasculaires.

Mots-clés: Antioxydant, Maladies cardiovasculaires, Hypercholestérolémie, Feuilles de l'olivier, Composés phénoliques

1. Introduction

Atherosclerosis, the principal contributor to the pathogenesis of myocardial and cerebral infarctions, is known to be one of the leading causes of morbidity and mortality worldwide and it is essentially caused by hyperlipidemia resulting from lipid metabolic changes (Aygustin (1999)). Phenolic compounds have been reported to prevent LDL oxidation *in vitro* and show marked hypolipidemic activity *in vivo*, suggesting the effectiveness of polyphenols for the prevention of atherosclerosis (Dufull et al (2003)). Olive leaves are considered as a cheap raw material which can be used as a useful source of high-added value products, especially phenolic compounds (Briante et al (2002)). The main phenolic compounds in olive leaves are the glycosylated forms of oleuropein and ligstroside (Amro et al (2002)). Numerous *in vitro* studies have shown that oleuropein and its derivatives have various biochemical roles (Mercier (1997), Amro et al. (2002)). Moreover, it was reported that these polyphenols are able to prevent low- density lipoprotein oxidation Amro et al (2002). Hydroxytyrosol is the principal oleuropein derivative; it is used to prevent diseases because it is endowed with an

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important antioxidant property (Capasso (1999)). Moreover, oleuropein aglycone is obtained by enzymatic hydrolysis of oleuropein by β-glucosidase (Konno (1999)). Oleuropein aglycone has many biological activities and it has been shown that it is more efficient than hydroxytyrosol and oleuropein as anti- proliferative and pro-apoptotic agent in the prevention of some cancer Menendez (2007). Therefore, the aim of this study was to investigate the effects of these olive leaf extracts on serum lipid levels and antioxidant enzymes activities in rats fed a cholesterol-rich diet.

2. Materials and methods

2.1. Preparation of the phenolic- rich extracts

Samples of fresh green leaves were dried and powdered (50 g), and extracted with a mixture of methanol and water (200ml, 4:1 v/v). Then, the extract was concentrated by evaporation to dryness. The acid hydrolysis was carried out as following: one gram of the ethyl acetate extract was dissolved in 10 ml of a MeOH/H_2O (4:1) mixture in sealed vial. The solution was hydrolysed at 100 ◦C for 1 h using 5ml of a 2 M HCl solution (Prolabo, France). After hydrolysis, the reaction was cooled and diluted with water (10 ml) and the hydrophobic fraction was extracted by a separatory funnel, three times with 25 ml of ethyl acetate, which was subsequently removed by evaporation. Finally, the enzymatic hydrolysis was carried out using β- glucosidase from almond (Sigma) as previously described (Bouaziz et Sayadi, 2005). A reverse-phase high-performance liquid chromatography technique was developed to identify and quantify the major phenolic compounds.

2.2. Experimental design

Fifty male Wistar rats weighing between 160± 10g were used. The animals were maintained in stainless steel cages under controlled conditions (23±1◦C, 12h light–dark cycle, and had access to a standard diet (SICO, Sfax, Tunisia) and drinking water. The animals were housed according to the EEC 609/86 Directives. They were divided into 5 groups (n=10). **Group 1** was fed a standard laboratory diet (CD). **Group 2** was fed a cholesterol- rich diet (HCD) (normal diet + 1% cholesterol and 0.25% bile salts). **Groups 3, 4 and 5** received HCD with, respectively; olive leaf, acid hydrolysate and enzymatic hydrolysate extracts (3mg/kg b.w oleuropein, hydroxytyrosol and oleuropein aglycone, respectively) which were dissolved in drinking water. The experiment was conducted over a period of 16 weeks and at the end, rats were killed by decapitation and blood samples were collected and stored at −80 ◦C.

2.3. Serum lipids

Concentrations of total cholesterol (TC), total triglycerides (TG), low-density lipoprotein (LDL-C) and high density lipoprotein HDL-C in serum were determined by enzymatic colorimetric methods using commercial kits (Kyokuto Pharmaceutical, Japan).

2.4. Antioxidant enzyme activities

CAT and SOD activities were evaluated in liver tissue homogenate. CAT activity was measured using the method of Regoli and Principato (Regoli et Principato, 1995) based on the spectrophotometric measure of the decomposition rate of H_2O_2 at 240 nm for 1 min. One unit of CAT was defined as the l mole H_2O_2 decrease/ ((mg protein) min). SOD activity was measured according to the method of Marklund and Marklund based on pyrogallol oxidation by superoxide anion (O_2−) and its dismutation by SOD (Marklund et Marklund, 1974). One unit was determined as the amount of enzyme inhibiting the oxidation of pyrogallol by 50%. The activity was measured at 440 nm expressed as units/mg protein.

2.5. TBARS assay

TBARS (thiobarbituric acid-reactive substances) concentrations were measured in the liver homogenates using the method of Park et al. (Park et al. (2002)). The homogenate solution was mixed with 600µl of distilled H_2O and 200µl of 8.1% (w/v) SDS. The reaction mixture was heated at 95 ◦C for 1 h after the addition of 1.5ml of 20% acetic acid (pH 3.5) and 1.5ml of 0.8% (w/v) TBA. After cooling the reaction, 5.0 ml of butanol: pyridine (15:1) were added. After centrifugation, the resulting coloured layer was measured at 532 nm using malondialdehyde (MDA) as standard.

2.6. Statistical analysis

Data are given as means± S.E. Statistical differences were calculated using a one-way analysis of variance (ANOVA), followed by Student's t-test. Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Olive leaves and hydrolysate extracts characterization

Fig.1 shows a high concentration of oleuropein (4.32 g/100g dry weight). The identification of oleuropein was based on of the chromatographic retention time. Various extraction methods were used to extract this compound and its amount varied with the olive cultivar (Bouaziz et Sayadi, 2005). Acid treatment of the leaf extracts induced hydrolysis of oleuropein. The most notable effect seen was the increase in hydroxytyrosol concentration. It is well known that acid hydrolysis of olive leaf extract leads to a high quantity of hydroxytyrosol (Bouaziz et al. (2006)). HPLC profile of acid hydrolysate extract showed that hydroxytyrosol was the major compound which concentration reached 1.4g/100 g dry weight.

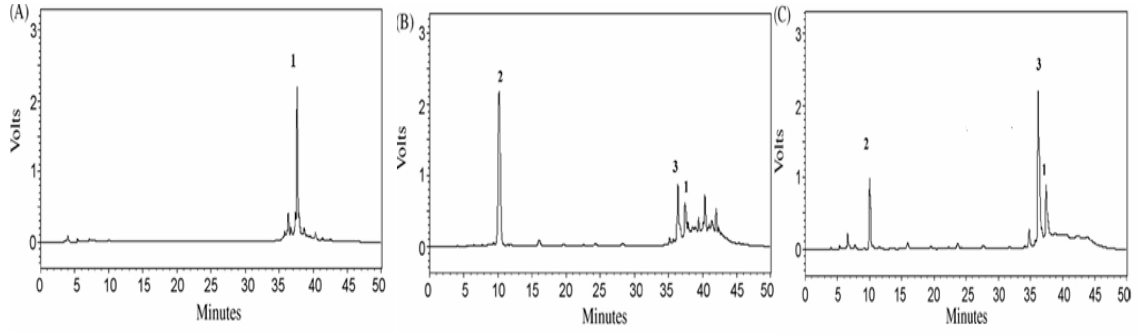

Figure 1: HPLC chromatograms at 280 nm of olive leaf extracts (A) olive leaf extract after acid hydrolysis (B) and olive leaf extract after enzymatic hydrolysis (C). (1): Oleuropein; (2) hydroxytyrosol; (3) oleuropein aglycone.

Moreover, the biotransformation of olive leaf extract by β- glucosidase carried out at 37 ◦C and pH 7 showed a small amount of hydroxytyrosol and high concentration of oleuropein aglycone after 2 h of incubation time. The concentration of the latter reached 3.82 g/100 g dry weight. The aglycone is well known as pharmacologically active molecule. In fact it is described for its potential application as an antioxidant and antimicrobial agent in some fairly common diseases of olive trees (Bouaziz et al. (2006)).

3.2. Serum lipids

After the treatment, the TC, TG and LDL-C concentrations of rats fed a cholesterol-rich diet (HCD) showed a significant increase compared with control group (CD) (Fig. 2). However, a decrease of HDL-C concentration of rats in the HCD group was observed ($P < 0.05$). Rats orally administrated with olive leaf, acid hydrolysate and enzymatic hydrolysate extracts had lower concentrations of TC, TG and LDL-C than rats receiving an HCD. Olive leaves extracts significantly reduced the TC, TG and LDL-C levels. Moreover, the treatment of HCD fed groups with phenolic extracts significantly re-established their HDL-C level ($P < 0.05$). Lowering levels of TC and LDL-C and improving level of HDL-C has been linked to a lower risk of CHD (Libby et al, 2000). In fact, it was reported that the decrease in LDL-C concentration and the increase of HDL-C level could fasten the removal of cholesterol from peripheral tissues to liver for catabolism and excretion (Young et al, 2004). In addition, the increase in HDL-C concentration could protect the LDL against oxidation *in vivo* because lipids in HDL are preferentially oxidized before those in LDL (Young et al. (2004)). Therefore, the results of the present study indicate that olive leaf phenolics may reduce the incidence of CHD.

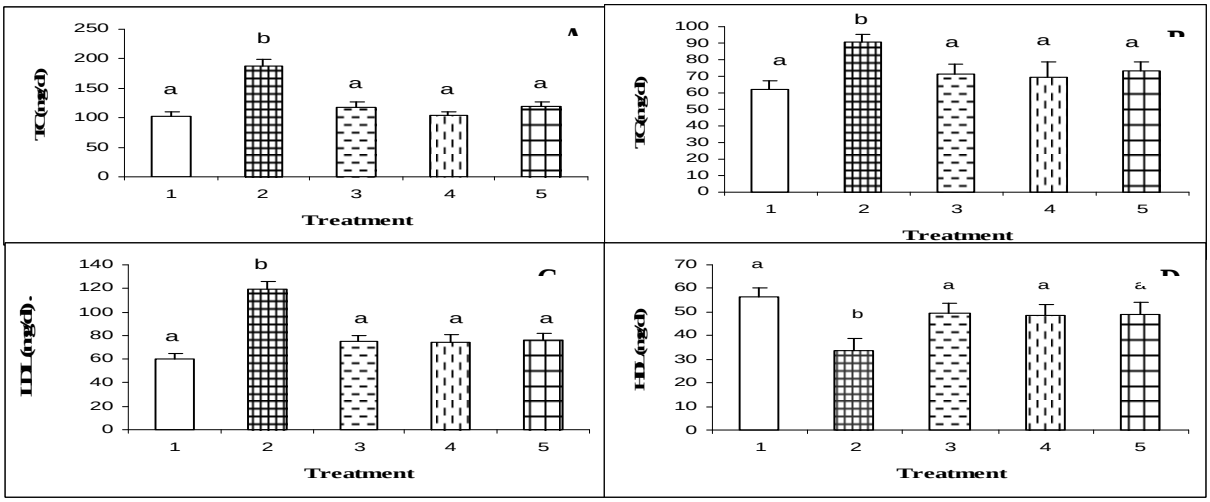

Figure 2: Effects of olive leaf extracts on rat total cholesterol (TC) (A), triglycerides (TG) (B), low-density lipoprotein cholesterol (LDL-C) (C), and high-density lipoprotein cholesterol (HDL-C) (D) levels. Group: 1, CD (standard diet); 2, HCD; 3, HCD+ olive leaf extract; 4, HCD+ acid hydrolysate extract; 5, HCD+ enzymatic hydrolysate extract. Each bar represents mean± S.E. from 10 rats. Bars with different letters differ significantly; $P < 0.05$.

3.3. Hepatic antioxidant enzyme activities

The CAT and SOD activities significantly decreased in livers of rats fed a cholesterol-rich diet compared to those fed a control diet. The decrease was significantly restored ($P < 0.05$) in the presence of the olive leaves and the hydrolysate extracts (Fig. 3). By the way, it has been reported that a high fat diet lowered the activities of antioxidant enzymes, which is possibly due to their increased implication in fighting excessive oxidative stress in hypercholesterolemic rats (Wang et al. (2008)). The increase of hepatic antioxidant enzyme activities and the serum antioxidant potential observed in olive leaf phenolic-supplemented rats may be due to the removal toxic reactive species resulting from the high cholesterol feeding.

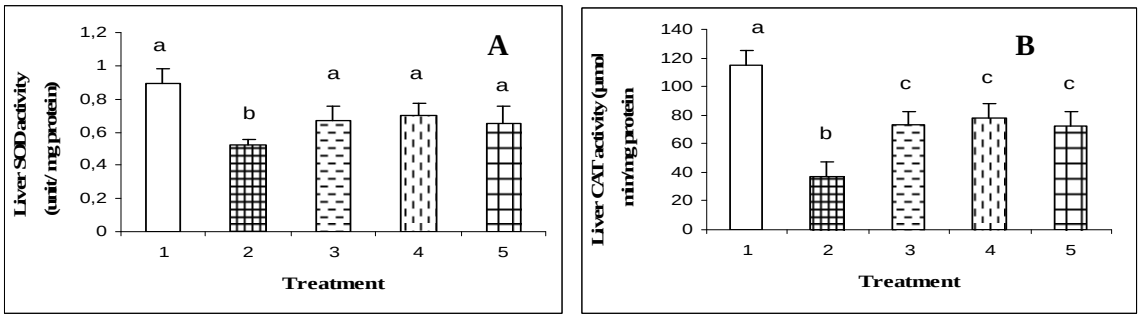

Figure 3: Effects of olive leaf extracts on SOD (B) and CAT (A) activities in liver.
Group: 1, CD (standard diet); 2, HCD; 3, HCD+ olive leaf extract; 4, HCD+ acid hydrolysate Extract ; 5, HCD+ acid hydrolysate extract. Each bar represents mean±S.E. from 10 rats.
Bars with different letters differ significantly ; $P < 0.05$.

3.4. TBARS levels

The olive leaf-deriving preparations were revealed to slow down the lipid peroxidation (Fig. 7). The TBARS levels were significantly increased ($P < 0.05$) in liver, heart, kidneys and aorta within animals fed high cholesterol diet compared to the control diet group. This increase was significantly reduced after treatment with phenolic extracts from olive leaves. TBARS are the major oxidation products of peroxidized polyunsaturated fatty acids; thus, increased TBARS content is an important indicator of lipid peroxidation (Prasad (1999)).

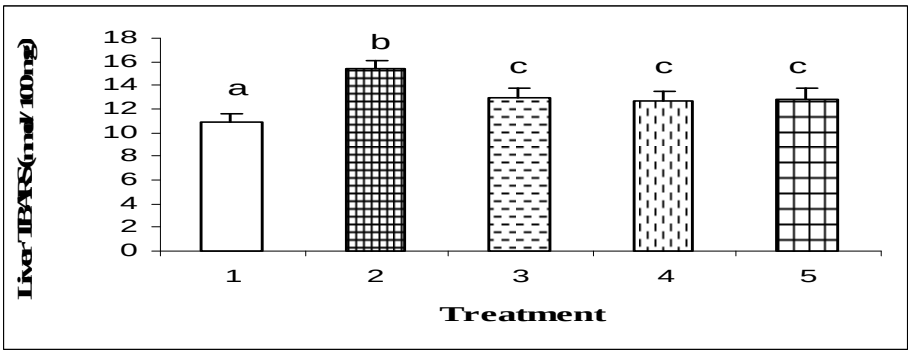

Figure 4: Effects of olive leaf extract (3) and hydrolysate extracts (4 and 5) on rat liver TBARS levels. Each bar represents mean± S.E. from 10 rats. Bars with different letters differ significantly; $P < 0.05$.

The reduced lipid peroxidation observed in the olive leaf extract-treated animals may be attributed to the important role of oleuropein, oleuropein aglycone and hydroxytyrosol as antioxidants. This power may be attributed to their ability to decompose free radicals by quenching reactive oxygen species and by trapping radicals before reaching their cellular targets (Srinivasan et al. (2007)).

4. Conclusion

By the presented results, we demonstrate that polyphenols recovered from olive leaf extracts, oleuropein, hydroxytyrosol as well as oleuropein aglycone, exhibited a pronounced hypolipidemic and antioxidant effects. Subsequently, olive tree by-products are a source of antioxidants able to reduce the frequency of cardiovascular diseases.

Acknowledgements

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S11-C3

Olive orchard amended with olive mill wastewater: Effects on olive fruit and olive oil quality

Mechri Baligh, Manel Issaoui

Abstract

The aim of this work was to study the effects of agronomic application of Olive mill wastewater (OMW) in a field of olive trees on olive fruit and olive oil quality. Agronomic application of OMW increased significantly the fungal: bacteria ratio, whereas the root colonization and the photosynthetic rates decreased significantly. Consequently, the oil content expressed as a percentage of dry weight, decreased significantly after agronomic application of OMW. Land spreading of OMW altered the relative proportion of individual olive fruit sugar and decreased significantly the nitrogen (N) and phosphorus (P) of the fruit. A significant increase was observed in total phenol content of oil after agronomic application of OMW. α-Tocopherol content, on the contrary, decreased with OMW application. The fatty acid composition of the oil was not affected by the treatments. To our knowledge, this is the first report of change in the olive fruit and olive oil quality following agronomic application of OMW.

Mots clés: Olive mill wastewater, olive oil, α-Tocopherol, fungal: bacteria ratio.

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S11-C4

Contrôle des pathogènes d'olivier en utilisant les eaux usées du moulin et quelques uns de ses dérivés chimiques et biologiques

A. Rhouma, M.A. Triki, K. Gargouri, T. Yangui, A. Dhouib and S. Sayadi

Résumé

La margine et certains de ses dérivés chimiques et biologiques ont été testé *in vitro* et *in vivo* contre quelques agents phytopathogènes de l'olivier (*Rhizoctonia bataticola, Fusarium solani, Verticillium dahliae et Pseudomonas savastanoi pv. savastanoi)*.
L'incorporation de la margine dans le milieu de culture a permis d'exercer un effet fongicide contre les champignons testés. Les polyphenols (hydroxytyrosol) possèdent des activités antifongiques et bactéricides.

Les bactéries isolées à partir de la margine se sont montrées efficaces contre les champignons et les bactéries phytopathogènes *in vitro* et *in vivo*. L'identification moléculaire basée sur le séquençage l'ARNr 16S a permis d'identifier plusieurs souches de *Bacillus* résistantes aux polyphenols.
Les essais menés *in vivo* ont mis en exergue l'effet de la margine dans la réduction des pourcentages d'attaque causés par *Rhizoctonia bataticola* et *Fusarium solani*. Les polyphenols se sont révélés très efficaces en inhibant complètement la formation de tumeurs causées par *Pseudomonas savastanoi* pv. *savastanoi*.

Mots clés : *Olea europea, margine, Polyphenols,* hydroxytyrosol*, agents phytopathogènes.*

Control of olive tree pathogens using olive mill waste water and some of its chemicals ans biological derivatives

Abstract

The olive mill waste water (OMW) and some chemical and microbial derivatives have been tested *in vitro* and *in planta* experiments against some plant pathogens of olive tree (*Rhizoctonia bataticola,* Fusarium *solani, Verticillium dahliae and Pseudomonas savastanoi pv. savastanoi)*. The incorporation of the OMW in the culture medium resulted in a fungicidal effect against the tested plant pathogenic fungi. Polyphenols (hydroxytyrosol) have shown a fungicide and fungistatic activity. On the other hand, the application of the volatile fraction resulted in an inhibition of the mycelia growth of the tested fungi as well as bactericide effect against *Pseudomonas savastanoi*. Indigenous bacterial strains isolated from Olive Mill Waste Water were proved efficient in the control of olive tree pathogens in vitro and *in planta*. Molecular identification using 16S sequencing has proved the identification of several strains of Bacillus resistant to polyphenols. The *in vivo* experiments have shown a very substantial effect of the OMW basing on the significant reduction of plants attacked by *Rhizoctonia bataticola*. and *Fusarium solani*. Polyphenols were proved very efficient in preventing tumour formation caused by *Pseudomonas savastanoi* pv. *savastanoi*

Keywords: *Olea europea,* Olive mill waste water, Polyphenols, Flavonoides, Plant pathogens.

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S12-C1

Ethanol Production from Olive Cake Biomass Substrate

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Abstract

The inexpensive production of sugars from lignocellulose is an essential step for the use of biomass to produce fuel ethanol. Olive cake is an abundant by-product of the olive oil industry and represents a potentially significant lignocellulosic substrate for bioethanol production in the Mediterranean basin. Converting olive cake to ethanol could add further value to olive production. In the present study, olive cake was evaluated as a feedstock for ethanol production. To this end, the lignocellulosic component of the olive cake was dilute-acid pretreated at 13.5% olive cake loading with 1.75% (w/v) sulfuric acid and heating at 160℃ for 10 min., followed by chemi cal elimination of fermentation inhibitors. Soluble sugars resulting from pretreatment were fermented using *E. coli* FBR5, a strain engineered to selectively produce ethanol. 8.1 g ethanol/L was obtained from hydrolysate containing 18.1 g soluble sugars. Increasing the pretreatment temperature to 180℃ resulted in failed fermentations, presumably due to inhibitory by-products released during pretreatment.

Mots clés: Ethanol, lignocellulose, fermentation, olive cake.

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S12-C2

Agrochemical characteristics of the compost made from “Alperujo” mixed with other plant and animal wastes

Juan Cegarra, J.A. Alburquerque, G. Tortosa, G. Ait-Baddi

Abstract

Around four millions tons/year of the wet solid lignocellulosic olive-mill waste called “alperujo” (AL) is generated by the olive oil extraction industry, due to the generalised use of the two-phase centrifugation method in Spain. Its high non-stabilised organic load includes phytotoxic compounds such as fats, organic acids and a short but active content of water soluble phenols, which make AL unsuitable for direct soil application.
Co-composting AL with other solid and liquid wastes, either from plant or animal origin, has been successfully performed, thus the procedure is nowadays considered a suitable alternative for AL disposal and leads to obtain non-phytotoxic soil organic amendments and fertilizers rich in partially humified lignocellulosic matter, as revealed by the maturity and humification indices. They usually contain abundant potassium, but also changeable amounts of other nutrients depending on the materials added to the AL.

Key words: Agrochemical characteristics, two-phase olive mill waste « alperujo », co-composting

Caractéristiques agrochimiques du compost fabriqué à partir du “Alperujo” mélangé avec autres déchets d’origine végétale et animale

Résumé

En Espagne, la production des déchets lignocellulosiques appelés communément “alperujo” (AL) générés par les huileries d’olives est trés importante (à peu prés 4 millions de tonnes/an). Ces déchets, résultent de l’extraction d’huile d’olive par des systèmes géneralisés de centrifugation à deux phases. Leur richesse en matière organique non stabilisée leur confère un caractère phytotoxique, ce qui empêche leur application directe au sol. Le co-compostage de ces déchets en mélange avec d’autres déchets organiques d’origine animale ou végétale présente une meilleure solution pour limiter le pourvoir toxique de ces déchets et pour produire un ammendement et un fertilisant organique riche en matière organique partiellement humifiée. A ces caractéristiques, s’ajoute aussi l’enrichissement en potassium et en d’autres nutriments, dépendamment de l’origine du matériel composté avec AL.

1. Introduction

The main by-product generated by the Spanish olive oil industry is “alperujo” (AL), which is annually produced in huge amounts (approximately four million tons/year) because the two-phase centrifugation method is used by more than 90% of olive mills. Apart from the treatments to recover its residual oil and further use for thermal and electrical energy generation, AL composting is increasingly considered a good way of improving profits of the olive oil production process, leading to obtain organic amendments and fertilizers containing “humic-like” compounds, which are known to be the most active fraction in compost and positively affect soil ecology, structure, fertility and productivity.

AL presents high contents of lignin, cellulose and hemicellulose, a rather scarce nutrient content, unbalanced C/N ratio, potentially phytotoxic compounds such as polyphenols, and slightly acidic pH values (Alburquerque et al., 2004). In addition, it is a scarcely porous material very susceptible to compaction, which is a result of the conjunction of its high moisture content, small particle size and high lipid content; all these properties being unfavourable for a correct distribution of air, water and dissolved substances during composting. Therefore, bulking agent addition is necessary to improve the AL physical structure and to amend its nutrient content, in order to ensure a proper composting performance.

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In this report, the most relevant results gained in successive co-composting experiments of AL mixed with several organic wastes as bulking agents, and the main characteristics of the composts obtained are summarized.

2. Materials and methods

2.1. Characteristics of raw materials

Three agricultural wastes and four animal manures were employed as bulking agents of the AL in several composting experiments (Table 1), the former had the highest C/N and the lowest pH and EC values whereas the latter generally showed the lowest lignin and the highest nutrient contents. These materials clearly showed lower moisture and lignin contents than AL (Table 3), but generally higher pH values.

Table 1: Main characteristics of bulking agents (g/kg, dry weight basis): CW, cotton waste ; GS, grape stalk ; OL, olive leaf ; FCB, fresh cow bedding ; HM, horse manure ; PM, poultry manure and SM, sheep manure.

Parameters	Agricultural wastes			Manures			
	CW	GS	OL	FCB	HM	PM	SM
Moisture (% f.w.)	11.5	5.9	7.3	46.1	19.5	20.1	38.5
pH[1]	6.8	4.4	5.6	7.5	7.5	7.5	8.5
EC[1] (dS m^{-1})	4.1	4.2	1.7	7.5	9.3	8.5	11.3
Organic matter	933.0	934.1	906.0	664.0	578.0	805.5	456.5
Lignin	232.0	262.3	319.1	185.0	201.1	129.7	211.4
Total-N	21.3	8.0	13.3	19.4	15.3	32.3	17.7
C/N ratio	22.4	60.1	39.4	19.0	20.4	12.3	14.3
P	1.8	0.6	0.8	2.5	2.3	2.2	2.2
K	17.4	20.0	5.7	35.8	21.2	13.5	16.5
Ca	23.0	6.3	26.0	63.7	58.6	47.5	100.9
Mg	4.2	1.5	2.4	8.8	14.9	5.5	18.7

[1] water extract 1:10 and EC: electrical conductivity.

2.2. Composting performance

Seven mixtures were made by adding the above bulking agents to the five selected AL samples according to the proportion shown in Table 2, later placed in eight trapezoidal piles. Aeration was first supplied by forced ventilation in the pilot-plant experiments (2-4 tons), which was only revealed to be effective when performed in conjunction with mechanical turning. As a result, only mechanical turning was used in the large-scale experiments (piles 6-8, 10-20 tons) in order to reduce operating costs.

Table 2: Composition and management of composting substrates.

Pile	Composition (%)		Turnings	Forced ventilation	Composting time (weeks)
	Fresh basis	Dry basis			
1	92.6 AL1 + 7.4 CW	(80/20)	1	yes	42
2	94.6 AL2 + 5 GS + 0.4 urea	(87/12/1)	3	yes	49
3	94.6 AL2 + 5 OL + 0.4 urea	(87/12/1)	3	yes	49
4 and 5	90 AL3 + 9 FCB + 1 AL compost	(87/11/2)	14	4 (no) 5 (yes)	36
6	91 AL4 + 9 HM	(85/15)	18	no	37
7	52 AL5 + 48 PM	(37/63)	7	no	38
8	65 AL5 + 35 SM	(57/43)	7	no	38

The active phase of composting was considered as finished when the temperature of the piles was close to ambient and re-heating did not occur, at which time air blowing and/or mechanical turning were stopped and the materials left to mature. Complete details of the composting performance and the analytical methods can be consulted in Alburquerque et al., (2009a, b).

3. Results and discussion

3.1. Composting progress

Both temperature and pH increases were considered clear indicators of the AL composting progress, low increases being related to deficient substrate aeration conditions. High thermophilic temperatures were quickly recorded in piles containing manures in contrast to piles made of agricultural wastes which showed a long lag-phase (data not shown). This fact must be related to the greater nutrient availability of manures and their inoculum effect, but also to the better oxygen distribution favoured by the more frequent turnings which enhanced the composting process by homogenising and re-distributing of microorganisms, moisture and nutrients. Thus, the composting times were considerably shorter in piles 4-8 (Table 2).

The longer time needed for AL composting in comparison to other substrates was due to the paramount predominance of the lignocellulosic fraction in AL substrate, its main component (lignin) is very difficult to be degraded by microorganisms (Whitney and Lynch, 1996). The two other components, cellulose and hemicellulose, were more extensively degraded than lignin (data not shown). The resultant OM-losses (Fig. 1), calculated as a percentage of the initial content, reflected clear differences between piles: pile 5 showed the highest loss (61%) while pile 3 the lowest (34%). The degradation process reduced the amount of non-humified compounds and also resulted in the release of compounds which probably were incorporated into the humification pathways, leading gradually to more humified and polymerized compounds.

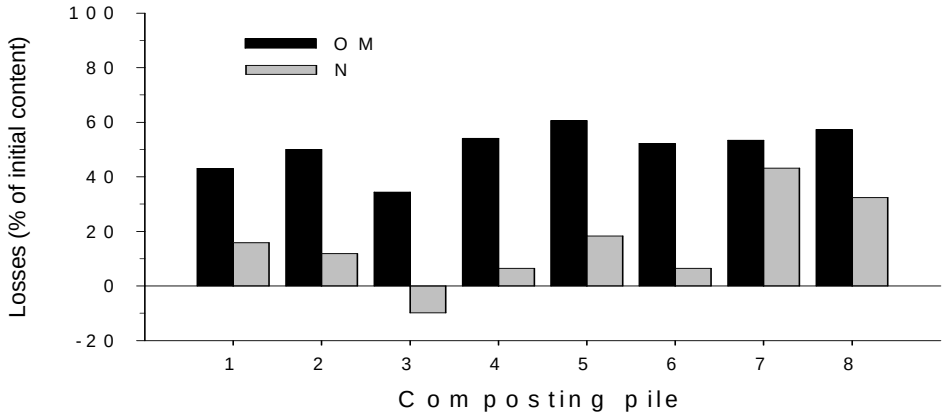

Figure 1: Total organic matter (OM) and nitrogen (N) losses at the end of the composting processes.

In all piles, a continuous nutrient concentration effect was recorded as composting progressed due to the mineralization of organic compounds and the subsequent OM-losses. This effect was equally evident for N, even if a clear ammonification process followed by intense ammonia formation and loss by volatilization was generally detected coinciding with the thermophilic period and high pH values (Jeong and Kim, 2001; Liang et al., 2000). As a result, in none of the experiments the NH_4-N decrease was accompanied by a clear increase in NO_3-N, which was only detected at low concentrations at the end of composting (Table 3). The highest total N-loss was detected in pile 7 (Fig. 1) due to the great proportion of poultry manure (rich in NH_4-N) added to the AL while a final N gain of 10% was registered in pile 3, where the mentioned lowest OM decomposition would have contributed to nitrogen immobilization and subsequent lower ammonia formation and volatilization.

3.2. AL compost characteristics

AL compost showed a pleasantly earthy smell and had alkaline pH, moderate salinity and high OM content, the half being lignin responsible of the relatively high C/N ratio. The end-values obtained for the DH (> 71%) and PAH (> 63%) indices suggest a substantial OM humification and polymerization,

respectively, whereas those of GI indicate a clear detoxification of the initial substrates. The compost also had an acceptable nutrient content, K, Ca and N (mainly organic) showing the most relevant values (Table 3).

In comparison to the raw AL, the compost obtained clearly shows higher nutrient content due to the AL amendment with the bulking agents and as a result of the above mentioned concentration effect. By the contrary, the C/N ratio and contents of fats and water-soluble phenols were much lower in AL compost, accordingly to the mentioned detoxification of the raw materials. When compared with manures of different origin (cow, pig, poultry, sheep and rabbit), organic and inorganic-N, P, Ca and Mg contents were considerably lower in AL compost but that of K was higher. Also, the C/N ratio was higher in compost than in manures in agreement to the higher lignin content in the former.

Table 3: Main characteristics of the raw AL, AL compost and manures of different origin (mean values, dry weight basis).

Parameters	AL (n=5)	AL compost (n=8)	Manures (n=10)
pH[1]	5.1	8.8	7.8
EC[1] (dS m^{-1})	4.4	3.1	4.6
Organic matter (g kg^{-1})	929.4	804.2	756.0
Lignin (g kg^{-1})	364.8	402.5	167.4
Fats (g kg^{-1})	95.6	5.0	-
Water-soluble phenols (g kg^{-1})	11.2	3.1	-
Total-N (g kg^{-1})	12.7	22.9	31.7
NH_4^+-N (mg kg^{-1})	-	130	5243
NO_3^--N (mg kg^{-1})	-	77	232
P (g kg^{-1})	1.0	1.9	3.2
K (g kg^{-1})	19.0	33.5	20.9
Ca (g kg^{-1})	5.9	26.7	56.4
Mg (g kg^{-1})	2.4	5.4	9.8
C/N ratio	42.0	18.2	12.5
GI (%)	0	81	-
DH (%)	-	75.5	-
PHA (%)	-	70.6	-

[1]water extract 1:10, EC: electrical conductivity, GI: germination index, DH: [(HAC+FAC)/TEC]×100, PHA: (HAC/TEC)×100, TEC: total organic carbon extracted in 0.1M NaOH, HAC: humic-like acid carbon and FAC: fulvic-like acid carbon. -: not determined.

The above characteristics make AL compost suitable for agricultural requirements and it has been shown (Alburquerque et al., 2006) that it can be better used than manures as an effective soil amendment due to the strong resistance of its lignocellulosic matrix to edaphic degradation. For plant growth, however, the low nitrogen mineralization rate of AL compost in the short term may require the addition of supplementary nitrogen fertilizers Alburquerque et al., 2007). In addition, these composts can be classified within the highest quality class according to the limits established for heavy metal concentrations of compost and fertilizer products made from wastes by the Spanish legislation (BOE, 2005), fulfilling the requirements of European Ecolabel criteria for soil improvers and growing media (European Commission, 2001).

4. Conclusions

Both selection of an appropriate bulking agent and mechanical turning were key factors for the proper AL composting performance. The best results were found by combining the use of manure as bulking agent with frequent turnings, which resulted in a clear reduction in the composting time. However, the lignocellulosic nature of AL led to long composting times. The resulting AL compost, free of toxicity, was rich in organic matter substantially humified and polymerized and had an acceptable nutrient content, but rather low in comparison to manures. In addition, AL compost can be considered as an effective product for soil amendment due to the great resistance of its lignocellulosic fraction to the edaphic degradation, but supplementary fertilization with mineral N is required, if used as fertilizer.

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S12-C3

Valorisation biotechnologique des sous produits de l'olivier par Fermentation en Milieu Solide

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Résumé

L'oléiculture et l'oléologie ont profondément évolué ces dernières décennies. Cette évolution a notamment entraîné des modifications importantes à tous les niveaux de la filière oléicole. Ainsi, au niveau agronomique, la culture de l'olivier s'oriente vers des productions biologiques avec un minimum d'entrants (engrais, pesticides…) ; au niveau technologique, les récoltes se sont mécanisées et automatisées permettant de réduire considérablement les délais entre la récolte et la trituration. La généralisation des systèmes de trituration continus a permis de traiter les olives sans délai d'attente. La conjugaison de toutes ces évolutions a conduit d'une part à des huiles d'olive vierges de grande qualité organoleptique et d'autres part à l'obtention de co-produits (margines, grignons, feuilles, bois…) de bonne qualité, ce qui permet d'orienter les recherches vers la valorisation biotechnologique de ces matières premières. Ces produits nouveaux constituent un SOS (Solid Olive Substrate) pour le traitement des margines utilisées comme substrat en biotechnologie pour la culture de champignons filamenteux en Fermentation en Milieux Solide (FMS). Quelques exemples d'applications (production de biopesticides, d'enzymes et des champignons comestibles médicinaux) seront détaillés pour démontrer le potentiel de ces matières premières pour un développement durable du secteur oléicole dans les pays Méditerranéens.

Mots clés : Grignons d'olive, margine, FMS, champignons, enzymes.

Abstract

Oleiculture and oleology have deeply progressed for these last decades. This progress has notably induced important changes in all olive chain levels. Also, in the agronomic level, olive tree culturing is directed towards biologic productions with a minimum of volumes (fertilizers and pesticides); in the technological level, harvests are mechanized and automatized enabling to reduce considerably the time between the harvest and tituration. The generalisation of continuous trituration systems has enabled to treat olives without time out. The conjugation of all these evolutions has led to virgin olive oils of a high organoleptic quality, on the one hand, and to the obtaining of co-products, in the other hand (margins, pomace, leaves, wood…) of good quality, enables to direct researches towards biotechnological valorisation of these raw materials. These new products constitute an SOS (Solid Olive Substrate) for the treatment of the margins used as substrate in biotechnology for the culturing of filamentarous fungi in fermentation in solid areas (FMS). Some application examples (biopesticides production, edible medicinal enzymes and fungi) will be detailed to demonstrate the potential of these raw materials for a sustainable development of the olive sector in the Mediterranean countries.

Key words: olive pomace, margins, FMS, fungi, enzymes.

1. Introduction: La culture de l'olivier dans les pays Méditerranéens

En l'absence de tout apport extérieur, l'exploitation d'une oliveraie se traduit par un appauvrissement progressif du sol en éléments (C, N, P, sels minéraux) nécessaires à la production des olives et à la biomasse des arbres (Therios, 2006). Une conduite intensive mais écologique et durable des oliveraies ne peut donc s'envisager qu'à la condition de rapporter au sol ces éléments sous la forme des sous-produits de l'exploitation, c'est-à-dire les déchets verts résultants de la taille et les résidus des moulins à huile qui peuvent être pâteux (margions) ou liquides/solides (margines/grignons) en fonction de la technologie utilisée pour extraire l'huile (biphasique ou triphasique).

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Les résidus bruts sortant des chaînes de trituration d'olives ne peuvent cependant être retournés aux champs directement que dans des conditions précises et limitées car ils contiennent des substances phytotoxiques et antimicrobiennes (phénols, acides gras et acides organiques). Leur fort contenu en matières organiques non stabilisées et en matières minérales (N, P, K) représente de toute façon un risque de pollution pour l'environnement (Kapellakis et al., 2008). La nouvelle loi cadre française sur l'eau et les milieux aquatiques parue en 2006 afin de répondre à la directive européenne 2000/60/CE sur l'eau impose d'ailleurs à l'industrie oléicole française de développer de nouvelles voies d'élimination de ces déchets.

Dans cette présentation, les différents procédés d'extraction d'huile d'olive à une ou à deux phases seront évoquées; la nature et la quantité des produits et sous produits obtenus seront décrites; les différents procédés de conservation et de biotransformation des sous produits seront brièvement présentés. Le but de cette tâche est donc de définir dans quelles conditions opératoires des techniques telles que l'ensilage, les fermentations en milieu solide, le compostage et le lombri-compostage permettent de stabiliser et détoxiquer les résidus de trituration en vue de leur réincorporation sans risque dans les oliveraies. Le degré de maturation des composts et lombri-composts sera déterminé avec précision afin de proposer des produits de qualité répondant à la norme et ne nécessitant ainsi aucune contrainte d'épandage.

2. La production d'olives, d'huiles d'olive et des sous produits dans le monde

La culture de l'olivier, à forte valeur culturelle et patrimoniale en région méditerranéenne, et la production d'huile d'olive, représentent environ 97% de la production mondiale (Fig. 1). Cette production s'accompagne de l'apparition de sous produits (restes de taille des oliviers, grignons et margines) peu ou pas valorisés à l'heure actuelle.

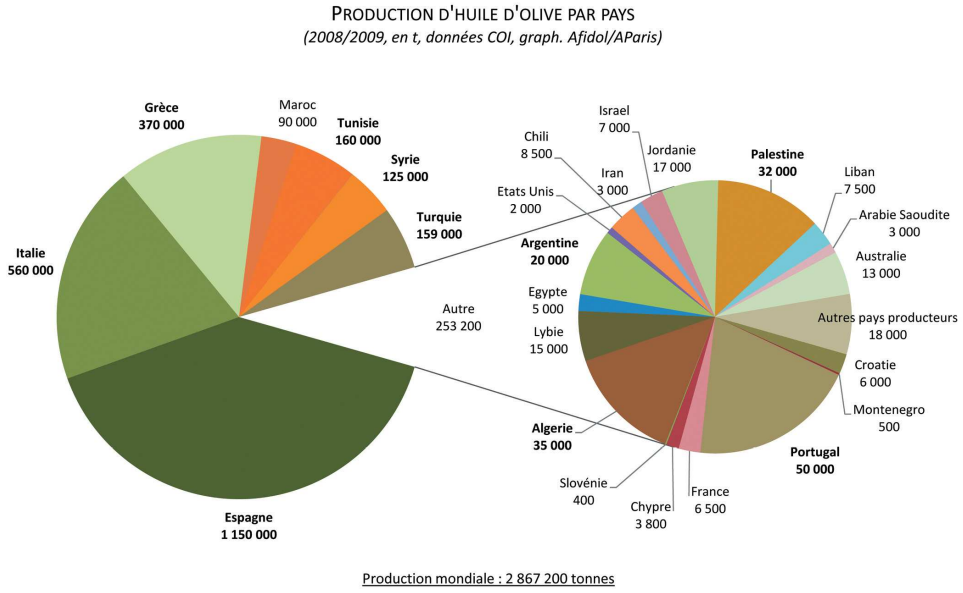

Figure 1: Production mondiale d'huile d'olive 2008/2009 (Source : AFIDOL).

3. Valorisation biotechnologique des sous produits de l'olivier pour une agriculture raisonnée

Les olives ne sont pas des fruits très riches en huile, comparées aux graines des oléagineux. Ainsi, la composition moyenne des olives est: eau 40 à 50% (eau de végétation ou margines) ; matières solides 25-35% (grignons) ; huile 20-25%. Les huiles d'olive vierges (HOV), obtenues uniquement par des moyens mécaniques, sont des systèmes chimiques complexes constitués de plus de 250 composés. Ils peuvent être classés en deux grands groupes : les substances saponifiables (de 96 à 98% de l'huile) et les substances insaponifiables (de 2 à 4% de l'huile).

Les composés phénoliques sont caractéristiques des huiles d'olives vierges et leur confèrent des propriétés particulières (stabilité oxydative, saveur…). La production d'huiles d'olive vierges nécessite un broyage des olives, suivi d'un malaxage de la pâte obtenue afin d'optimiser le rendement

d'extraction. La séparation des phases liquides (huile et margines) de la phase solide (grignons) est réalisée à l'aide de différents équipements au sein desquels la pâte d'olive est soumise à l'action de forces diverses qui, en fonction du système employé, peuvent être : la pression (système discontinu ou traditionnel) ou la force centrifuge (système continu).

Le système discontinu sépare les deux phases liquides de la phase solide. Les deux phases liquides sont ensuite séparées par décantation ou centrifugation. Le système continu se subdivise en trois modes de fonctionnement possible : (i) le mode trois phases qui permet de séparer en une seule opération les deux phases liquides de la phase solide mais qui nécessite l'ajout d'eau dans la pâte ce qui conduit à environ 120 L de margines/100 kg d'olive, (ii) le mode deux phases qui sépare l'huile des margines plus les grignons et qui généralement ne nécessite pas d'ajout d'eau. Ainsi, il est produit un grignon dit humide (contenant 60-70% d'eau) mais plus de margines, (iii) le mode deux phases et demie nécessite un ajout d'eau de 5 à 15 L d'eau/100 kg d'olives. Ce mode permet de séparer l'huile d'un grignon moins humide que précédemment (contenant 53 à 58% d'eau) et une production réduite de margines allant de 5 à 20 L/100kg d'olives. L'addition d'eau dans les procédés d'extraction centrifuge entraîne une diminution de la teneur en composés phénoliques de l'huile, une augmentation de leur teneur dans la phase aqueuse et un accroissement du volume des margines. En effet, ces composés se répartissent lors du malaxage et de la centrifugation entre les phases huileuse et aqueuse.

L'industrie oléicole mondiale, en plus de sa production principale qui est l'huile (huile d'olive vierge et huile de grignons) qui a été de 2.867.200 tonnes pour année 2008-2009, génère deux résidus : l'un liquide (les margines) et l'autre solide (les grignons). De plus, l'olivier à travers la taille (annuelle, bisannuelle, de rajeunissement, etc.) ou la récolte mécanique engendre des résidus tels que du gros bois et selon les estimations de nombreux pays, 25 kg de feuilles et brindilles (diamètre inférieur à 4 cm) sont produits par an et par arbre. Ceci se traduit par une production annuelle dans le monde d'environ 20 millions de tonnes de feuilles et brindilles fraîches qui pourraient être utilisées en alimentation animale (Nefzaoui, 1988), ou compostées sur place en les mélangeant avec des margines.

En Espagne, premier pays producteur d'huile d'olive, le système biphasique a été introduit vers les années 1970 et actuellement il y a une production de 4.000.000 de tonnes de « alperujo », un sous produit solide, composé essentiellement de lignine (31%), d'hemicellulose (24%), de cellulose (14%) de matières grasses (11%) de protéines (6%) de sucres solubles (6,5%), de phénols solubles (1,5%) et de nombreux sels minéraux étant donné que les cendres représentent 6% de matière sèche (Alburquerque et al. 2004).

Les sous-produits de l'olivier sont donc nombreux, de compositions différentes et d'utilisations très variées suivant les différents pays. Les grignons d'olive sont des sous produits solides essentiellement ligno-cellulosiques contenant la pulpe d'olive et du bois mais aussi des matières grasses, des sucres, des aminoacides, des polyphénols et des sels minéraux. La valorisation des grignons se fait dans diverses applications suivant les pays et le contexte (Tomati et Goli, 2006):

3.1. **Valorisation des grignons d'olive en alimentation**

Il convient avant toute alimentation de séparer les noyaux éclatés de la pulpe. En 2006, la Société Pieralisi a mis au point de nouvelles machines capables de séparer à partir des grignons d'olive d'une part la pulpe d'olive et d'autre part le bois des noyaux d'olives (Digiovacchino et Prezinso, 2006). Les produits ainsi obtenus peuvent être valorisés séparément, la pulpe pour l'alimentation, les noyaux en biocombustible ou autre usage.

3.2. Valorisation biotechnologique des grignons d'olive

Les grignons d'olive ont été utilisés comme substrat pour la culture de champignons filamenteux thermophiles par fermentation en milieu solide pour la production de lipases thermostables de *Rhizopus oligosporus* (Projet PRAD 02/13). Les matières grasses résiduelles des grignons d'olive favorisent la production importante de biomasse et des enzymes comme les lipases (Ismaili-Alaoui et al. 2002).

3.3. Compostage des grignons d'olive

Les résidus solides ou pâteux générés de l'extraction de l'huile d'olive sont riches en matière organique et constituent un aliment de choix pour la croissance de microorganismes.

Le compostage est la méthode la plus utilisée pour la préparation des amendements organiques et pour la fertilisation des sols. Il permet de détoxiquer ces résidus solides contenant des substances phytotoxiques et antimicrobiennes à cause de la présence de phénols, des acides gras et des acides organiques. Souvent pour le compostage efficace des grignons on y ajoute des déchets végétaux ou des déchets urbains. Il existe deux sortes de compost obtenus avec un mélange de déchets de l'olivier (Feuilles oliviers +biomasse de taille d'olivier + margines +grignons d'olive) ou d'un mélange de déchets urbains verts+ Pailles de céréales+ pailles de céréales+ déchets de l'olivier). L'AFIDOL (Association Française Interprofessionnelle de l'Olive) a déjà expérimenté en France des compostages à grande échelle avec succès (Tableau 1).

Tableau 1: Composition moyenne des mélanges pour le compostage à grande échelle des sous produits liquides et solides de l'industrie oléicole.

Caractéristiques	Mélange 1	Mélange 2	Mélange 3
Composition	•9 tonnes (9m^3) de grignons humides issus d'un décanteur à 2 phases •9,5 tonnes (30m^3) de déchets verts broyés	• 9,9 tonnes (9,9 m^3) de grignons humides issus d'un décanteur à 2 phases 10,2 tonnes (28 m^3) de déchets verts broyés 40 kg d'urée à 46%	12,8 tonnes (13,5 m^3) de grignons humides issus d'un décanteur à 3 phases 10,2 tonnes (28m^3) de déchets verts broyés 6 m^3 de margines issues moulin à 3 phases
Densité	0 ;50	0,55	0,62
Teneur en eau des mélanges	49%	49%	56%

Source AFIDOL

La Durée approximative du compostage est compris entre 6 à 8 mois, voire davantage pour un compost plus mûr. Par contre pour la réussite d'un compostage, il est impératif de travailler avec des teneurs en eau faibles pour les différents mélanges, d'où l'intérêt d'ajouter de déchets verts broyés.

3.4. Ensilage et lombricompostage des grignons d'olive

Une recherche sur *ISI web of knowledge* montre que plusieurs articles ont été publiés tant sur l'ensilage que le lombri-compostage des résidus de trituration des olives. Il en est de même pour la filtration des margines sur membrane. Ces modes prometteurs de détoxification et de valorisation des sous-produits de l'industrie oléicole sont donc pratiquement vierges et les conditions optimales de leur mise en œuvre ainsi que leur viabilité restent à définir.
Les quelques essais d'ensilage réalisés jusqu'ici n'ont porté que sur les grignons et avaient exclusivement pour but la production d'aliment pour le bétail (Weinberg et al., 2008) sans entrevoir la possibilité d'utiliser les produits ensilés à d'autres fins comme, par exemple, en tant que substrat pour la culture de champignons.

Les résultats disponibles dans la littérature sur le lombri-compostage aboutissent à la conclusion qu'il n'est envisageable que sur le résidu solide généré lors de la récupération au moyen de solvants de l'huile résiduelle présente dans les grignons et les margions préalablement séchés (Moreno et al., 2000). Les autres sous-produits de trituration (grignons, margines, margions) ne permettraient pas par contre pour des raisons de toxicité un développement optimal des vers de terre, du moins dans leur état brut. Rien ne permet cependant de présager d'un échec du lombri-compostage sur ces produits après un prétraitement comme l'ensilage ou après mélange à d'autres résidus agricoles.
Plus récemment, la société Cabries-lombricompostage, a utilisé avec succès, les grignons d'olive pour l'élevage de vers de terre (http://desbois-lombriculture.info/1.html). Les essais de co-compostage et de lombri-compostage des sous-produits seront conduits selon la technique classique des andains retournés. Des andains expérimentaux (hauteur 1,50 m x largeur 4 m x longueur 30 m) ont été utilisés pour tester les meilleures proportions entre sous-produits des moulins et pailles de céréales compostés. Dans un second temps, des andains pilotes de plus grande taille (50 m de longueur) seront mis en place.

3.5. Conservation par ensilage

L'ensilage des résidus saisonniers de l'industrie oléicole, permet d'envisager une utilisation annuelle pour diverses valorisations tels que la lombriculture, la production de champignons supérieurs ou encore alimentation animale. L'ajout de bactéries lactiques endogènes sélectionnées permettra d'obtenir des ensilages contrôlés (Perraud-Gaime et al. 2009).

4. Les margines

Comprennent deux fractions, l'une insoluble (matière organique 64,6%) est essentiellement constituée de pulpes d'olives, l'autre soluble et contient les sucres (12%), les lipides (4,2%), les sels minéraux (7,2%) et les composés phénoliques (2,2%). Des études de toxicité et de biodégradabilité de ces margines ont montré que la toxicité est due à la fraction soluble et que les composés phénoliques de type anthocyanes et monomères aromatiques sont très toxiques mais biodégradables. Par contre, les composés phénoliques responsables de la coloration noire sont peu toxiques et très difficilement biodégradables (Hamdi, 1991). Pour des quantités importantes, le compostage des margines est la manière la plus simple pour les recycler et consiste à les mélanger avec des substrats agricoles solides (Paredes et al. 1999 ; Paredes et al. 2002). Cependant la tendance actuelle vise à récupérer d'abord des molécules antioxydantes comme l'hydroxytyrosol, avant d'orienter les margines vers le compostage ou le traitement d'épuration de l'eau (Fki et al. 2005). Les margines sont utilisées en épandage comme fertilisant, et elles servent surtout à d'autres procédés biotechnologiques (extraction de composés aromatiques et phénoliques). Elles interviennent également dans la fabrication de compost pour la production de champignons (Paredes et al. 2005 ; Olivieri et al. 2006 ; D'Annibale et al. 2004).

5. Description du SOS ou COS

Un mélange de coproduits d'olivier (grignons d'olives, bois de la taille, margines) a été mis au point pour fabriquer le COS (Coproduits de l'Olivier Solides) et l'utiliser à la fois comme substrat et support solide, pour la culture des microorganismes par Fermentation en Milieu Solide, en particulier des champignons filamenteux entomopathogènes, nématophages pour la production de biopesticides ou pour la culture du mycélium de champignons comestibles et médicinaux comme le Shiitake (Lakhtar 2009).

5.1. Production de lipases

Des grignons d'olive mélangés avec de la bagasse de canne à sucre ont été utilisés pour la croissance de champignons filamenteux thermophiles pour la production de lipases thermostables(> 80℃). La technique de Fermentation en Milieu Solide s'avère plus adaptée pour la croissance des champignons thermophiles que la culture liquide, la production de lipases est plus élevée (10 à 20 fois) et ces enzymes sont extracellulaires. La présence dans les grignons d'olive d'acide oléique, utilisé comme inducteur, augmente la production de lipases en FMS (8 fois). L'addition à la bagasse de canne à sucre, de grignons d'olive augmente la production de lipases (26 fois). Ces enzymes sont stables pendant la culture (Cordova et al. 1998).

5.2. Production de biopesticides

Les grignons d'olive mélangés à d'autre déchets agroindustriels (margines, feuilles d'olivier, autres substrats lignocellulosiques) constituent un excellent substrat solide pour réaliser des cultures de champignons filamenteux entomopathogènes ou nématophages (Hassouni 2007). Dans la mesure où des conditions optimales de culture sont obtenues pour chaque microorganisme, le produit fermenté obtenu, enrichi en mycélium de ce type de champignon, pourrait s'avérer, après retour au champ, être un excellent agent pour une lutte biologique contre certains des agresseurs de l'olivier. Les procédés les plus prometteurs sont validés à une échelle pré-industrielle.

5.3. Production de champignons comestibles médicinaux

Une étude récente réalisée dans notre laboratoire vise à la détermination d'une formule originale d'un mélange (20/30/50) pour la préparation des différents composants des coproduits de l'industrie oléicole (bois d'olivier, grignons d'olive et des margines d'olives) afin de réaliser des cultures des

champignons comestibles et médicinaux sur un Substrat Oléicole Solide (SOS) par fermentation en milieu solide (Lakhtar 2009). Cette culture permet d'assurer d'une part la production de la biomasse fongique pour l'alimentation humaine et d'autre part la détoxification des coproduits (margines) pour servir à l'alimentation animale. La saisonnalité de substrats d'oliviers (les grignons d'olives et les margines) a été remédiée par le séchage solaire sous serre, une étude qui sera présentée en détaille dans une autre présentation (Lakhtar et al. 2010). Un test de criblage des souches de *Lentinula edodes* (champignons comestible et médicinal, connu sous le nom de Shiitake) a été effectué afin d'obtenir une souche efficace et capable de dégrader les polyphénols dans les margines. La préparation d'inoculum de la souche sélectionnée a été optimisée par l'induction du mycélium à la production des laccases. Les conditions de culture du champignon ont été optimisées et la cinétique de croissance de shiitake sur le mélange ainsi que la transformation de la matière organique ont été étudiées.

6. Conclusion de l'existant et du potentiel

On peut dire que l'olivier par son huile d'olive, est une source de produits alimentaires et cosmétiques, par ses grignons une source alimentaire pour le bétail, mais aussi une source de carbone et d'énergie par ses résidus et ses tailles, pouvant servir de substrat pour la croissance de microorganismes dans les procédés biotechnologiques, en particulier pour la production de biopesticides (champignons filamenteux entomopathogènes, nématophages ou des antagonistes de phytopathogènes). Ainsi, ces actions s'inscrivent dans une politique de développement durable et devraient pouvoir être généralisées à divers sous-produits ligno-cellulosiques, Roussos et al. (2006). Les moulins ont tout intérêt à valoriser ces sous-produits de façon d'une part à obtenir des coproduits ayant une valeur marchande et d'autre part ne pas devoir payer la redevance de l'Agence de l'Eau. On rappelle que suivant les rejets, l'Agence de l'Eau peut percevoir une redevance (principe pollueur –payeur) pouvant atteindre environ 300 €/par tonne d'huile d'olive. Parmi les pays producteurs d'huile d'olive, l'Espagne et la Tunisie sont les pays pionniers quand à la valorisation industrielles des sous produits solides et liquides de l'industrie oléicole. L'Italie, est le seul pays européen ayant développé une législation lui permettant d'utiliser l'épandage des margines dans les champs d'oliviers, sous des conditions bien réglementées. En conclusion, les sous produits de l'olivier représentent des tonnages plus importants que l'huile d'olive, leur composition les destine à une multitude d'usages, en particulier comme matière première dans les procédés biotechnologiques et dans l'avenir, ils vont jouer un rôle primordial pour un développement durable du secteur oléicole dans les pays Méditerranéens ; il est donc important de les considérer comme des matières premières au même titre que l'huile.

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S12-C4

Extraction par solvant de l'huile de grignon d'olive assistée par micro-ondes. Comparaison avec l'extraction conventionnelle

Fatiha Amarni , **Hocine Kadi** * , Ramdane Moussaoui

Résumé

L'extraction par solvant assistée par micro-ondes (ESAM) a été utilisée pour la récupération de l'huile à partir du grignon d'olives. Comparée à l'extraction par solvant conventionnel (ESC), cette nouvelle technique produit de meilleurs rendements en des temps très courts. Les différentes analyses réalisées (acidité, indice de peroxyde, teneur en polyphénols…) ont également montré que l'huile extraite par ESAM est de meilleure qualité. La composition en acides gras est identique pour les deux méthodes.

Mots clés: micro-ondes, extraction de l'huile, solvant, olive cake, caractérisation de l'huile

Microwave-assisted solvent extraction of oil from olive cake. Comparison with the conventional extraction

Abstract

The microwave-assisted solvent extraction (MASE) was used for the recovery of oil from the olive cake. Compared with the conventional solvent extraction (CSE), this new technique provided better yields in very short times. Various analyses carried out (acidity, peroxide index, polyphenol content…) also showed that the oil extracted by ESAM is of better quality. The fatty acids composition was identical for the two methods.

Key words: Microwave, oil extraction, olive cake, solvent, oil characterization.

1. Introduction

Le grignon d'olive dont la production mondiale a été estimé par Conseil Oléicole International (COI, 1989) à 3000 000 tonnes/an est un sous-produit oléicole qui peut générer des dommages à l'environnement en raison des composés phénoliques (Martinez-Garcia et al, 2006) qu'il contient. Sa valorisation passe par la récupération par solvant de son huile résiduelle (Méziane et Kadi, 2008) qui peut atteindre jusqu'à 8%. Dans la majorité des travaux relatifs à cette extraction solide-liquide, l'hexane apparaît comme le solvant le plus utilisé (Pérez-Serradilla et al, 2007).

Ces dernières années, l'utilisation de l'extraction par solvant assistée par micro-ondes (ESAM) semble être une alternative à l'extraction conventionnelle par solvant (ECS) de l'huile à partir de matériaux végétaux (Molins et al, 1996 ; Chen, 2007 ; Spigno et De Faveri, 2009). En effet, les micro-ondes ont l'avantage de générer la chaleur au cœur du matériau offrant ainsi de nombreux avantages : rapidité de traitement, rendements élevés, sélectivité, réduction de la consommation de solvant.

Le but de ce travail est d'appliquer cette technique à l'extraction de l'huile à partir d'un grignon d'olive pour étudier l'influence de la puissance de la radiation sur le rendement et sur la qualité de l'huile. Les résultats obtenus sont comparés à ceux donnés par l'extraction conventionnelle.

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2. Matériel et méthodes

2.1. Matériau et réactifs

Le grignon d'olive utilisé a été prélevé de l'huilerie « Ifriolive » située à Béjaïa (Algérie). Son humidité initiale qui était de 45.8% a été ramenée par séchage à l'étuve à 6.2%. Le taux d'huile résiduelle déterminée par extraction épuisante sur soxhlet en utilisant l'hexane est de 7.0 %.
Le grignon a été utilisé à l'état brut c'est-à-dire sans subir aucun broyage. Toutes les extractions sont réalisées avec de l'hexane d'une pureté de 95 %.

2.2. Extraction par solvant assistée par micro-onde (ESAM)

Le système ESAM utilisé se compose d'un four micro-onde, d'un réacteur et d'un agitateur en verre (fig.1). Le four micro-onde (COBRA, Model: CB-200-28L-G) a été modifié dans notre laboratoire. Sa puissance maximale fonctionnant à 2450 MHz est de 900 W variable avec un pas de 180 W. Les dimensions de sa cavité interne sont 240 mm x 354 mm x 358 mm.
Les extractions sont réalisées dans un réacteur cylindrique en verre de 600 ml équipé d'un agitateur mécanique. Les solides sont séparés du miscella par filtration sous pression réduite. L'huile est récupérée par distillation du miscella à l'aide d'un évaporateur rotatif. Les traces de solvant sont éliminées par séchage dans une étuve à 103℃.

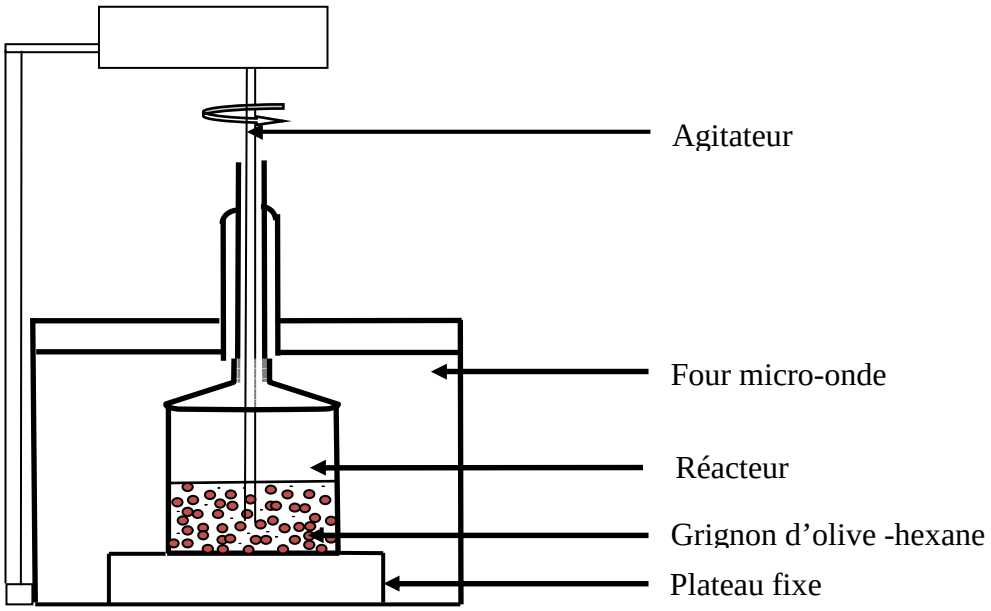

Figure 1: Installation de l'extraction assistée par micro-ondes

Chaque valeur de rendement reportée correspond à une moyenne de trois essais. La masse de grignon utilisée pour chaque essai est de 50 g. Les conditions opératoires sont les suivantes:

- Rapport liquide-solide, (L/S= 3 cm^3/g)
- Vitesse d'agitation, Va= 480 tours/mn
- Temps d'extraction, t= 1 et 2 mn
- Puissance de la radiation, P= 180 et 720 W

2.3. Extraction par solvant conventionnelle (ESC)

Le système conventionnel ESC est le même que celui utilisé en extraction assistée par micro-onde excepté le fait que la température est contrôlée par immersion du réacteur dans un bain thermostaté. Les conditions expérimentales sont aussi identiques, seule la température est fixée à 25 et 45℃.

Il faut signaler que sous micro-onde, on ne peut pas dépasser un temps d'extraction de 3 minutes surtout pour les puissances de radiation élevées car le solvant entre en ébullition. Pour P= 720 W, le temps maximum possible est seulement de 2 minutes.

2.4. Analyses des huiles extraites

L'huile extraite a été soumise aux analyses suivantes : acidité, absorbance spécifique, indice de peroxyde, indice d'iode, indice de saponification, teneur en insaponifiables et teneur en polyphénols.

Acidité:

La méthode décrite dans la norme française (NF T60-204, 1968) a été utilisée pour déterminer l'acidité des huiles extraites. Elle est basée sur la mise en solution d'une prise d'essai dans un mélange d'éthanol et d'éther éthylique (1/1, v/v) suivie d'un titrage des acides gras libres à l'aide d'une solution de soude.

Absorbance spécifique:

L'absorbance à 270 et 232 nm de l'huile extraite dissoute dans l'hexane pur a été déterminée à l'aide d'un spectrophotomètre à double faisceau suivant la norme française (AFNOR, 1978).

Indice de peroxyde:

La méthode utilisée est celle décrite par la norme française (NFT 60-220, 1968). Le principe consiste à traiter le corps gras en solution dans de l'acide acétique et du chloroforme par une solution d'iodure de potassium. L'iode libéré est titré par une solution de thiosulfate de sodium en présence d'empois d'amidon comme indicateur.

Indice d'iode:

La méthode utilisée est celle décrite par le règlement N° 2568 / 91 de la CEE dont le principe consist e à mettre en solution une prise d'essai dans un solvant et addition du réactif de Wijs. Au terme d'un laps de temps déterminé, on ajoute une solution d'iodure de potassium et de l'eau avant de procéder au titrage de l'iode libéré par une solution de thiosulfate de sodium.

Substances insaponifiables:

La teneur en substances insaponifiables a été déterminée par la méthode dite à l'oxyde diéthylique. Cette méthode décrite par française homologuée NF T60 – 205 (1975) consiste d'abord en la saponification du corps gras par traitement à l'ébullition sous reflux avec une solution éthanolique d'hydroxyde de potassium. Les substances insaponifiables sont extraites ensuite de la solution de savon par l'oxyde diéthylique.

Indice de saponifiation:

Cet indice est déterminé suivant la méthode donnée par la norme NF 60-206 (déc. 1968). L'échantillon est soumis à une ébullition sous réfrigérant avec reflux avec une solution éthanolique d'hydroxyde de potassium. L'excès d'hydroxyde de potassium est titré avec une solution aqueuse d'acide chlorhydrique.

Composés phénoliques:

Le dosage des composés phénoliques totaux des extraits obtenus a été effectué conformément à la méthode de Gutfinger (Gutfinger, 1981) dont le principe est le suivant: Cette méthode utilise le réactif de Folin – Ciocalteu qui est constitué par un mélange d'acide phosphotungstique ($H_3PW_{o12}O_{40}$) et d'acide phosphomolybdique ($H_3PM_{o12}O_{40}$) qui est réduit lors de l'oxydation des phénols en un mélange d'oxydes bleus de tungstène (W_8O_{23}) et de molybdène ($M_{O8}O_{23}$) (Swain et Goldstein, 1964). La coloration produite estimée par spectrophotométrie visible à 750 nm est proportionnelle au taux de composés phénoliques.

Acides gras:

Les acides gras ont été transformés en esters de méthyle (NFT 60 – 23, 1977) et analysés par chromatographie en phase gazeuse (AFNOR NF T 60 – 234. 1977). Le chromatographe utilisé est de type Chrompack CP 9002, celui-ci est équipé d'un injecteur Split 1/100, d'un détecteur à ionisation de

flamme et d'une colonne capillaire DB 23 de 30 m de longueur et de 0,32 mm de diamètre intérieur. Les pics ont été identifiés par référence aux temps de rétention des esters de méthyle connus.

Les conditions opératoires de travail sont : Débit du gaz vecteur (azote): 1ml/mn, température de colonne : 190℃, température de l'injecteur : 250℃ et température du détecteur : 250℃. La quantité injectée est 0,2 µl.

3. Résultats et discussions

3.1. Etude du rendement

Le tableau 1 montre que la température dans l'extraction conventionnelle et la puissance de la radiation dans l'extraction assistée par microonde influent positivement sur le rendement en huile.
En effet, quand la température passe de 25 à 45℃, le rendement augmente de 5.9% pour un temps d'extraction t= 1 mn. Pour t= 2 mn et pour une même variation de la température, le rendement augmente de 6.4%.

S'agissant de la méthode ESAM, quand la puissance de la radiation passe de 180 à 720 W, l'augmentation du rendement est 16.5% pour t= 1 mn. Pour une même variation de la puissance et mais avec t= 2 mn, l'augmentation est de 17.7%.

Comparée à l'extraction traditionnelle, l'extraction par micro-onde donne de meilleurs rendements. On observe sur le tableau 1 que pour un temps d'extraction t=1mn, le rendement qui est de 3.71 % à 25℃ et 4.37% à 45℃ pour la CSE croit à 4.25% à P =180 W et à 4.95 à P= 720W. Pour 2 minutes, il ya toujours une augmentation mais elle est moins importante. Cependant, on observe que pour 2 minutes le rendement est plus élevé avec la ESC à 45℃ qu'avec la méthode ESAM à P= 180 W.

Tableau 1: Rendement en huile extraite par ESC et ESAM en fonction du temps (L/S=3, Va=480 tours/mn).

Temps (mn)	ESC		ESAM	
	T= 25 ℃	T= 45℃	P=180 W	P= 720 W
1	3.71	4.37	4.25	4.95
2	4.21	4.58	4.40	5.18

3.2. Analyses des huiles

Les huiles soumises aux différentes analyses sont extraites dans les conditions suivantes : L/S=3, t= 2 mn, Va=480 tours/mn. Tous les résultats des analyses sont consignés dans le tableau 2 exceptés ceux relatifs aux acides gras.

Acidité :

Les huiles extraites de manière conventionnelle présentent une acidité qui ne semble pas varier avec la température. Dans le cas de l'extraction ESAM, la puissance de la radiation influence légèrement cette acidité qui n'augmente que de 6,3 % quand la puissance passe de 180 à 720W.

Les huiles récupérées par l'extraction ESAM sont moins acides que celles obtenues par la méthode conventionnelle. La diminution de l'acidité varie de 10,7 à 16,8 %

Ces huiles dont les valeurs d'acidité sont supérieures à 3,3 % correspondent à des huiles d'olive lampantes (COI, 2003).

Tableau 2: Analyses des huiles extraites (L/S=3, t= 2 mn et Va=480 tours/mn).

		Extraction conventionnelle		Extraction assistée par micro-onde	
		T= 25 °C	**T= 45 °C**	**P= 180 W**	**P= 720 W**
Acidité (%)		7.4	7.5	6.3	6.7
UV	232 nm	3.9998	3.9998	1.0462	1.0511
	270 nm	0.6550	0.7357	0.1492	0.1611
	R	6.11	5.44	7.01	6.69
Indice peroxyde (meq/kg)		28.8	30.5	22.2	26.1
Indice d'iode (g I_2/100 g d'huile)		85.6	63.7	95.5	88.4
Indice de saponification (mg KOH/g d'huile)		166.9	167.1	189.0	190.4
Teneur en insaponifiables (g/100 g d'huile)		1.0	1.2	1.1	1.3
Teneur en polyphénols		562.6	647.9	718.8	1158.5

Absorbance spécifique:

En ESC, l'augmentation de la température n'a pas d'influence sur l'extinction à 230 nm. L'huile présente donc la même oxydation primaire à 25°C com me à 45°C. La croissance de l'extinction à 270 nm indique que l'huile extraite à 45°C contient plu s de produits secondaires d'oxydation. La variation du rapport R montre que l'augmentation de la température a une influence négative sur la qualité de l'huile.

S'agissant de l'extraction ESAM, on observe que l'oxydation primaire est accentuée par l'augmentation de la puissance de la radiation qui engendre aussi plus de produits secondaires d'oxydation. La variation du rapport R montre que l'huile est moins oxydée à 180 W.

Les huiles extraites par les deux procédures sont de qualité médiocre. Cependant, l'extraction par micro-onde donne des huiles de moins mauvaise qualité.

Indice de peroxyde:

Les huiles extraites par micro-onde présentent des indices de peroxyde inférieurs à celles obtenues que celle obtenues par ESC.

L'indice de peroxyde des huiles extraites augmente avec la température (extraction conventionnelle) et avec la puissance (extraction micro-onde).

Toutes ces huiles dont les valeurs de l'indice de peroxyde sont supérieures à 20 méq d'O_2 /Kg d'huile, sont de mauvaise qualité.

Indice d'iode:

On observe que les huiles extraites par microondes ont un indice d'iode supérieur à celles obtenues de manière conventionnelle. On remarque également que la température (ESC) et la puissance (ESAM) provoquent l'altération de l'huile.

Indice de saponification:

On observe que la température comme la puissance de radiation ne semblent pas affecter l'indice de saponification. Il faut cependant remarquer que l'huile obtenue par extraction assistée par micro-onde présente un indice plus élevé que dans le cas conventionnel. Cet indice dont la valeur est voisine de 190 mg de KOH/g d'huile se situe dans les limites fixées (184-196) par COI pour les huiles d'olive.

Teneur en insaponifiables:

L'extraction par micro-onde permet de récupérer plus de matières insaponifiables. On observe également que la température (ESC) et la puissance (ESAM) favorisent cette récupération.

Teneur en polyphénols:

L'extraction ESAM conduit à une huile plus riche en polyphénols que celle obtenue par l'extraction ESC. La récupération de ces polyphénols semble favorisée par l'augmentation de la puissance de la radiation et par la température.

Acides gras:

Les résultats de l'analyse qualitative et quantitative des acides gras des huiles de grignon extraites de façon conventionnelle et micro-ondes sont donnés par le tableau 3.

On remarque que le profil en acides gras des huiles extraites à partir du grignon d'olive semble ne pas être affecté par la nature du procédé d'extraction utilisé, par la puissance de la radiation micro-onde et par la température.

Ce tableau montre également la prédominance de l'acide oléique. Les acides gras essentiels (linoléique et linolénique) sont présents en quantité acceptable. Il faut aussi noter que la teneur en acide gras linoléique est supérieure aux valeurs trouvées dans le cas des huiles d'olive vierges (Talantikite et Ait-Amar, 1988 ; Sánchez Casas et al, 1999). Les autres composés représentés par les acides (palmitoléique, margarique, stéarique et arachidonique) se trouvent en faible quantité.

Tableau 3: Analyse des acides gras.

Acides gras	ESAM		ESC	
	P=180 W	P=720 W	T= 25℃	T=45℃
Palmitique C16 : 0	12,90	12,92	13,09	12,89
Palmitoléique C16 : 1	1,53	1,54	1,53	1,58
Margarique C17 : 0	0,08	0,08	0,08	0,08
Stéarique C18 : 0	2,45	2,45	2,49	2,43
Oléique C18 : 1	68,50	68,43	68,48	68,44
Linoléique C18 : 2	13,10	13,17	12,93	13,20
Linolénique C18 : 3	1,00	1,00	0,98	0,98
Arachidonique C20: 0	0,28	0,29	0,28	0,26
Somme des Saturés	15,71	15,74	15,94	15,66
Somme des Insaturés	84,13	84,14	83,92	84,20

4. Conclusion

Les deux méthodes de récupération de l'huile présentent le même profil en acides gras. Comparée à l'extraction conventionnelle, l'extraction assistée par micro-onde présente permet d'obtenir:

- De meilleurs rendements. Ce phénomène peut s'expliquer de la manière suivante : le chauffage au cœur du matériau induit par les radiations provoque l'éclatement des cellules qui libèrent plus d'huile ;

- une huile de meilleure qualité et plus riche en substances insaponifiables et en composés phénoliques.

Les résultats obtenus permettent de penser que l'extraction assistée par micro-onde peut constituer une alternative intéressante à la récupération de l'huile à partir du grignon d'olive.

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S12-C5

Conservation des résidus de l'agro-industrie oléicole par ensilage : de l'isolement de bactéries lactiques endogènes à l'étude de faisabilité

Isabelle Perraud-Gaime, Y. Labrousse, S. Roussos

Résumé

Pour envisager une valorisation annuelle (production de champignons comestibles, lombriculture) des sous produits saisonniers de l'industrie oléicole (grignons, margines, margions), il faut pouvoir les conserver. Cette conférence propose d'exposer les avancées et les résultats sur la faisabilité de la conservation des résidus par ensilage, ensilage naturel ou ensilage contrôlé par l'inoculation par des bactéries lactiques endogènes sélectionnées.

Mots clés : résidus oléicoles, conservation, ensilage, inoculation, bactéries lactiques.

Presrvation of the residus of olive agro-industry by silaging: from lactic endogenous bacteria isolation to feasibility study

Abstract

To consider an annual valorization (edible fungi production, lombiculture) of seasonal under-products of olive industry (pomace, margins, margion), we should be able to preserve them. This conference suggests to expose the advances and results on the feasibility of residus preservation by silaging, natural silage or controlled by the inoculation through selected endogenous lactic bacteria.

Key words: olive residus, preservation, silaging, inoculation, lactic bacteria.

1. Introduction

La superficie oléicole mondiale est estimée à 8 600 000 hectares, dont 95% se situent dans le bassin méditerranéen. La production moyenne en olive est de 10 millions de tonnes par an dont 92% sont utilisés pour l'extraction d'huile, le reste étant consommé en tant qu'olives de table (Roussos et al., 2006).

L'industrie oléicole, dont le produit final est l'huile d'olive, laisse par le système classique de pressage deux résidus, l'un liquide: les margines (eaux contenus dans l'olive, eaux de lavage, eaux liés au processus de traitement), l'autre solide: les grignons (peaux, résidus de la pulpe, fragments de noyaux). Un nouveau type de résidu apparaît, les margions (terme né de la contraction de margines et de grignons) avec l'essor d'un nouveau système continu utilisant la centrifugation Ces déchets polluants de l'industrie oléicole sont produits en très grande quantité (Mulinacci et al. (2005), Chimi, 2006).
La transformation des olives et la production de l'huile d'olive sont des activités saisonnières de Novembre à Mars suivant les régions. Le stockage des sous produits, en vu d'une valorisation annuelle, est un problème à résoudre.

Des recherches en cours au sein du laboratoire EMB-IMEP montrent que l'on pourrait utiliser ces résidus pour la production de champignons supérieurs (Lakhtar (2009)), une autre utilisation pourrait être la production de lombricompost. Mais pour cela il faut, avant tout, conserver ces déchets pour avoir une disponibilité annuelle. Une technique de conservation serait l'ensilage. Cette méthode fait intervenir des microorganismes les bactéries lactiques.

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Des travaux antérieurs réalisés sur un autre résidu agro-industriel (la pulpe de café) au cours d'un projet européen Biopulca ont démontrés qu'il était possible de conserver ce type de résidus par la technique de l'ensilage (Perraud-Gaime (1995)) et permettre ainsi une possible utilisation annuelle de ce résidu devenu ainsi substrat potentiel à diverses valorisations tels que la lombriculture, la production de champignons supérieurs (Biopulca (2001)).

D'où l'idée d'appliquer cette technologie simple et peu coûteuse aux résidus saisonniers de l'agro-industrie oléicole pour une valorisation annuelle soit par lombriculture, soit par production de champignons supérieurs comestibles et/ou médicinaux, soit pour l'alimentation animale (suivi de la désamérisation), soit pour la production de molécules à haute valeur ajoutée (enzymes et autres molécules).

La finalité de ce travail sera de déterminer la faisabilité de la conservation par ensilage de ces résidus par l'isolement et la caractérisation de la microflore endogène à partir d'ensilages naturels puis la réalisation d'un ensilage contrôlé.

2. Matériels et methods

Cette étude porte sur 2 types de sous produits issus de 2 systèmes d'extraction d'huile d'olive du Maroc : le système traditionnel par pressage de Beni Mellal-Maroc qui permet d'obtenir les margines et les grignons séparés et le système moderne par centrifugation de Sidi Bou Othman-Maroc qui permet d'obtenir les margions (margine et grignons mélangés).

Les grignons (35% d'humidité) et les margines issues du système par pressage ont été mélangés pour arriver à une humidité de 63% puis sont mis en microsilos et stockés à l'obscurité (Figure I).
Les margions (humidité supérieure à 80 %) ont été directement mis en microsilos (Figure II).

Les micro-silos ont été réalisés entre Décembre 2007 et Février 2008 en fonction des différents sous produits (Tableau I)

Figure 1: Montage des micro-silos (naturels) des sous produits issues du système par presse.

Figure 2: Montage des micro-silos (naturels) des margions.

Tableau I: Récapitulatif des microsilos réalisés au Maroc.

Microsilos n°	Système	Type sous produit	Origine	Date	T°Stockage
D1BM - D2BM	Tri-Phasique Pressage	Grignon + Margine	Beni-Mellal	12/2007	T° ambiante
BM1 - BM3 - BM5	Tri-Phasique Pressage	Grignon + Margine	Beni-Mellal	02/2008	25℃
BM2 - BM4 - BM6	Tri-Phasique Pressage	Grignon + Margine	Beni-Mellal	02/2008	T° ambiante
C1SBO - C2SBO -C3SBO	Tri-Phasique Centrigugation	Margion	Sidi Bou Othmane	12/2007	T° ambiante
OTH1 - OTH3 - OTH5	Tri-Phasique Centrigugation	Margion	Sidi Bou Othmane	02/2008	25℃
OTH2 - OTH4 - OTH6	Tri-Phasique Centrigugation	Margion	Sidi Bou Othmane	02/2008	T° ambiante

Après ouverture, des observations sur l'aspect, l'odeur et la couleur ont été réalisées pour chaque micro-silo. Le pH, l'humidité ont été déterminés.

Un isolement de la microflore endogène anaérobie a été réalisé sur milieu MRS à 30℃. Les souches pures ont été caractérisées par description morphologique et biochimique, par identification sur Galeries API 50CH et par profil fermentaire par analyse par HPLC des surnageant de culture après 24h à 30℃.

Les ensilages contrôlés par inoculation d'un ferment d'ensilage ont été réalisés dans des micro-silos de laboratoire dont la capacité est de 1300g de matière humide. En utilisant des grignons séchés et des margines congelées obtenus sur le site de Beni Mellal (structure traditionnelle par pressage) en janvier 2008 (A partir d'un lot homogène de 600 kg d'olives, il a été obtenu : 253 kg de grignon ; 220 litres de margines ; 123 litres d'huile)

Le ferment d'ensilage a été mis en culture 24 heures à 30℃ dans des flacons de 150ml contenant 90ml du milieu MRS liquide. La culture a été centrifugée pendant 15 min à 14500 rpm ensuite le culot est récupéré et mélangé avec les margines.

Les grignons (conservés sec) ont été réhydratés avec les margines contenant le ferment d'ensilage à une concentration de 2.10^8 bactéries par gramme MS.

Les pots d'ensilage sont remplis en tassant la pulpe afin de limiter la quantité d'air. Ils sont fermés puis scellés. Les microsilos sont incubés à l'obscurité et à l'étuve à une température de 25℃.

3. Résultats et discussion

Après un an de conservation, les ensilages naturels présentent des caractéristiques acceptables : pH inférieur à 5, humidité entre 50 et 60 %, (Figure III), pas d'odeur de putréfaction.

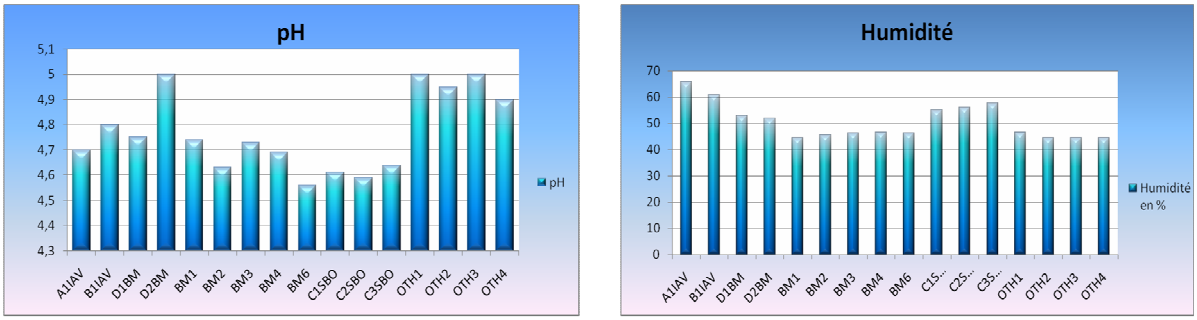

Figure 3: pH et Humidité des ensilages naturels.

L'analyse des jus d'ensilages, après centrifugation et filtration, par l'HPLC permettra d'apporter des informations complémentaires sur la concentration en sucres résiduels et sur la concentration en acide lactique et acétique. Une différence entre les ensilages à partir desquels on a pu isoler les bactéries lactiques avec ceux contenant majoritairement des levures pourra être ainsi faite et apporter des réponses plus précises sur la faisabilité des ensilages.

Ce travail a permis d'isoler 66 souches à partir des différents ensilages, 25 souches ont été identifiées comme des bactéries. Elles sont toutes Gram (+) et Catalase (-). L'analyse par HPLC des surnageants de cultures des 25 souches bactériennes isolées, a mis en évidence le métabolisme fermentaire de chaque souche, il en résulte la présence de 3 souches homofermentaires (IF 0.96/0.97) dont la production d'acide lactique est nettement supérieur à ceux des autres acides, 5 souches hétérofermentaires facultatives produisant moins d'acide lactique et plus d'acide propionique et acétique (IF 0.84/0.85), une seule souche est hétérofermentaire stricte (IF 0.68). On note l'absence de production de l'éthanol pour toutes les souches. (Tableau II).

L'isolement et la caractérisation a permis de mettre en évidence la présence des bactéries lactiques dans des ensilages des sous produit de l'olivier. Il ressort de ce travail que la présence des bactéries lactiques semble dépendre de la technique d'extraction de l'huile d'olive utilisé. En effet, les résultats d'analyses montrent que les bactéries qui produisent le plus d'acide lactique proviennent du site de

Beni Mellal où l'on utilise la méthode de pressage sur des olives non lavées pour obtenir des grignons et des margines.

Les ensilages conservés à 25℃ ont permis d'isoler le plus de bactéries lactiques tandis que les levures ont été récupérées principalement sur les ensilages conservés à température ambiante. L'isolement des levures n'est pas caractéristique d'un type de résidu comme les bactéries puisqu'on les retrouve sur les grignons/margines et sur les margions.

Au vu des regroupements faits en fonction de la production d'acides organiques, de la taille des colonies, de leur aspect et de leur profil sur les galeries API, on peut supposer que l'on a 9 souches de bactéries différentes. Ces souches appartiennent aux genres : *Lactobacillus plantarum* et *Lactobacillus pentosus*.

Après une caractérisation des bactéries lactiques, la mise en place d'un ensilage a été réalisée avec une souche préalablement isolée des ensilages naturels. Après 18 jours, le processus de fermentation n'a pas été réalisé au sein des ensilages inoculés et témoin, cela a été mis en évidence par le suivi de la microflore qui a montré la prédominance des levures et les champignons (Tableau III). Les bactéries dénombrées dans l'ensilage 2 ne sont pas des bactéries lactiques.

La mise en place des ensilages inoculés avec la souche NE022 n'a pas abouti aux résultats souhaités, cela est du à plusieurs raisons tels que l'homogénéité du mélange, la capacité et la résistance de la souche et la nature du substrat.

Tableau II: Détermination du métabolisme de différentes bactéries lactiques.

Groupe	Souches	Glucose résiduel	Acide lactique g/L	Acide acétique g/L	Acide propionique g/L	Indice fermentaire	Genre et espèce	Origine
1	NE031	0,98	25,03	10,55	1,29	0,68	*L.plantarum*	BM1
2	NE055	0,62	61,96	10,28	1,26	0,84	*L.plantarum*	OTH1
	NE058	0,59	61,53	10,34	1,26	0,84	*L.plantarum*	OTH1
	NE059	0,70	61,05	10,51	1,30	0,84	*L.plantarum*	OTH1
	NE053	0,65	61,25	10,46	1,26	0,84	*L.plantarum*	OTH1
3	NE052	1,21	61,18	10,50	1,24	0,84	*L plantarum*	BM4
	NE037	1,11	61,16	10,59	1,26	0,84	*L.plantarum*	BM3
	NE034	1,01	61,01	10,40	1,26	0,84	*L.plantarum*	BM3
	NE033	0,88	59,82	10,09	1,25	0,84	*L.plantarum*	BM3
	NE036	0,63	58,82	9,87	1,22	0,84	*L.plantarum*	BM3
4	NE039	6,03	57,67	10,45	0,00	0,85	*L. pentosus*	BM3
5	NE038	1,50	61,30	10,52	1,28	0,84	*L. pentosus*	BM3
6	NE030	3,29	58,84	10,46	0,98	0,84	*L. pentosus*	BM1
	NE032	3,00	60,09	10,62	0,86	0,84	*L. pentosus*	BM1
	NE035	6,83	56,82	10,26	0,90	0,84	*L. pentosus*	BM3
7	NE015	0,64	25,17	0,43	0,48	0,96	*L plantarum*	D1BM
	NE022	0,44	24,87	0,39	0,52	0,96	*L plantarum*	D2BM
	NE018	0,91	24,73	0,49	0,50	0,96	*L plantarum*	D1BM
8	NE013	0,51	25,03	0,15	0,60	0,97	*L plantarum*	D1BM
9	NE016	1,20	24,73	0,69	0,50	0,95	*L. pentosus*	D1BM
	NE017	3,22	23,38	0,47	0,44	0,96	*L.pentosus*	D1BM
	NE014	0,54	25,06	0,65	0,41	0,96	*L. pentosus*	D1BM
	NE029	3,85	22,70	0,55	0,45	0,96	*L. pentosus*	BM1
	NE019	3,92	22,63	0,27	0,46	0,97	*L. pentosus*	D1BM
	NE020	3,42	22,93	0,32	0,45	0,97	*L. pentosus*	D1BM

Tableau III: Analyse de la microflore totale, du pH et de l'humidité dans les ensilages inoculés.

Paramètres	Témoin		Ensilage Inoculé 1		Ensilage Inoculé 2	
Temps (jours)	**T0**	**T18**	**T0**	**T18**	**T0**	**T18**
Bactéries totales (ufc/g PMS)	1,35 10^4	<3,31 10^2	2,54 10^4	<3,3210^2	2,54 10^4	<3,92 10^2
Champignons (ufc/g PMS)	2,11 10^2	<3,31 10^2	5,97 10^3	<2,3210^2	5,97 10^3	<2,36 10^3
Levures (ufc/g PMS)	<2,11 10^2	**1,73 10^6**	<2,13 10^1	**1,5510^5**	<2,1310 ^{1}	<2,62 10^3
Bactéries MRS (ufc/g PMS)	<2,11 10^2	<3,31 10^3	3,26 10^3	<3,310^2	3,26 10^3	**1,86 10^6**
Levures MRS (ufc/g PMS)	<2,11 10^2	**1,57 10^6**	<2,13 10^1	**2,4310^5**	<2,13 10^1	<3,93 10^3
pH	5,10	4,96	5,10	5,04	5,04	5,01
Humidité %	40,81	35,53	41,35	37,59	41,35	36,34

Cependant, ces travaux préliminaires ouvrent de nouvelles perspectives quant à la valorisation des sous-produits de l'industrie oléicole par voie microbienne. En effet, ces « déchets » de l'olivier posent des problèmes de pollution car ils sont déversés dans l'environnement sans moyen de ré-utilisation et/ou de stockage adapté. L'ensilage des ces résidus saisonniers permettrait d'envisager une utilisation annuelle pour diverses valorisations tels que la lombriculture, la production de champignons supérieurs ou encore alimentation animale. L'ajout de bactéries lactiques endogènes sélectionnées permettra d'obtenir des ensilages contrôlés.

La suite de ce travail sera de continuer les essais pour arriver à avoir un ensilage correct qui répond aux critères biologiques et biochimiques d'un bon ensilage, par exemple en utilisant un ferment mixte, des substrats frais.

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S12-C6

Culture des champignons comestibles et médicinaux sur un coproduit oléicole solide pour le développement durable du secteur oléicole au Maroc

Hicham Lakhtar[1,2] , M. Ismaili-Alaoui[2] , S. Roussos[1]

Résumé

L'industrie oléicole, une activité concentrée dans la région méditerranéenne, connait actuellement une évolution à tous les niveaux. Cette industrie cause, en absence de traitement de ses rejets, d'énormes problèmes environnementaux. Les perspectives actuelles s'orientent vers la valorisation de la biomasse totale de l'olivier pour assurer un développement durable du secteur oléicole au Maroc. Notre étude vise à la détermination d'une formule originale de préparation des différents composants des coproduits de l'industrie oléicole (bois d'olivier, feuilles d'olive, grignons d'olive et des margines d'olives) pour la culture des champignons comestibles et médicinaux sur un Substrat Oléicole Solide (SOS) en fermentation en milieu solide. Cette culture a pour but d'assurer à la fois la production de la biomasse fongique pour l'alimentation humaine et la détoxification des coproduits (meule) pour servir à l'alimentation animale. La saisonnalité de substrats d'oliviers (les grignons d'olives et les margines) a été remédiée par le séchage solaire sous serre, une étude qui sera présentée en détaille dans une autre présentation. Un test de criblage des souches de *Lentinula edodes* (champignons comestible et médicinal, connu sous le nom de Shiitake) a été effectué afin d'obtenir une souche efficace et capable de dégrader les polyphénols dans les margines. La préparation d'inoculum de la souche sélectionnée a été optimisée par l'induction du mycélium à la production des laccases. Les conditions de culture du champignon ont été optimisées par le développement des modèles mathématiques. La cinétique de croissance de shiitake sur le mélange ainsi que la transformation de la matière organique ont été étudiées. L'activité laccase, qui a été observée dominante pendant la croissance des champignons sur le SOS a été purifiée pour des applications biotechnologiques ultérieures.

Mots clés : Substrat Oléicole Solide (SOS), *Lentinula edodes*, Fermentation en Milieu Solide (FMS), Polyphénols, Enzymes, Laccases.

Edible and medicinal mushroom cultivation on solid olive substrate (SOS) for sustainable olive field in Morocco

Abstract

The olive industry, an activity concentrated in the Mediterranean area, is nowadays under development at all levels. This industry has, until now, lack of processing of their discharges, resulting in major environmental issue. The current research is directed towards a recovery of the total biomass of trees for a sustainable industry. Our present study aims to broaden the horizon of recovery of the so-called discharges by culturing edible and medicinal mushrooms on olive SOS biomass (olive tree wood, olive leafs, olive pomace and olive mill wastewater OMWW) to produce biomass for both food and feed, and also for the detoxification of OMWW, the main determinant of environmental pollution in the olive industry. Seasonality substrates olive (olive cake and OMWW) has been remedied by drying solar in greenhouse, a study which will be detailed in another presentation. A screening of strains of *Lentinula edodes* (edible and medicinal mushroom, known as Shiitake) was carried out to obtain an efficient strain capable of degrading the polyphenols in olive waste. The preparation of the spawn has been optimized by the induction of mycelium. Experimental plans have been developed to optimize the cultivation of shiitake by mixing of the entire biomass SOS. The kinetics of growth of shiitake was studied. The enzymes used in the detoxification of olive oil have been listed. The laccase activity which dominated during the growth of fungi on the SOS has been purified to use for further application.

Keywords: Solid Olive Substrate (SOS), *Lentinula edodes*, Solid State Fermentation, polyphenols, enzymes, Laccases.

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1. Introduction

L'oléiculture représente une des plus anciennes activités agricoles dans le bassin méditerranéen. Toutefois, elle présente l'inconvénient de générer d'énormes quantités de déchets liquides dont la fraction organique complexe leur confère un pouvoir fortement polluant (Sayadi et al., 2000). A côté des margines, l'industrie oléicole produit deux coproduits solides, les grignons d'olive et le bois de taille.

De nombreuses études ont été menées en vue de trouver des solutions pour le traitement des déchets oléicoles, en particulier la réduction de la charge phénolique des margines par la culture de champignons (Zervakis et al., 1996, Kalmis et Sargin, 2009, Lakhtar et al., 2010a). Les perspectives de ces études ont mis l'accent sur la valorisation des déchets de l'oléiculture par la culture des basidiomycètes en fermentation en milieu solide (FMS). Les basidiomycètes de pourriture blanche, en particulier *Lentinula edodes* (Berk.) Pegler, sont capables de se développer en présence de la lignine, et leur développement est associé à la production des enzymes phénoloxydases non spécifiques (Mata et Savoie, 1998). Ainsi, les basidiomycètes ont été considérés comme de bons candidats pour permettre à la fois une valorisation des coproduits oléicoles lignocellulosiques et la réduction des polyphénols des margines (Zervakis et al., 1996, Kalmis et Sargin, 2009).

L'objectif de l'étude est d'évaluer la transformation de la composition chimique et la réduction en polyphénols du mélange de coproduits de l'olivier solide (COS) constitués de bois de taille, grignons d'olive et margines durant la croissance du mycélium de *L. edodes* Le119. Une attention particulière a été consacrée à l'évaluation de la production de CO_2 durant la croissance mycélienne de la souche Le119. Le changement de l'abondance relative des fractions de carbone, à la fois simples et complexes analysés par la technique de la CPMAS RMN 13C soutenue par les analyses biochimiques a été étudié pour relier l'activité métabolique du champignon à la transformation des résidus oléicoles.

2. Matériel et méthodes

2.1. Origine des échantillons

Les échantillons des grignons et margines d'olive ont été prélevés dans une unité de trituration triphasique avec le système de presse. Les standards phénoliques ont été fournis par Extrasynthese. Tous les solvants utilisés pour l'extraction étaient de qualité HPLC (Sigma, France).

2.2. Microorganisme et préparation du spawn

La souche de *L. edodes* Le119 a été préalablement sélectionnée pour sa capacité de dégradation des polyphénols de margines (Lakhtar et al., 2010a). La souche a été régulièrement repiquée sur un milieu de PDA contenant 10% des margines. L'inoculum (spawn) a été préparé sur les gains de blé contenant 10% de margines (Lakhtar et al., 2010b). La préparation du spawn a été effectuée dans les colonnes de « Raimbault » (De Araujo et al., 1997) et l'incubation a été faite à 25℃ pendant 10 jours .

Figure 1: Dispositif de FMS connecté au Système de Respirometrie PNEO.

2.3. Préparation des substrats COS

Le bois de taille a été broyé et tamisé pour récupérer des particules comprises entre 2 mm et 0,8 mm. Les particules, ayant avec une taille inférieure à 0,8 mm, ont aussi été utilisées dans la composition du substrat représentant 30% du poids total du bois de taille. Le mélange du COS optimal est composé de 20% de bois de taille, 30% de grignons d'olive et 50% de margines. Il a été stérilisé à 121℃ pendant 30 minutes.

2.4. Inoculation du COS par le spawn de *L. edodes*

L'inoculation a été faite à raison de 10% (poids humide: poids humide), et le remplissage des colonnes a été procédé en alternant des couches de substrat et de spawn, pour assurer une croissance homogène du mycélium. Les colonnes ont été incubées dans l'étuve sans aération forcée à 25℃ pendant 2 jours et par la suite ont été plac ées dans le dispositif de la FMS (De Araujo et al., 1997) pour une durée d'incubation d'un mois. Deux colonnes témoins non inoculées ont été préparées et incubées dans les mêmes conditions.

2.5. Analyse des échantillons

L'activité respiratoire a été mesurée chaque jour à l'aide du PNEO, qui permet de mesurer à la fois quatre paramètres ; le débit d'aération, l'humidité de l'air, la température de l'air et la teneur de l'air en CO_2 a la sortie de la colonne. Le débit de l'aération a été fixé a 20 $mL.min^{-1}$ La biomasse fongique a été estimée par la quantification de l'ergostérol (Lakhtar et al., 2010b). Les sucres réducteurs et sucres totaux ont été déterminés par la méthode de Miller (1959) en utilisant le réactif de DNS et celle de DuBois et al. (2002) en utilisant de l'anthrone respectivement. L'extraction de la fraction phénolique a été réalisée dans la solution eau-méthanol (40-60) ; à un gramme du substrat fermenté lyophilisé et finement broyé, on ajoute 10 ml de la solution organique. Le mélange a été homogénéisé et incubé pendant 1h à 4℃. L'extraction a été répétée trois fois. La quantification des phénols totaux a été faite suivant la méthode de Folin Ciocalteu (Bärlochar et Garça, 2005) en utilisant l'acide caféique comme standard.

2.6. Analyses chromatographiques

L'extraction des monomères a été effectuée en deux étapes. La première étape consiste à l'extraction de la fraction phénolique par la solution eau-méthanol (40-60). La deuxième étape consiste en la purification des monomères phénoliques par l'extraction avec l'acétate d'éthyle et l'analyse des monomères a été réalisée par l'HPLC suivant la méthode de De Marco (2007). La plateforme HPLC est composée d'un dégazeur (1200 Series Degasser, Agilent technologies), d'une pompe quaternaire (1200 Series Quaternary Pump, Agilent technologies), d'un échantillonneur liquide automatique ou passeur (1200 Series Automatic Liquid Sampler, Agilent technologies) et d'un détecteur à barrette de diodes (1200 Series Diode-Array Detector, Agilent technologies). La séparation des composés a été opérée sur la colonne Atlantis d18 (5µm, 4,6 x 250 mm) Waters gardée à une température constante (25±2℃). La détection des composées a été effectué e sur une longueur d'onde de 280 nm. Les pics ont été identifiés par comparaison avec les standards.

2.7. Activités enzymatiques

2.7.1. Extraction d'enzymes

L'extraction des enzymes a été effectuée sur 3 gr du substrat fermenté frais décongelé mélangé avec 10 ml du tampon d'acétate (0,1M, pH 5,0) contenant 5 mM de CaCl2, 0,05% du Tween 80 et 3% du polyvinylpolypyrrolidone insoluble (PVPP) (Sampedro et al., 2007). Le mélange a été incubé à la température ambiante pendant 1h et l'extrait aqueux a été récupéré par centrifugation (4500 rpm pendant 10 min) suivie d'une filtration sur papier Watman N°2.

2.7.2. Activités enzymatiques

L'activité endoglucanase (cellulase, EC 3.2.1.4) a été déterminée selon la méthode de Mandels et al. (1976). L'activité enzymatique est exprimée en unité par g du substrat poids sec (SPS) (1U= 1 µmole de sucres réducteurs formés par minute). L'activité lipase a été déterminée suivant à la technique rapportée par Goujard et al., (2009), en utilisant *p*- laurate de nitrophenyl comme substrat. L'activité tannase a été mesurée suivant la technique de Sharma et al. (2000). Les activités de Mn peroxydase, Lignine peroxydase et laccase ont été mesurées suivant la technique rapportée par Lakhtar et al. (2010a). La technique décrite par Santos et Linardi (2004) a été utilisée pour la détermination de l'activité enzymatique de catéchol-1,2-dioxygénase et protocatéchuate-3,4-dioxygénase. L'activité est exprimée en unité par g SPS (1U= 1 µmole de produit formé par minute).

2.8. Analyses élémentaires : C et N

Les teneurs en C et N, dans les échantillons de culture de *L. edodes*, ont été déterminées à l'aide d'un analyseur élémentaire CHN (Flash EA 1212 Elemental Analyzer, France). L'analyseur CHN a été calibré avec l'acide aspartique à différentes concentrations.

3. Résultats et discussions

3.1. Croissance de *L. edodes* sur COS

Le remplissage des colonnes de Raimbault avec le substrat COS prétraité a été alterné avec l'ajout du spawn, qui permet un envahissement homogène et uniforme du substrat par le mycélium. L'incubation des colonnes pendant deux jours dans l'étuve nous a permis d'éviter les contaminations par les moisissures. Après deux jours d'incubation, on a remarqué que tous les grains du spawn inoculés ont démarré par le développement du mycélium. L'envahissement total du substrat a été remarqué après la troisième semaine d'incubation (24 jours d'incubation). Par ailleurs, aucune contamination n'a été observée sur les deux colonnes témoins non inoculées.

3.2. Analyses des effluents gazeux par PNEO

A la sortie des colonnes de Raimbault, les effluents gazeux ont été collectés afin de suivre l'évolution de quatre paramètres (débit d'air, humidité relative, température et la production de CO_2) en fonction du temps (Fig. 1). L'humidité relative et la température de l'air à la sortie des colonnes fluctuent entre deux valeurs (50 et 58 %) pour la l'humidité et (23 et 27℃) pour la température de l'air. Avant la mesure de CO_2 à la sortie de la colonne, le débit de l'air traversant la colonne, a été ajusté à 20 ml/min.

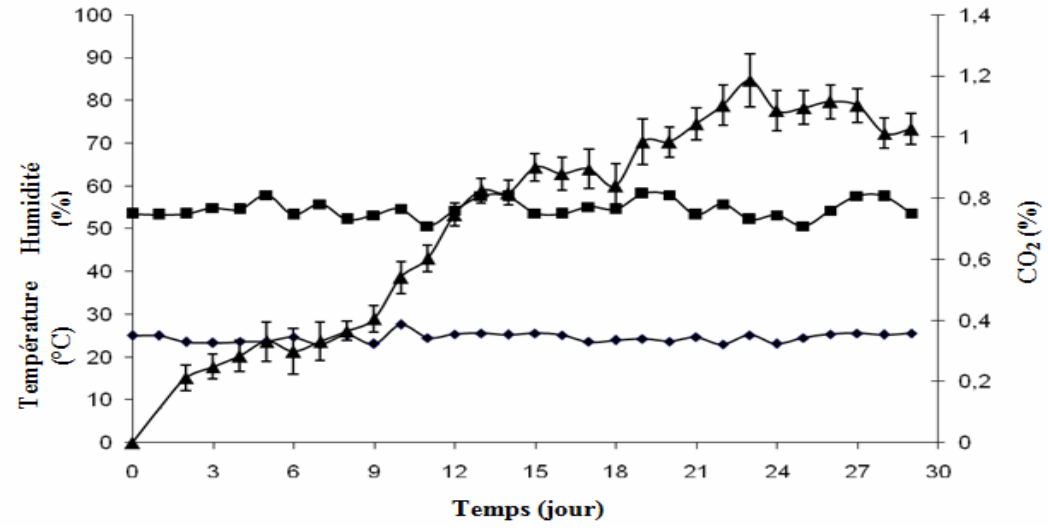

Figure 1: Evolution de trois paramètres; (▲) CO_2 produit, (◆) température de l'air, (■) humidité relative de l'air, au cours de 30 jours de culture de *L. edodes* sur COS à 25℃.

Sur la Figure 1, on remarque que l'évolution de CO_2 s'effectue selon quatre étapes alternées. Tout d'abord, durant les 6 premiers jours d'incubation sur le dispositif FMS, une augmentation continue de CO_2 a été remarquée passant de 0 à 0,23%. Ensuite, entre le 6 et 8ème jour d'incubation, un premier plateau de stabilisation du CO_2 située à 0,23% de CO_2 produit. Par contre, entre le 8ème et le 14ème jour d'incubation, une augmentation importante de la respiration du mycélium de Le119 a été remarquée allant de 0,23 à 0,7. Un deuxième plateau de stabilisation de CO_2 a été observé entre le 14ème et 16ème jour de FMS. Enfin, un plateau haut de respiration a été obtenu à partir du 23ème jour de FMS et reste constant pendant une semaine avec un taux élevé de CO_2 obtenu (1,18%).

3.3. Evolution de la biomasse de *L. edodes* sur COS:

L'estimation de la biomasse a été déterminée indirectement par la quantification de l'ergostérol contenu dans le substrat fermenté par la souche Le119 de *L. edodes*. Le coefficient de conversion de l'ergostérol en biomasse de *L. edodes* a été évalué à 3.80 mg de l'ergostérol pour 1 g de biomasse pure. L'évolution de la biomasse au cours de la FMS (Fig.2) suit relativement l'allure de production de CO_2 mesuré dans les effluents gazeux (Fig.1).

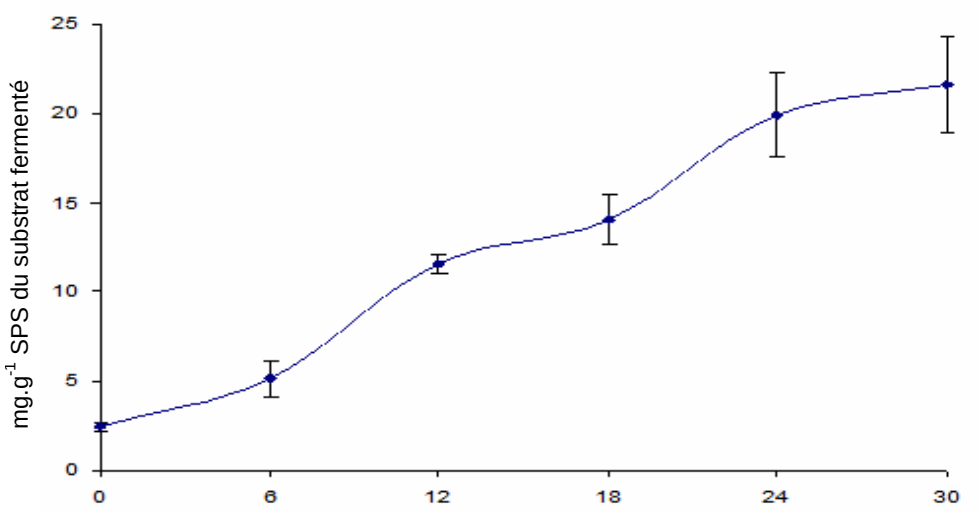

Figure 2: Evolution de la biomasse de la souche Le119 produite au cours de 30 jours d'incubation sur COS à 25℃.

L'évolution de la biomasse au cours de la FMS passe par trois phases de croissance rapide alternées par deux phases de croissance relativement lente. La biomasse évolue de 2.47 à 5 mg/g SPS du COS pendant les 6 premiers jours d'incubation. Ensuite entre le 6ème et le 12ème jour d'incubation une augmentation importante de la biomasse produite a été remarquée, allant de 5 à 11,4 mg.g^{-1} SPS du COS. Par contre entre le 12 et 18ème jour d'incubation, la biomasse passe de 11,4 à 14,5 mg.g^{-1} SPS, et cette évolution de biomasse correspond au deuxième plateau de CO_2 produit durant le développement du mycélium (Fig.1). Entre 18 et 24 jours d'incubation une augmentation importante a été de nouveau observée, allant de 14,5 à 20,5 mg.g^{-1} SPS du COS. Cette augmentation correspond également à l'augmentation de CO_2 observé dans la Figure 1. Enfin, un plateau de biomasse a été obtenu à partir de 24 jours de FMS pour arriver à une biomasse de 21,61 mg.g^{-1} après 30 jours d'incubation. Après un mois d'incubation, la biomasse estimée dans notre étude dépasse 2,5 fois la biomasse du *Pleurotus ostreatus* produite sur les coques de café avec le même taux d'inoculation (10%) (Sobal, 2002).

3.4. Evolution des sucres réducteurs, sucres totaux, pH du substrat fermenté

L'évolution de sucres réducteurs, des sucres totaux et du pH du substrat inoculé avec la souche Le119 de *L. edodes* est présentée dans la Figure 3.

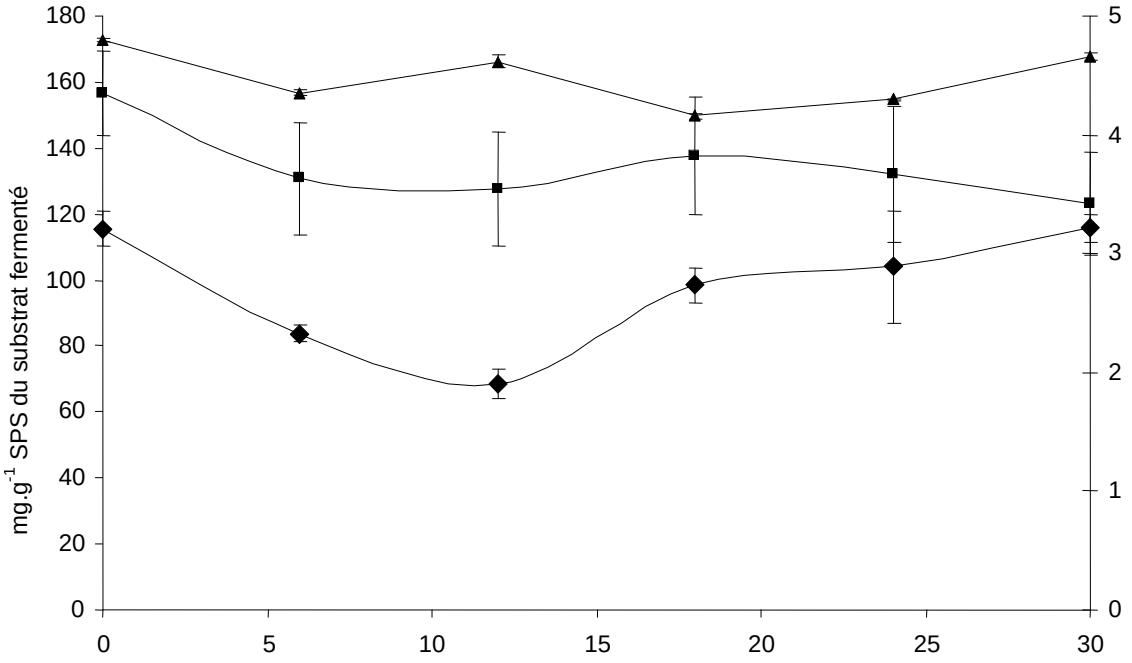

Figure 3: Evolution des (♦) sucres réducteurs, (■) sucres totaux et du (▲) pH du substrat fermenté au cours de 30 jours de culture de la souche Le119 sur COS à 25℃.

On remarque une diminution de la teneur en sucres réducteurs et totaux dans le milieu après 12 jours d'incubation, suivie d'une augmentation graduelle pour arriver à 108 et 120 $mg.g^{-1}$ SPS du SOS respectivement. Accompagné de cette évolution, on remarque que les valeurs du pH du substrat fermenté restent très stables tout au long de la FMS de la souche de *L. edodes* sur COS et fluctuent de 4,5 à 5.

3.5. Evolution des phénols totaux

Les phénols totaux ont été déterminés par la méthode standard de Folin Ciocalteu pour pouvoir comparer nos résultats aux ceux de la littérature. La décoloration de l'extrait organique des phénols totaux a été déterminée à 280 nm. Selon Chin et al., (1994), l'absorbance à 280 nm est une mesure approximative du degré d'aromaticité des matières organiques dissoutes. Une forte corrélation entre la dégradation des phénols et la décoloration du substrat a été observée. Le développement du champignon a permis une dégradation de 80% des phénols totaux et 85% de décoloration du substrat (Fig. 4).

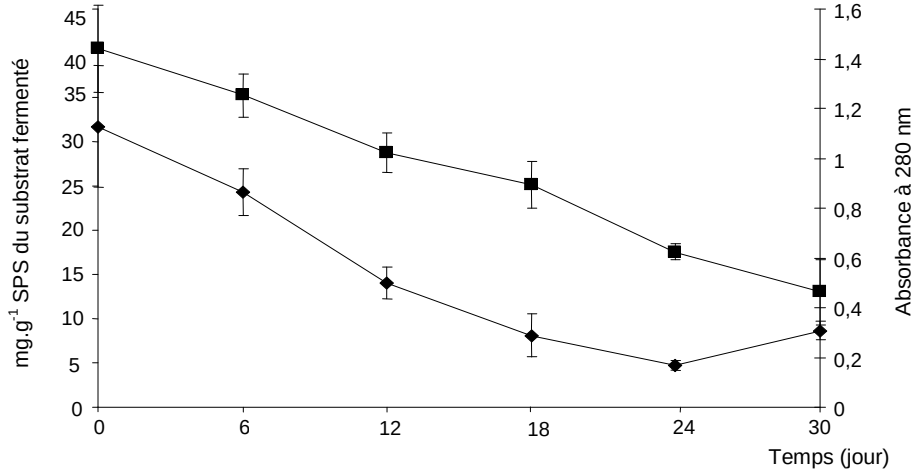

Figure 4: Evolution de (■) la décoloration de l'extrait phénolique et (♦) des phénols totaux du substrat fermenté au cours de 30 jours de culture de la souche Le119 sur COS à 25℃.

Les résultats confirment ceux obtenus à partir de la décoloration et montrent l'apparition des molécules absorbant aux alentours de 500 nm. Ces molécules peuvent être responsables de l'augmentation légère des phénols totaux observée après 24 jours d'incubation. Sampedro et al. (2007) ont mesuré de semblables chutes de teneurs en phénol totaux des grignons humides, issues du système de trituration à deux phases, par la culture de *Paecilomyces farinosus* après 20 semaines d'incubation. Selon Aloui et al. (2007), l'ajout de la bagasse de canne de sucre aux grignons humides favorise la dégradation des phénols totaux. En effet une réduction des phénols totaux de 60% a été observée après l'ajout de 30% de la bagasse de canne à sucre pour la culture de *Phanerochaete chrysosporium* après 6 jours d'incubation.
Les spectres UV-vis des extraits d'échantillons prélevés durant la croissance du champignon sont présentés dans la Figure 5.

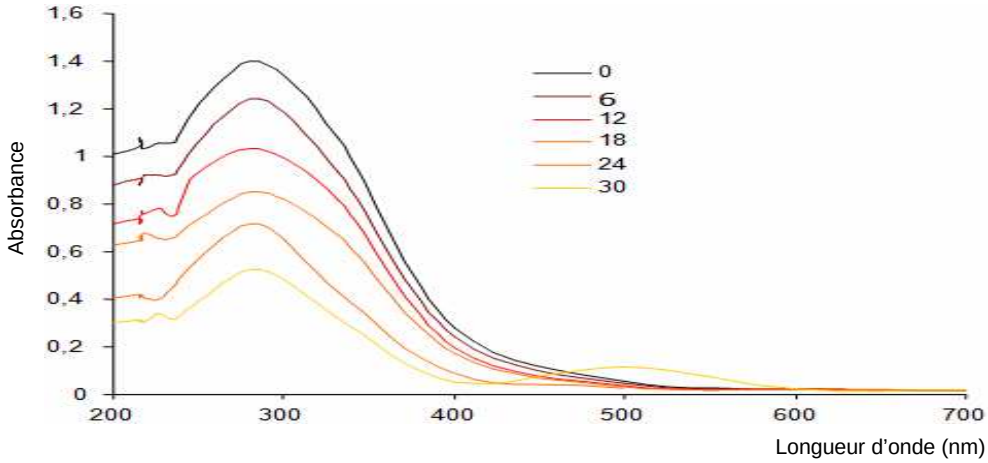

Figure 5: Spectres UV-visible de la fraction phénolique extraite des échantillons du substrat fermenté au cours de 30 jours de culture de la souche Le119 sur COS à 25℃.

3.6. Les monomères phénoliques

La disparition des monomères phénoliques est présentée dans le Tableau 1. Six monomères ont été identifiés dans l'extrait éthylique obtenu à partir du substrat fermenté. Leur quantification, déterminée par l'HPLC, a montré que l'Oleuropéine est le composé le plus abondant dans le COS avant la fermentation (6,54 µmole.g^{-1} SPS du SOS), suivie par l'acide *p*-coumarique et l'hydroxytyrosol (4,51 et 4,32 µmole.g^{-1} SPS du SOS respectivement). Apres un mois de culture de la souche Le119, à l'exception de l'acide *trans*-cinnamique qui présente une faible diminution de sa teneur (15 %), tous les composés analysés, ont subi une réduction de leur teneur avec des rendements variables (92 et 26 % de réduction pour l'hydroxytyrosol et l'acide *p*-coumarique respectivement). Dans le COS témoin, non inoculé et incubé dans les mêmes conditions que celles de la culture, on a remarqué une réduction partielle de tous les composés analysés, après 30 jours d'incubation avec un débit d'aération de 20 mL.min^{-1}. Leur réduction peut être expliquée par l'oxydation non enzymatique qui pourrait être causée par le passage forcé de l'air dans le substrat durant son incubation dans le dispositif de la FMS. El Hajjouji et al. (2008) ont reporté qu'une augmentation de réduction des composés phénoliques est favorisée avec l'application d'une aération forcée sur les margines.

Tableau 1: Concentration des monomères phénoliques (µmole.g^{-1} SPS du COS) durant la culture de *L. edodes* pendant 1 mois d'incubation à 25℃.

Temps (jours)	Oleuropéine	Hydroxy-tyrosol	Acide caféique	Tyrosol	Acide trans-cinnamique	Acide *p*-coumarique
0	6,54	4,32	3,22	2,32	2,32	4,51
6	4,21	2,35	3,21	2,12	2,23	3,54
12	3,21	3,21	3,15	1,63	2,35	4,16
18	2,54	2,32	2,36	1,33	2,16	5,54
24	1,02	0,32	1,06	1,03	2,03	3,21
30	0,95	0,32	1,01	0,03	1.97	3,32
Témoin	5.98	4.45	3.11	2.22	2.25	4.02

L'écart type a été estimé à 5%.

3.7. Evolution de la composition élémentaire du substrat fermenté :

L'évolution de cinq paramètres conventionnels (humidité du substrat, C, N, C/N, Perte en matière) a été effectuée à partir des prélèvements du substrat fermenté au cours de 30 jours d'incubation à 25℃. Les résultats sont présentés dans le Tableau 2.

Tableau 2: Evolution de différents paramètres chimiques mesurés des échantillons du substrat fermenté au cours de 30 jours de culture de *L. edodes* sur COS à 25℃.

Temps (jours)	C (%)	N (%)	C/N	Humidité (%)	Perte en Matière (%)
0	48.52±1,3	0,95±0,10	51,07	44.30±0.49	-
6	46.17±0,7	0.95±0,06	48,60	45.14±2.61	1.87±0.67
12	43.89±1,4	0,96±0,04	45,72	45.94±1.14	4.99±1.01
18	42.89±1,7	1,09±0,07	39,35	46.47±0.92	6.73±1.80
24	41.32±1,2	1,31±0,08	31,54	48.39±0.37	9.48±1.59
30	40.32±0,6	1,52±0,1	26,53	46.41±1.07	12.18±1.39

Un test d'analyse de variance a été effectué pour l'ensemble des paramètres étudiés. Les résultats montrent une différence significative entre les prélèvements (p= 0.05). Le passage de l'air humidifié à travers les colonnes (volume utile de 200 mL) avec le débit de 20 mL.min^{-1} a permis de maintenir une humidité de 46±2 %. Les résultats des analyses élémentaires, montrent que le carbone diminue en passant de 48.52 à 40,32 % pendant la durée d'incubation, alors que l'azote présente une augmentation de 0.95 à 1.52% de la matière sèche. La diminution de carbone est caractéristique de la dégradation des matières organiques. En effet, de semblables profils de diminution de carbone ont été rapportés pour la dégradation des matières organiques (Mena et al., 2003, Huang et al., 2004). Le ralentissement de la diminution du carbone peut être expliqué par la nature lignocellulosique du substrat qui est dégradé beaucoup plus lentement. Par ailleurs, l'augmentation d'azote est due à sa concentration engendrée par la dégradation des composés carbonés réduisant la masse totale du substrat durant la culture. De ce fait, le rapport C/N diminue au cours de la culture compris entre 51,07 et 26.53. Cette diminution reflète le développement du champignon accompagné d'une part avec un dégagement de CO_2 et d'autre part de la décomposition de la matière organique, ainsi que l'enrichissement du substrat en matières azotées.

3.8. Activités enzymatiques

Les activités enzymatiques mesurées durant la croissance du champignon sur le COS sont présentées dans le Tableau 5. Parmi les activités enzymatiques impliquées dans la décomposition de la matière organique, l'activité des cellulases a été mesurée. Il y a apparition de cette activité après 6 jours d'incubation et elle atteint sa valeur maximale après 18 jours (1,13 U.g^{-1} SPS). L'activité lipase présente une augmentation au début de la culture (de 0,01 à 0,02 U.g^{-1} SPS), et diminue après 18ème jours de culture pour disparaître vers la fin de l'incubation. Les activités peroxydases, Mn peroxydase et Lignine peroxydase, présentent une augmentation pour arriver à sa valeur maximale (0,231 et 0,321 UI.g^{-1} SPS du SOS après 18 et 24 jours d'incubation respectivement). Ensuite, les activités peroxydase diminuent.

Les activités laccases suivent relativement la même allure avec une augmentation de 1,52 à 8,5 UI.g^{-1} SPS après 18 jours d'incubation, puis une diminution pour arriver à une valeur de 4,63 UI.g^{-1} SPS. D'après les résultats, on a remarqué que l'activité laccase est l'activité majeure parmi les activités enzymatiques impliquées dans la transformation des polyphénols. A partir de l'ensemble des résultats, on a également remarqué que les activités cellulases et les deux activités phénoloxydase présentent des tendances similaires avec une augmentation au début de la culture pour atteindre un maximum puis une diminution et stabilisation jusqu' à la fin d'incubation. Cette tendance peut être expliquée par une complémentarité entre ces activités pour aboutir à la dégradation du complexe lignocellulosique. Par ailleurs, les activités catéchol-1,2-dioxygénase et protocatéchuate-3,4-dioxygénase n'ont été pas détectées dans les conditions de culture.

Tableau 5: Mesure des activités (UI.g^{-1} SPS) cellulase, lipase, laccase, Mn peroxydase, lignine peroxydase et tannase de *L. edodes* Le119 durant un mois d'incubation à 25°C.

Temps jours	Cellulase	Lipase	Laccase	Mn peroxydase	Lignine Peroxydase	Tannase
0	0.02	0.001	1,52	0,04	0.001	0.004
6	0.4	0.002	5,8375	0,124	0,121	0.121
12	0.82	0.0014	8,055	0,142	0,245	0.225
18	1.13	0.0018	8,5175	0,231	0,214	0.154
24	0.76	0.010	7,6025	0,1789	0,321	0.174
30	0.41	nd	4,63	0,1245	0,281	0.11

4. Conclusion

Les résultats de l'étude ont montré une bonne croissance de *L. edodes* Le119 sur le COS en FMS. L'inoculation du COS avec un mycélium produit sur les grains de blé avec des margines à 10% (v/v) a permis d'éviter la contamination de culture par des antagonistes. Le suivi respiromètrique de *L. edodes* Le119 cultivée sur le COS a mis en évidence la présence de trois phases de croissance. L'étude des paramètres de sucres réducteurs, sucres totaux, phénols totaux ainsi que les spectres RMN du ^{13}C (résultats non publiés) a montré que la première phase de croissance a été attribuée à la minéralisation des matières facilement dégradable, suivie par la phase de dégradation des polysaccharides (hémicelluloses, celluloses). La dernière phase de croissance correspondait à la dégradation légère de la lignine provenant du bois de taille. L'activité enzymatique a mis en évidence la présence d'un cocktail enzymatique qu'on pourrait utiliser pour des applications biotechnologiques.

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S12-C7

Pyrolysis kinetics of olive residue/plastic mixtures by non-isothermal thermogravimetry

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Abstract

The TGA studies of a pyrolytic decomposition of mixtures of olive residue/plastic were carried out. The investigation was made at the temperature ranging from 300 to 1273 K in the nitrogen atmosphere at four heating rates β=2, 10, 20 and 50 K min^{-1}. High density polyethylene (HDPE), low density polyethylene (LDPE), polypropylene (PP) and polystyrene (PS) were selected as plastic samples. Based on the results obtained, three thermal stages were identified during the thermal degradation. The first two were dominated by the olive residue pyrolysis, while the third was linked to the plastics pyrolysis, which occurred at much higher temperatures. Discrepancies between the experimental and calculated TG/DTG profiles were considered as a measurement of the extent of interactions occurring on pyrolysis olive residue/plastic mixtures. The maximum degradation temperatures of each component in the mixture were higher than those of the individual components. These experimental results indicate a significant synergistic effect during olive residue and plastic co-pyrolysis at the high temperature region. In addition, a kinetic analysis was performed to fit thermogravimetric data. A reasonable fit to the experimental data was obtained for all materials and their mixtures.

Keywords: Pyrolysis: Kinetics; Olive residue; Plastic; Olive residue/plastic mixtures.

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Thème 7

Economie et marchés oléicoles : évolution, prospective et stratégies d'acteurs

S13-C1

The Global Market for Olive Oil: Trends, Policies and trade liberalisation in the Mediterranean basin

Samir Mili

Abstract

Future competitive scenario for olive oil will be shaped primarily by market dynamics (developments in production, consumption and trade), business strategies (innovation, promotion, concentration, internationalisation), and the ongoing agricultural and trade policy reforms (CAP reform, WTO negotiations, regional and bilateral trade agreements). Based on this premise, the purpose of this contribution is to address market change and policy reform processes in the world olive oil industry, pointing out the main factors associated with their present and expected evolution. It is assumed that although some of these factors act on a global level, others may vary across countries and even within countries amongst different supply chain agents. This implies that there is a wide spectrum of possible strategies and courses of action for the future.

Keywords*:* Market dynamics, business strategies, policy reforms, olive oil.

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S13-C2

Olive Oil Economics & Markets in Turkey

Renan Tunalioglu

Abstract

Turkey, the Mediterranean in geography and the olive crop is grown for creating one of the lucky country. Fourth in the world olive oil production, export share of 5% to fifth place. Olive oil technology is developing and packaged and branded olive oil exports are supporting to directly in Turkey since the last decade. Olives always was presented in Ottoman Empire Republic of among the agricultural products. The first official olive policies was initiated in 1929 and has continued until today. In recent years, the existing policy of bilateral agreements with organizations such as the IMF, World Trade Organization and etc (some of the relations economic integration EU, FAO, WTO, IOC, etc) referring to some as the content has changed the scope of compliance work. In this study purpose, Changes of Turkey olive and olive oil sector will assessed as economy (production and marketing) policy developments.

Key words: Olive oil, Marketing, Politics, Changing, Turkey.

1. Introduction

Turkey has the world's olive trees number of 17%, olive areas of 9%, olive production of 8%, olive oil production and exports of 5% and 10% (Figure 1, 2, 3 and 4). Turkey is one the important producer country in the other olive producer's countries. Olive cultivation is one of the major agricultural activities in Turkey and this importance is increasing every year. It is effectived the changing agricultural policies in Turkey and the increasing the importance of olive cultivation in the world.

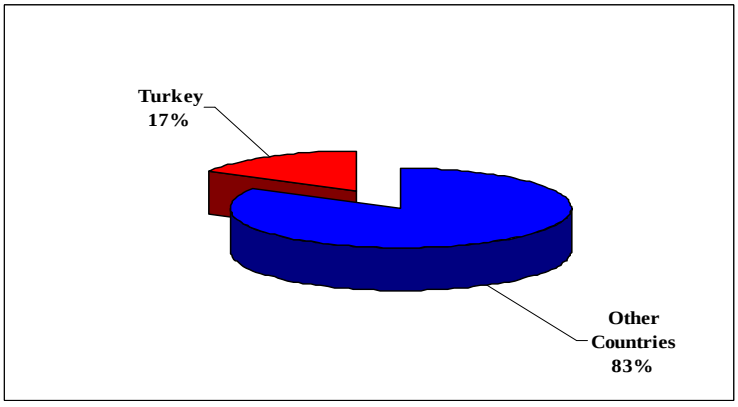

Figure 1: Olive Trees in the Turkey and World. **Source:** www.fao.org,2009

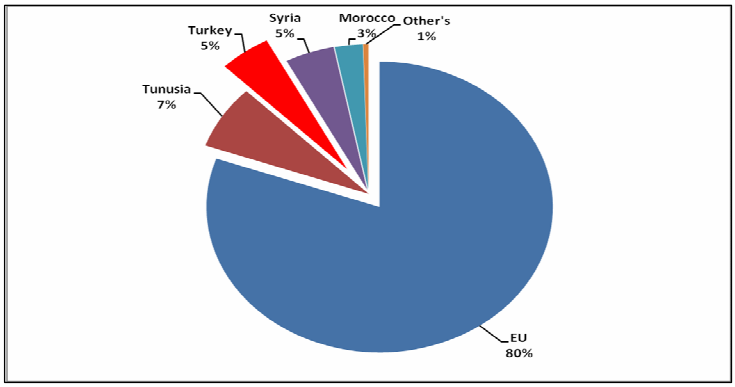

Figure 2: World Olive Oil Production. **Source:** www. www.internationaloliveoil.org,2009**.**

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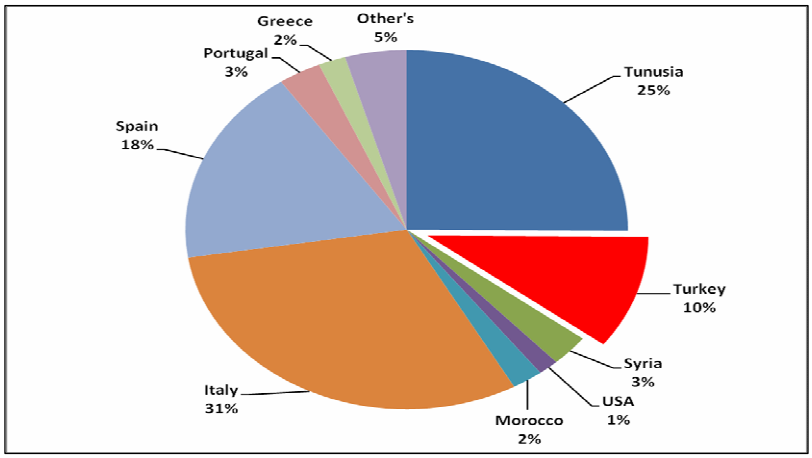

Figure 3: World Olive oil Exportations.
Source: www.internationaloliveoil.org,2009

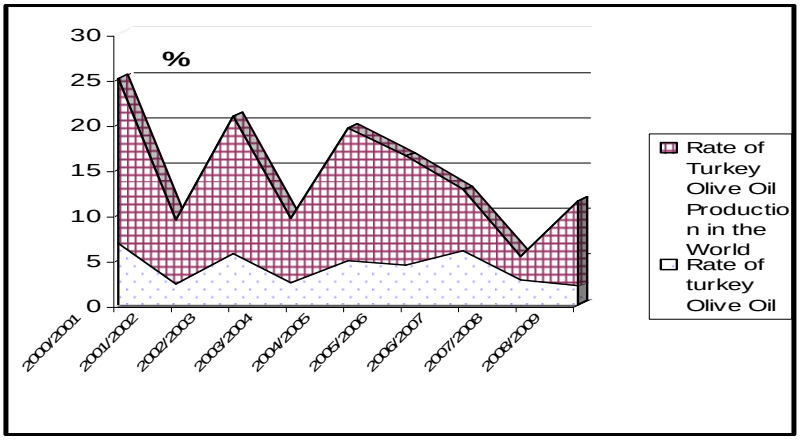

Figure 4: Rate of Turkey's Olive Oil Production and Export in the World
Source: is preperad by Aegean Exporter Union's datas in Turkey, 2008.

Olive cultivation were taken into culture in ancient Mesopotamia in BC 4000's which belongs to the Republic of Turkey in Anatolia today. Olives and olive oil, when Turks come to Anatolia 11. century and then established Ottoman Empire 12. century and of the Republic of Turkey has always been important product (Blázquez, 1997) (TGNA, 2008) (Unsal, 2003).

A large number of mono-species olive orchards established during the Ottoman Empire and even some benevolents donated them to foundations. And after then young Republic of Turkey was founded in 1923, leader and founder Atatürk was started a big olive cultivation campaign

2. Olive Oil Economıcs and Marketing Policies in Turkey

2.1. Economics

Olives is always a valuable sector in Turkey. This value has increased that applied through agricultural policy since 2000. Olive areas has increased 21% in the last decade in Turkey. Olive areas has reached of agricultural land within 3.4% (www.tuik.gov.tr) (Figure 5).

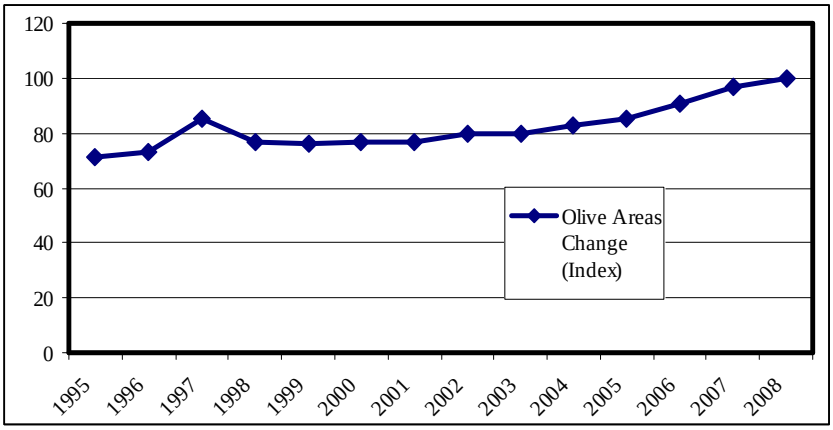

Figure 5: Turkey's Olive Growing Area in the Agricultural Area (Thousand ha)
Source: www.tuik.gov.tr

There are 774 thousand hectares olive areas, 151 million olive trees in Turkey. This tree has occurred 70% of bearing, 30% non bearing. The total olive production's 62% is olive for oil and rest into table olive (Figure 6 and 7). Turkey is produced approximately 112 thousand tons of olive oil. Today, Turkey's olive and olive oil'net trade volume is $ 250 million. This is 5.7% of world olive oil net trade volumes (www.tim.org.tr, 2009) (Net Trade Volume is calculated Exports + Imports (value or quantity)

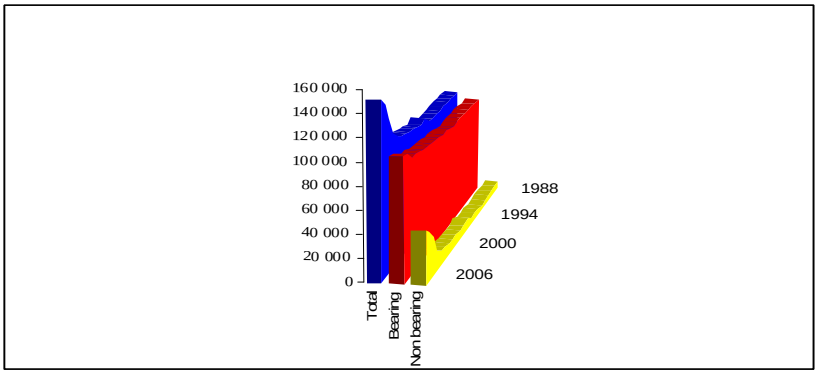

Figure 6: Olive Trees Number in Turkey. **Source:** www.tuik.org.tr

2.2.1. Policies and Marketing

Private sector is more effective than cooperatives in olive oil marketing in Turkey because olive oil production and exports of 80% belongs to private sector (Tunalioglu and Ozdogan, 2008).

Production:

Republic of Rural Affairs and Ministry of Agriculture is responsible for agricultural and olive cultivation policy in Turkey. But in practice, there are many responsible authorities; Ministry of Agriculture and Rural Affairs, Industry and Trade Ministry and Interior Ministry. That is experiencing confusion.

In Turkey, the price of olive oil is the most important agricultural policy instruments for production. Apart from input and orchard plant support are also important in recent years.

- 1929-1939 period: olive campaign and in 1939 "a special olive law" was removed and breeding efforts begin.
- 1939 - 1960 period: olives in the new planting and maintenance of provincial support.
- 1960-1966 period: It was taken place in fist Development Planning and exported olive oil in the first time.
- 1966 - 1972: period: olive oil was supported

• in 1973: olive oil was not supported

• 1974 - 1978 period: olive oil was supported.

• in 1979 year: olive oil was not supported

• 1980 - 1986 period: olive oil was supported

• 1987 - 1990 period: olive oil was not supported

• 1991 - 1993 period: olive oil was supported.

• 1994 - 1997 period: olive oil was not supported

• in 1998: Premium support was applied.

• In 1999: olive oil was not supported

• From 2000 until today: premium support and stability was achieved in changing agricultural policies (Tunalioglu, 2006 and TGNA, 2008) (Figure 1).

Applied olive oil production policies in Turkey is stable with two main reasons in recent years. Firstly, same negotiations are made to the EU, WTO, IMF, World Bank agreements. Second is affect by Grand National Assembly of Turkey's (TGNA) agricultural and marketing policies laws (in 2004 Food, in 2004 Organic Agriculture, in 2005 Agricultural Insurance, in 2006 Agriculture Law)
Turkey's agriculture policies, in January 1996 Customs Union Agreement with the EU has entered a new process. The production and marketing policies have changed in this process.

- 2001 May, with the IMF stand-by agreement to be signed,
- 2001 July, World Bank International Bank for Reconstruction and Development (IBRD) was initiated with the approval of the Agricultural Reform Implementation Project (ARIP)
- 2004 July, adoption of the text for the WTO framework,
- October 2005, begin to negotiations with the EU and the opening up of the agricultural chapter,
- December 2008, end of the Agricultural Reform Implementation Project (ARIP)

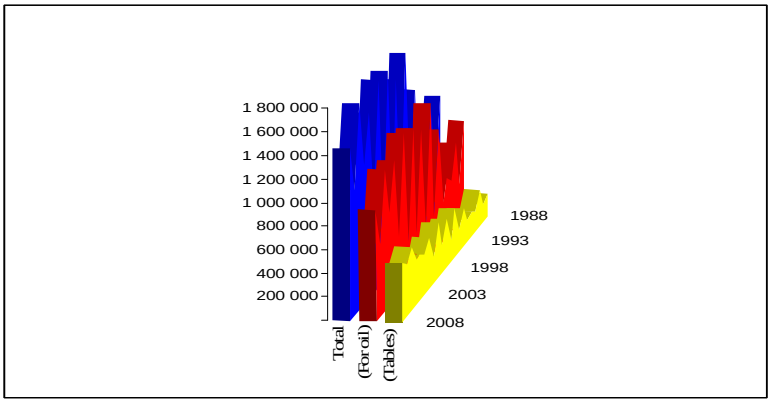

Figure 7: Olive Production in Turkey (Thousand tons). **Source:** www.tuik.org.tr

This project was carried out by the Ministry of Agriculture and Rural Affairs. Industry and Trade Ministry has also provided support. ARIP is a project which was affecting agricultural and olive policies in Turkey. This project has been registered with the producer, restructered of cooperatives and have been changed production support policies. Olives in this context, input, drip irrigation, orhard plant, R & D and machine renewal has benefited from support (Table 1).

Table 1: Applications of Support Prices in Olive Oil in Turkey.

1960									
0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
-	**-**	**-**	**-**	-	-	SPP	SPP	SPP	SPP
1970									
0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
SPP	SPP	SPP	PAP	SPP	SPP	SPP	SPP	SPP	PAP
1980									
0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
SPP	SPP	SPP	SPP	SPP	SPP	SPP	PAP	PAP	PAP
1990									
0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
PAP	SPP	SPP	SPP	PAP	PAP	PAP	PAP	PS	PAP
2000									
0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
PS	PS	PS	PS	PS	PS	PS	PS	PS	PS

Source: Tunalioglu, 2006, SPP= Support of the Purchase Price, PAP=Producer Association's Price, PS=Premium Support

2.2.2. Trade:

Foreign trade policies are carried out by Undersecretariat of the Prime Ministry for Foreign Trade. These policies of WTO and EU negotiations continued commitment to innovation will be open as long (Table 2).

Table 2: Subsidies of Olive and Olive Oil Production and Exports.

• **PRODUCTION's support**
• **Olive Oil Premium Support**
• Input (fertilizer, pesticid etc.) • Agricultural Research Development • Machinery Equipment • Drip Irrigation • Environmental Protection • Plant Insurance • Certified Nursery with Orchard Plant • Agricultural Loans
• **EXPORT's and IMPORT's support**
• Trade Fairs • TURKQUALITY
• Customs Tax

Source .is preperad to www.tarim.gov.tr and www.egebirlik.org.tr

Olive Oil Marketing policy in Turkey;

• Between 1972-1994: Bulk and barrels of olive oil with export is prohibited.

• From 1994 - till today: Bulk and barrels of olive oil exports is free.

• From 1997-till today: As part of export refunds for agricultural products are packaged olive oil subsidies.

• From 2006-till today: are given to both branded and packaged olive oil exports (Table 1).

To support exports in Turkey, within the framework of the WTO commitments and the quality of packaged classes are based. Turkey also introduced the world to olive oils "Turkquality" program under the "Olive to live" and "Olive and Olive oil from Turkey" studies have been initiated. On the other hand Turkey has still a big problem today. Turkey's olive oil can not be exported which is the largest market to the European Union, because of high customs duties branded-packaged olive oil. Therefore, Turkey's exports of oil barrels and bulk is higher than packaged (Figure 8).

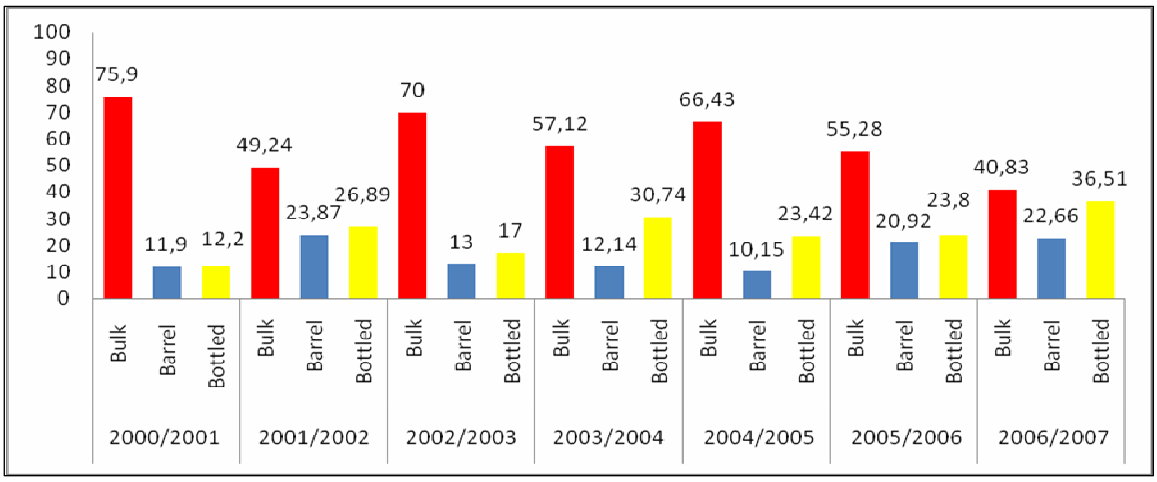

Figure 8: Olive Oil Exportations of Turkey. **Source:** Aegean Exporter Unions, 2008.

3. Results and Summarize

Olive oil economy and marketing have changed and developed since last ten years in Turkey. Because agricultural policies based on olive policy also has changed. This change, the National Olive Council to leave some inaccuracies and omissions, despite are positive.

Prices is preferred olive policies most the way in all methods. In Turkey, price supports increase to olive production and exports but unfortunately structural problems can not solve. Completely.
Olive genetic problems (alternans), small size olive holding, cultural applications, and lack of organization of producers enable to continuity and quality in production difficulties. These difficulties, also negatively affects the exports and exporters.
Turkey can be solved genetic, structural and marketing problems in olive sector but in the long term and permanent policies. These situations is directly related to directly policies of Turkey's changing and renewed agricultural policies

3.1. Current Situation in Turkish Olive Oil Sector

3.1.1. Increase

- Mono-olivepecies orchardsand olive areas,
- Olives production quantity,
- Olive oil production and quality,
- Packaged and branded olive oil exports,

3.1.2. Development

- Developments of olive oil technology process,
- Turkish Food Codex, FAO and EU legislation in compliance with the olive oil production,
- Geofigureical indications in olive oil,
- Organization of olive oil tasting panels,
- Supported wiht laws (legislation and regulations) (Tunalioglu, 2009)

3.2. Trends and Strategies in Turkish Olive Oil Sector

- The continuity of production support,
- Enable effectiveness of cooperatives,
- Training licensed trade excange in olive oil
- Increasing of boutique olive oil production,
- Increasing of the consumption Turkish people's,
- Membership of IOC again,
- Increasing packaged and branded olive oil exports.

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S13-C3

Compétitivité des exportations tunisiennes de l'huile d'olive face à la nouvelle concurrence sur le marché mondial : Analyse par l'approche *shift-share*

Aicha Mokrani[1], Mohamed Béchir Sai[2], **Boubaker Dhehibi[3]**

Résumé

L'exportation de l'huile d'olive occupe une place très importante dans l'économie de la Tunisie en maintenant l'équilibre de la balance commerciale agricole. En effet, elle représente 3,5% des exportations totales du pays en 2005. En effet, nous constatons qu'au cours de ces dernières années l'émergence de nouveaux pays producteurs/ exportateurs concurrents à la Tunisie et qui essayent de conquérir le marché de l'huile d'olive en se développant à une croissance et un rythme important. A cet égard, il est impératif d'étudier le positionnement de la Tunisie et de la situer parmi ces nouveaux pays émergents qui menacent sa position sur le marché international. L'objectif, donc de cet travail, s'insère dans le cadre de cette préoccupation et s'intéresse à l'évaluation de la situation actuelle du positionnement de la Tunisie sur le marché mondial de l'huile d'olive afin d'aider les orientations du pays à établir une stratégie plus efficace face à cette nouvelle tendance. Dans cette optique, il s'agit d'évaluer la position compétitive des exportations tunisiennes de l'huile d'olive par rapport à ses nouveaux concurrents moyennant l'approche d'analyse shift-share sur deux périodes 1997-2001 et 2002-2006. Les résultats empiriques montrent que la Tunisie a su maintenir sa première place sur le marché américain et canadien en comparaison avec les autres concurrents qui sont la Syrie, l'Argentine, l'Australie et le Chili, tout en augmentant sa part du marché. Pour le marché européen, la Tunisie reste toujours le premier fournisseur de l'UE mais avec une part du marché qui a diminuée au cours des années et qui est compensée par l'augmentation de la part de la Syrie.

Mots clés : Huile d'olive, compétitivité, analyse shift-share, marché mondial, Tunisie.

Competitiveness of Tunisian olive oil exports against the new competitors on the international market: A shift-share analysis approach

Abstract

The olive oil export sector occupies an important place in the Tunisian economy by maintaining agricultural trade balance. In 2005, it account for 3.5 % of total country exports. However, during these last years, we observe the emergence of new competitors for Tunisia which try to conquer the olive oil market by an important development growth rhythm. For this reason, it is imperative to study the position and competitiveness of Tunisian olive oil exports among these new emerging countries inn the international market.

The main objective of this research is to the evaluate the current position of Tunisian olive oil sector exports among theses new competitors in the international market in order to draw an appropriate strategies to this new trend. To this end, the method used is based on the shift share approach analysis for the periods 1997-2001 and 2002-2006.

Empirical results show that Tunisia continue increasing its market share and maintaining its leader position in the American and Canadian market on comparison with the new competitors such as Syria, Argentina, Australia and Chile. In the European Union market, Tunisia always remains the first supplier but with a decreasing market share during the last years which is compensated by the increasing Syrian market share.

Key-words: Olive oil, competitiveness, shift-share analysis, international market, Tunisia.

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S13-C5

La signalisation de la qualité est elle une solution pour la valorisation de l'huile d'olive tunisienne sur les marchés extérieurs ?

Oueslati Meriem, Rastoin Jean-Louis ; Daviron Benoit ; Khaldi Raoudha

Résumé

En Tunisie, la compétitivité de l'huile d'olive à l'exportation n'est plus à prouver. Cependant, ce produit n'est évalué que sur ses attributs matériels alors que les attributs symboliques sont accaparés par les pays importateurs. Cet article a pour objectif de mettre en évidence les principales contraintes à la valorisation de l'huile d'olive tunisienne en termes de signes de qualité (appellation d'origine ; indication géographique) à travers une enquête réalisée auprès des exportateurs. L'absence d'une action collective pour la promotion de l'huile d'olive, l'inexistence d'appellation d'origine reconnue, la faible notoriété de la qualité de ce produit, le manque d'unités de conditionnement sont autant d'obstacles évoqués par les exportateurs. Les politiques quantitatives adoptées devraient être accompagnées par des mesures spécifiques tout au long de la filière pour la valorisation du produit.

Mots clés: Signalisation- Qualité-Huile d'olive-Indications d'origine.

Is signaling quality a good argument for promoting Tunisian olive oil on foreign markets?

Abstract

Exported Tunisian olive oil has proven itself to be highly competitive. It is however promoted solely on the basis of its physical characteristics, and it is only importing countries that point out its symbolic or intangible advantages. This article seeks to put forth the main problems that need to be solved before Tunisian olive oil can effectively use signaling of quality [designation of origin and geographical indication (GI)] as a selling point. These constraints have been established on the basis of information gathered through a survey of exporters. Indeed, exporters agree that the absence of a joint effort to promote Tunisian olive oil, the lack of an acknowledged system for designation of origin, the low level of awareness of the quality of this product, and the scarcity of processing units are all obstacles to signaling the quality of Tunisian olive oil. Policies that focus on quantity will need to be complemented by specific measures based on quality at all levels of the industry if export of this key product is to reach its full potential.

Keywords: constraints to export, quality, olive oil, valorization

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S13-C6

Filière oleicole au Maroc : formes et développements alternatifs du monde rural

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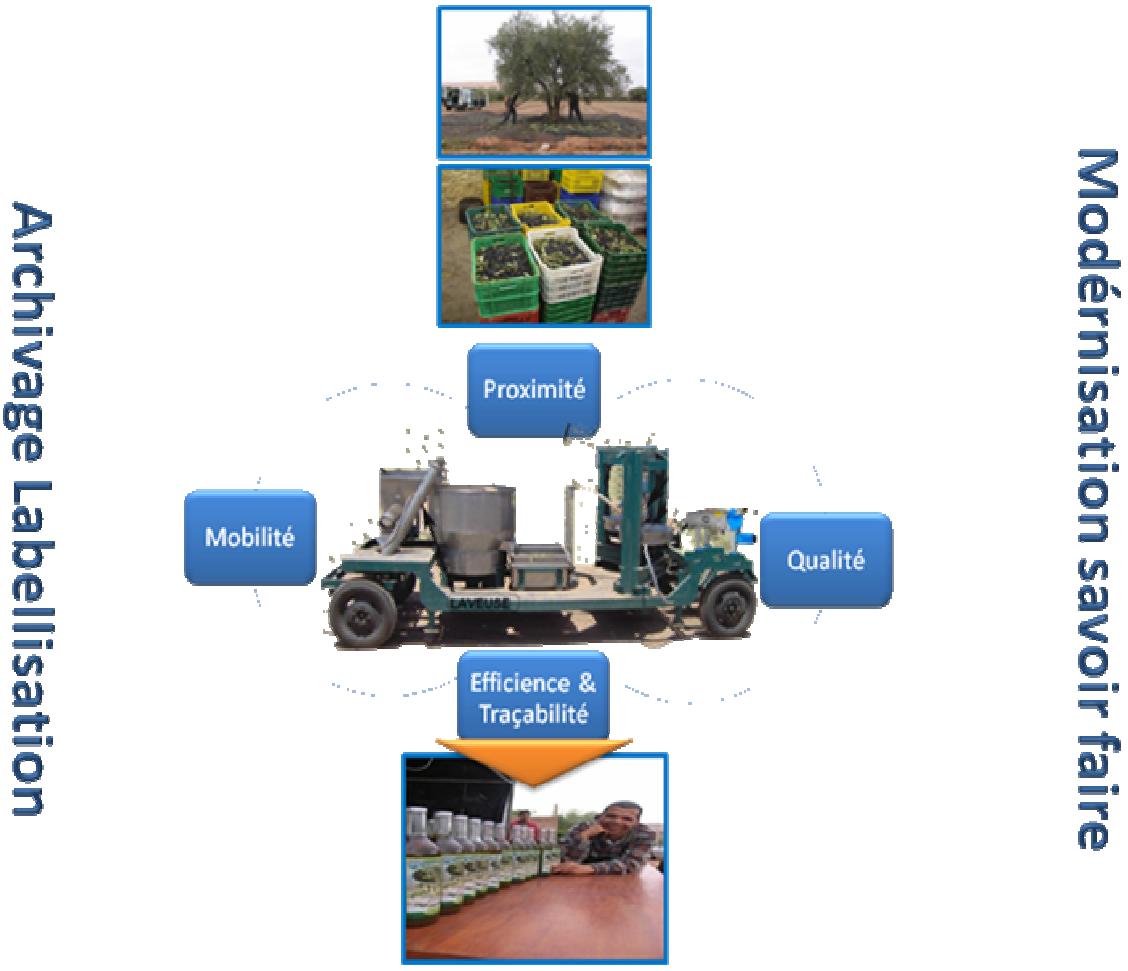
Résumé

L'Oléiculture Marocaine participe à l'autosuffisance du pays en huiles alimentaires à près de 18 % des besoins. La production marocaine est d'environ 600 000 tonnes d'olives dont 60% de la production est réalisée par des petits producteurs pratiquant l'extraction dans des unités artisanales (maasra).
Elles produisent de l'huile d'olive dans des conditions d'extraction fastidieuses avec un faible rendement qui ne dépassent pas 14 % contre 21 à 23 % du potentiel de la variété « Picholine marocaine », ce qui engendre une perte qualitative et quantitative pouvant atteindre 25 % de la production nationale, soit environ 10 000 t/an. Les raisons qui expliquent une telle situation ont trait aux multiples opérations dans la chaîne de production (récolte, transport, stockage, Hygiène, conduites des opérations, qualification etc.) au niveau des maasras.

L'objectif du projet est d'apporter de nouveaux outils de compétitivité et d'innovation capables de produire des huiles d'olives de bonnes qualité sur les lieux de production, d'annuler les coûts et les effets de transport, de stockage, d'Hygiène et de conduites des opérations par la mise en place d'une unité mobile adaptée aux réalités socio-économique du milieu rural marocain. Cette nouvelle technologie devrait permettre le soutien du pilier II du plan Maroc Vert, la réhabilitation de la filière oléicole dans les zones enclavées et le développement d'entreprises rurales innovantes qui vont s'approprier des technologies nationales modernes développées sur la base du savoir faire local.

Mots clés : Filière oléicole, Maroc, maasra, unité mobile, réhabilitation.



Abstract

Moroccan olive culturing participates in the self-sufficiency of countries'edible oils of about 18% of the needs. Moroccan production is about 600 000 tons of olives in which 60% of the production is realised by small producers practicing extraction in craft units (maasra).

They produce olive oil in tedious extraction conditions with a weak yield which does not exceed 14% versus 21 to 23 % of the variety potential "Moroccan Picholine", which generates qualitative and quantitative loss which can reach 25% of national production (crop, transport, storage, hygiene, operations passages, qualification etc.) on the level of maasras.

This project goal is to bring new tools of competitiveness and innovation capable of producing olive oils of good quality in the production areas, to cancel the costs and effects of transport, storage, hygiene and operations conduct by the implementation of a mobile unity adapted to the socio-economic realities of the Moroccan rural area. This new technology should enable the support of the pillar II of the Green Morocco plan, the rehabilitation of the olive chain in the landlockedareas et the development of the innovative rural companies which will appropriate modern developed national technologies on the base of the local know-how.

Key words: olive chain, Morocco, maasra, mobile unit, rehabilitation.

1. Introduction

Le Maroc cherche depuis longtemps à travers des plans stratégiques à promouvoir et à moderniser son agriculture pour subvenir aux besoins alimentaires des populations. Le patrimoine oléicole national s'étend sur une superficie totale de 600.000 Ha répartie sur 400.000 exploitations agricoles. La "Picholine Marocaine" représente plus de 90% des plantations. Le secteur permet de générer une valeur ajoutée de l'ordre de 1,5 Milliard de Dirhams et contribue à la création d'environ 15 Millions de journées de travail/an, soit l'équivalent de 70.000 emplois permanents. Elle permet de garantir l'approvisionnement de 300 unités industrielles ou semi industrielles de transformation des olives et de 15.591 unités traditionnelles de trituration (maâsras) détenant des capacités de près de 700.000 T et 170.000 T, respectivement (DPV 2008) (Fig. 1). En plus de son rôle nutritionnel (résorption de plus de 16 %, du déficit du pays en matière d'huiles alimentaires particulièrement en milieu rural), le secteur joue un rôle déterminant dans la consolidation de l'équilibre de la balance commerciale et permet de placer le Maroc au 3ème rang des pays exportateur des olives de table, après l'Espagne et la Grèce, avec une moyenne annuelle de près de 60.000 T. Le secteur génère en chaque fin de compagne environ 250.000 m^3 de margine et 200.000 tonnes de grignons d'olive.

L'objectif du présent travail et d'apporter de nouveaux outils de compétitivité et d'innovation capables de produire des huiles d'olives de bonnes qualité sur les lieux de production, d'annuler les coûts et les effets de récolte, de transport, de stockage, d'hygiène et de conduites des opérations par la mise en place d'une unité mobile adaptée aux réalités socio-économique du milieu rural marocain. Cette nouvelle technologie devrait permettre le soutien du pilier II du plan Maroc Vert, la réhabilitation de la filière oléicole dans les zones enclavées et le développement d'entreprises rurales innovantes qui vont s'approprier des technologies nationales modernes développées sur la base du savoir faire local.

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2. Etat des lieux de l'oléiculture en milieu rural au Maroc

- Grande biodiversité, climats, végétale et humaine
- Population agricole oléicole en croissance extension des oliveraies
- Activité oléicole liée aux aléas climatiques
- Persistance d'une petite agriculture familiale (peu compétitive).
- Existence d'exploitations et industries familiales sous dimensionnées et sous exploitées
- Micro exploitation sous forme de clôture
- Faible valorisation de produits oléicole
- Grande capacité de trituration des unités dans les grandes villes (Fig. 1)
- Le milieu rural à besoin de plus d'infra structures « plusieurs chaînes sont coupées : Routes, Electrification, Froid, Encadrement technique .etc.

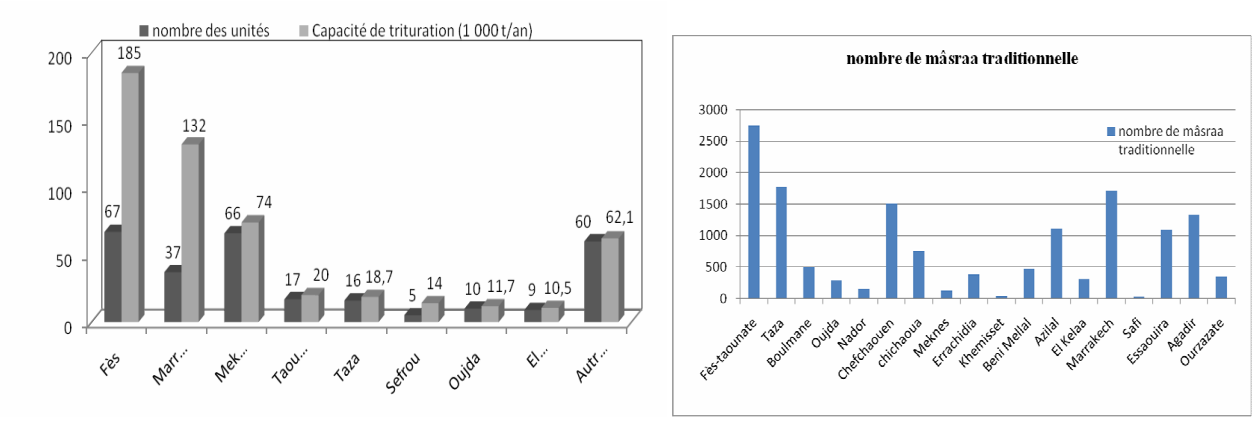

Figure 1: Répartition des unités de trituration industrielles et traditionnelles au niveau des principales zones oléicoles (DPV 2008).

L'analyse des prix des olives et des huiles d'olives, mises à part les fluctuations annuelles constatées durant les années 90 (Tableau 1), ont amorcé une tendance marquée à la hausse et particulièrement durant la période 1998-2006 où les prix ont pratiquement doublé (Rapport MCC 2009).

Tableau 1: Evolution des prix des olives et huiles d'olive pendant les compagnes 1998/99 et 2005/06.

Produits	1998/99	2005/06
Olives de trituration (DH/Kg)	2	4,75
Olives de conserverie (DH/Kg)	3	5,7
Huile d'olive (DH/L)	22,5	37,5

Source : Rapport MCC 2009.

3. Contraintes de la filière oléicole

Nombreuses sont les contraintes endogènes et exogènes qui pèsent sur l'oléiculture marocaine :

- La vulnérabilité de la production vis-à-vis des variétés retenues (90 % de la picholine marocaine), de la conduite culturale, de la rareté des ressources hydriques et de l'accompagnement professionnelle des oléiculteurs.
- La faible productivité au niveau des exploitations (1,5 tonnes à l'hectare) en raison de l'inadéquation du système de production, de l'exigüité des exploitations oléicole, de la variété employée dans la majorité des exploitations, et de formation aux exigences de bonnes pratiques de production et de transformation en matière de maîtrise des technologies modernes et leur mise au service du développement de la filière.
- La faiblesse de l'organisation professionnelle
- Les mauvaises conditions de récolte, de collecte et de transformation
- La faible maîtrise des paramètres régissant la maturité des produits
- L'inadaptation du matériel utilisé pour le ramassage et le transport des olives
- L'omniprésence du secteur traditionnel de trituration qui triture de 30-40% de la production nationale.

- La concentration de l'activité des unités de trituration sur la prestation de service
- La concentration des grandes unités dans les grandes villes, alors que la production est en montagnes.
- La vétusté des équipements et la non conformité aux règles de bonnes pratiques de production de fabrication au niveau de plusieurs unités industrielles
- La faible valorisation du savoir-faire traditionnel en matière de la conservation des olives.
- Faible productivité qualitative au niveau des unités de trituration traditionnelles (maasra)

4. Opportunités

Plusieurs opportunités réelles sont présentement à saisir notamment par l'application des formes alternatives adaptées aux réalités socio-économiques des petits oléiculteurs à l'amont et à l'aval de la filière. Ces formes doivent tenir compte des contraintes ci-dessus mentionnées et des programmes de développement du gouvernement en la matière notamment ; le Plan National Oléicole (PNO) et le plan Maroc Vert (MAMVA, 1998 et 2009).

En effet, le plan National élaboré en 1998 avait comme objectifs essentiels de doubler les superficies des plantations oléicoles pour les faire passer de 500.000 ha à 1.000.000 ha en 2010 et restructurer la filière sur le plan culturale et technologique. Malgré les efforts déployés, ce denier n'a pu augmenter la superficie que de 100.000 à 150.000 ha soit 20% à 30 % de l'objectif initial, alors qu'on matière de restructuration des maasras traditionnelles le PNO n'a restructuré que 2.5 % des unités traditionnelles. En montagne et oasis l'olivier continue d'être utilisé clôture. Le plan vert est venu pour palier ces contraintes avec deux piliers.

Le Plan Maroc Vert table sur la création d'un million d'entreprises agricoles et s'attend à un PIB agricole supplémentaire de 70 à 100 milliards DH. Tout en diagnostiquant le secteur, il oriente vers des pistes de relance après l'élaboration de plans régionaux de développement agricole (MAMVA, 1998).

Ces plans permettront d'asseoir les bases d'une utilisation optimale des ressources naturelles qui varient selon les régions et d'où découlent diverses possibilités de production. Ils sont fondés sur des paramètres dictés par le potentiel et les contraintes du milieu naturel, les acquis de l'expérience agricole régionale et l'environnement humain.

Le programme d'action pour l'optimisation de la mise en valeur agricole dans chaque région retenue à l'horizon 2020 est décliné sous formes de deux piliers à mettre en œuvre.
L'objectif du pilier I est de développer une agriculture performante, adaptée aux règles du marché, grâce à une nouvelle vague d'investissements privés, organisés autour de nouveaux modèles d'agrégation équitables.

Par contre, l'objectif du pilier II est de développer une approche orientée vers la lutte contre la pauvreté, en augmentant significativement le revenu agricole des exploitants les plus fragiles, notamment dans les zones périphériques (par exemple en bour défavorable). En termes de chiffres, le pilier I a pour but de générer un maximum d'investissements privés sur ces filières avec 10 milliards de dirhams par an pour les relancer, autour de nouveaux modèles d'agrégation, portés par des investisseurs à forte capacité managériale.

Ces modèles d'agrégation seront équitables, par le biais d'une double contractualisation, entre l'Etat et l'agrégateur d'une part, et entre l'agrégateur et l'exploitant agrégé d'autre part.
Le pilier II, vise également la mise en œuvre de projets sociaux autour de trois programmes. Ce pilier prévoit un volet financement innovant. Il s'agit ainsi de traiter les bailleurs des fonds sociaux comme des investisseurs à part entière, au moyen d'une «Offre sociale Maroc».

Par ailleurs, des opportunités réelles sont également à saisir notamment pour les créneaux suivants :

- Les produits de terroir labellisés, étant donné que l'oléiculture marocaine présente la caractéristique d'être pratiquée dans des contextes de production très diversifiés (montagne, plaines, oasis, bour, irrigué) (Fig. 2) et travers des signes distinctifs d'origines et de qualité (SDOQ loi 25.06) ;

- les produits biologiques, sachant qu'une part importante de l'oliveraie nationale s'apprête parfaitement à ce mode de production avec le faible recours des agriculteurs à l'usage des produits chimiques pour la conduite et l'entretien de leurs plantations ;
- les produits de haute gamme dans la mesure où la qualité de la production oléicole nationale est intrinsèquement supérieure pouvant facilement être valorisée à travers l'instauration d'un système de production sauvegardant cette qualité et la valorisant par l'accroissement de sa valeur ajoutée et la promotion de son image de marque auprès des consommateurs par le recours notamment aux techniques de marketing.

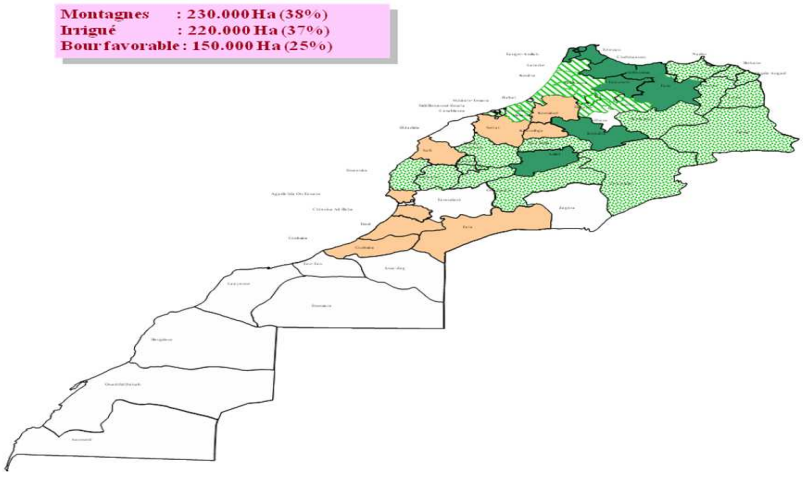

Figure 2: Répartition des zones oléicole à l'échelle nationale au Maroc (Ismaili-Alaoui, 2006) .

5. Mesures d'accompagnement de la filière

Les mesures d'accompagnement proposés pour les développements alternatifs sont basés sur la mise en place des économies de qualité qui sont supposées se fonder sur l'utilisation durable des ressources naturelles et humaines de la région, promouvoir une perspective à long terme et tendre vers une stabilité économique basée sur l'efficacité, la diversité et l'équité. Elles sont « douces » envers la nature et les gens, respectent les connaissances et les cultures traditionnelles et apportent une valeur ajoutée aux populations. Il s'agit de :

- Améliorer les technologies de récolte, de stockage et de transformation des matières premières
- Appuyer les plans et la stratégie de l'état en matière de développement : PNO et Plan Maroc Vert
- Appuyer le management de la qualité dans les IAA
- Chercher la maîtrise de la qualité (40 % des pertes) : la maîtrise de la qualité passe par la maîtrise des opérations production et de transformation
- Spécialiser les ressources humaines et la terre sur les choses qu'on sait faire.

Appliquer les systèmes de traçabilité : La traçabilité et la labellisation dans le monde rural passe par le transfert et l'adaptation des réglementations, des innovations technologiques et biotechnologiques auprès des gardiens de ce savoir. En effet, les itinéraires techniques des produits de terroirs sont bien adaptés aux réalités socio-économiques des milieux, tous les segments s'y retrouvent.
Les développements alternatifs proposés visent de redresser voir annuler les contraintes qui handicapent le développement de la filière et valoriser au mieux les ressources disponibles et les opportunités offertes. Elles intéressent l'ensemble des maillons, depuis la production, la commercialisation et la valorisation des sous produits de l'olivier, ce qui permettra d'une part l'accroissement de la productivité et ainsi de la valeur ajoutée sur les lieux de production et d'autre part une l'élimination des contraintes liées à la mauvaise gestion au sein des petites exploitations et unités de trituration (maasra), notamment, la qualification des agriculteurs (an alphabétisation professionnelle), le gaulage, le mode de transport des olives, le stockage sur les lieux de production et au niveau des unités de trituration, le mode de trituration, les conditions d'hygiènes et la labellisation etc.

6. Unité mobile d'extraction des huiles d'olive

Les unités artisanales de trituration au nombre 15591 unités, ne valorisent que partiellement la production des huiles d'olive. En effet, avec des rendements en huiles d'olive qui ne dépassent guère 14 % contre un rendement potentiel de 23 % pour la picholine marocaine en pleine maturité. La perte en huile dans les grignons d'olives est estimée de 8000 à 10.000 t/an. Cette perte représente entre 18 à 25 % de la production nationale en huile d'olive dans ces unités, sans tenir compte des pertes en huile dans les margines[1].

La conception des unités mobiles est basée sur le savoir faire locale est sur le principe de la mobilité et de la proximité. Le principe part de l'idée de la moissonneuse-batteuse. Concrètement, l'idée est que c'est la machine qui va vers le fruit et non l'inverse. Car, «la qualité dépend du temps passé entre la cueillette et la trituration. Il ne faut pas qu'elle dépasse les 24 heures. La Figure 3 résume le schéma de réalisation du prototype. Deux modèles ont été réalisés/ unité mobile sur roues et plate forme transportable en monobloc. Cette unité d'extraction d'huile d'olive devrait réduire la durée de stockage et la fréquence de broyage des olives 2 h au lieu de 2 mois parfois au niveau des unités artisanales, ce qui contribuera à l'amélioration de la qualité du produit, du rendement de l'environnement écologique des oliveraies.

Figure 3: La mobilité et la proximité par les unités mobiles d'extraction des huiles d'olive (Ismaili Alaoui, 2004).

7. Enrichissement en protéines des grignons d'olive

Les grignons générés pendant l'extraction du système à trois phases sont des matières organiques riches en fibre, cellulose en matières grasses et pauvres en protéines (Tableau 2).

Tableau 2: Composition chimique indicative des différents types de grignons en % de matières sèches (Ismaili-Alaoui et al. 2003).

Type	Matière sèche	Matières minérales	Matière azotée totale	Cellulose brute	Matières grasses
Grignon brut	75 - 80	3 - 5	5 - 10	35 - 50	8 - 15
Grignon gras p. d.*	80 - 95	6 - 7	9 - 12	20 - 30	15 - 30
Grignons épuisés	85 - 90	7 - 10	8 - 10	35 - 40	4 - 6
Grignon épuisé p.d.	85 - 90	6 - 8	9 - 14	15 - 35	4 - 6
Pulpe grasse	35 - 40	5 - 8	9 - 13	16 - 26	26 - 33

* p.d. = partiellement dénoyauté

Leur enrichissement par fermentation en milieu solide a permis d'augmenter leur taux protéiques de l'ordre de 14 % (Ismaili Alaoui et Cheheb, 2004). La Figure 4, résume le procédé de Fermentation en milieu solide sur plateaux à l'échelle pilote pour la préparation d'aliment pour bétail à partir des grignons d'olive.

[1] Transfert de technologie en agriculture (2001), Qualité des huiles d'olive au Maroc, Rabat, avril.

Figure 4: Enrichissement desgrignons d'olive par Fermentation en Milieu Solide (FMS). Préparation du substrat (A) ; Inoculation avec une suspension de spores de champignons filamenteux (B) ; Répartition du substrat inoculé sur des plateaux (C) et Transport des plateaux dans le ferementeur pilote (D).

8. Conclusion:

Ce Travail a permis d'apporter de nouveaux outils de compétitivité et d'innovation capables de produire des huiles d'olives de bonnes qualité sur les lieux de production, d'annuler les coûts et les effets de récolte, de transport, de stockage, d'hygiène et de conduites des opérations par la mise en place d'une unité mobile adaptée aux réalités socio-économique du milieu rural marocain. Cette nouvelle technologie devrait permettre le soutien du pilier II du plan Maroc Vert, la réhabilitation de la filière oléicole dans les zones enclavées et le développement d'entreprises rurales innovantes qui vont s'approprier des technologies nationales modernes développées sur la base du savoir faire local.

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S13-C7

Prospective de la production et de l'exportation de l'huile d'olive biologique et de l'huile d'olive conditionnée

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Résumé

Cette recherche, entamée en mai 2008, fournit une estimation de la production et de l'exportation tunisiennes de l'huile d'olive biologique et de l'huile d'olive conditionnée à l'horizon 2020 et procure une évaluation globale des facteurs internes (forces et faiblesses) et externes (opportunités et menaces) qui régissent les exportations tunisiennes.Elle utilise la méthode Delphi qui constitue un outil courant populaire de prospective à moyen et long terme et d'aide à la décision et la méthode SWOT qui constitue un cadre de diagnostic et de réflexion stratégique et un outil universel, simple et populaire d'aide à la décision. Les informations utilisées proviennent des résultats d'une enquête effectuée en face à face auprès d'un échantillon e 20 experts qui assurent des fonctions différentes au niveau de la filière huile d'olive en Tunisie.

Mots clefs: Huile d'olive, production, exportation, expert, DELPHI, SWOT

Abstract

This research started on May 2008, provides an estimate about the production and the Tunisian exportation of biologic olive oil conditioned by 2020, and it provides a global evaluation of the internal factors (strengths and weaknesses) and external ones (opportunities and threats) which govern Tunisian exports. It uses Delphi method which constitutes a popular tool of medium and long term perspective helping the decision and SWOT method which constitutes a frame of diagnostic and strategic reflexion and a universal, simple and popular tool helping the decision. The used information coming from the results of an investigation carried out with a sample of 20 experts who ensure different functions at the Tunisian olive oil chain.

Key words: olive oil, production, exportation, expert, DELPHI, SWOT

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S13-C8

L'importance de l'origine géographique dans le choix des produits agroalimentaires : le cas d'huile d'olive en Espagne

Erraach Yamna, Sayadi Samir

Résumé

La référence à l'origine du produit est devenue un facteur de différenciation et de valeur ajoutée dans le secteur agroalimentaire, où les nouvelles exigences vis à vis la sécurité et la qualité alimentaires provoquent, de plus en plus, des changements dans les habitudes d'achat et le comportement de consommation. Le secteur oléicole ne fait pas une exception à cette tendance. Ainsi, il parait intéressant estimer la structure des préférences des consommateurs en ce qui concerne l´huile d'olive, en mettant l'accent sur l'importance relative de l'origine géographique et / ou de sa certification. L´information utilisée dans cette recherche proviennent d'un sondage dirigé à 500 consommateurs d'huile d'olive en Andalousie (Sud de l'Espagne), en appliquant la technique de l'Analyse Conjointe (AC) dont on a considéré quatre attributs d´huile d'olive: Le type l'emballage, le prix d'huile d'olive, sa couleur et son origine géographique.

Mots clés: Huile d'olive, origine géographique, structure des préférences, Analyse Conjointe, Espagne.

The importance of origen in the choice of agro-food products: the case of Spanish olive oil

Abstract

The reference to the origin of the product has become actually a factor of differentiation and added value in the Agro-food industry, where the new requirements of consumer to safety and quality are increasingly causing a change in the habits of purchasing and consuming. The olive oil sector is not an exception to this tendency. In this context, it is of great interest to estimate the consumer preferences structure regarding olive oil, paying special attention to the relative importance of geographic origin and / or its certification. The information handled in this research were obtained from a survey performed to 500 olive oil consumer's in Andalusia (South of Spain), applying the Conjoint Analysis method (CA) and considering four attributes of olive oil: The type of package, the price of olive oil, its color and the geographic origin.

Key words: Olive oil, geographic origin, preference structure, Conjoint Analysis, Spain.

1. Introduction

L'évolution des productions agroalimentaires au cours des dernières décennies, surtout dans les pays développés, ainsi que la stagnation qui marque la demande alimentaire ont engendré une certaine saturation des marchés (Sánchez, 2006 ; Ulloa et Gil, 2007). Par conséquent, les consommateurs et les entrepreneurs se trouvent immergés dans un processus de croissance et de développement global de l'économie, qui leurs impose à son tour un rythme d'adaptation à tous les niveaux (Dawar et Frost, 1999 ; Espejel et al., 2007). Parallèlement à tous ce qui précèdent, les consommateurs sont à chaque fois plus exigeants et expriment leurs préférences à la consommation et l'achat des produits innovants, de qualité et surtout avec des garanties sanitaires. Ceci rend les producteurs et les agents des différentes filières agroalimentaires obligés à adapter leurs manières d'organisation, stimuler la demande existante, de la créer dans des nouveaux marchés potentiels et se différencier de la compétence et acquérir des avantages compétitifs dans ce contexte de mondialisation.

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Dans le cas espagnol, une des stratégies de différentiation la plus soutenue, par le secteur de la production et les administrations locales, régionales, nationales et voir même européennes, est l'emploi des Appellations d'Origine Protégée (AOP) ou les Indications Géographiques Protégées (IGP). Il s'agit d'un ensemble des marques génériques attribuées à des produits spécifiques avec une identité propre soutenue par la qualité et l'origine de la matière primaire et tout de même par la spécification de la méthode de production employée (ou le savoir-faire) (Haucap et al., 1997 ; Ulloa et Gil, 2007).

Les Appellations d'Origine Protégée, comme stratégies d'organisation de la qualité différentielle liée au territoire, ont pour objectif la création de produits alimentaires, comme le cas d'huile d'olive, avec une forte composante patrimoniale et de typicité par rapport à des attributs de qualité différentielle (Bonnet et Simioni, 2001 ; Van der Lans et al., 2001 ; Steiner, 2004 ; Sanz Cañada, 2009).

Le secteur d'huile d'olive espagnol jouit d'une place importante aussi bien à l'échelle nationale qu'internationale, étant donné que l'Espagne se classe en premier rang de point de vue production et exportation d'huile d'olive sur le marché international. Ainsi qu'à partir d'une production annuelle moyenne de 800.000 tonnes, une moyenne de 300.000 tonnes est destinée à l'exportation à plus de 100 pays durant la dernière décennie (ICEX, 2009). En dehors de son rôle économique, le secteur d'huile d'olive joue un rôle vital dans l'articulation du milieu rural (création des postes de travail et maintien de la communauté locale dans son lieu d'origine) ainsi que dans le maintien du patrimoine culturel, régional et environnemental du pays. On dénombre actuellement en Espagne environ 31 AOP d'huile d'olive (MARM, 2009) parmi lesquelles environ la moitié (13 AOP) est accaparée par l'Andalousie. Avec 1,5 million d'hectares d'olivier l'Andalousie est considéré le majeur producteur d'olives et d'huile d'olive du pays ainsi que dans le monde. Environ la moitié du territoire olivier andalou, est protégé avec des AOP, lui procurant la possibilité de commercialiser une grande partie des huiles d'olive extra vierge.

L'objectif de ce travail est d'estimer l'importance relative de la certification liée à l'origine géographique et au territoire, plus concrètement les Appellations d'Origine Protégée, par rapport à d'autres attributs comme l'emballage, la couleur et le prix, dans la formation des préférences des consommateurs andalous vis à vis l'huile d'olive.

2. Méthodologie

Dans cette étude nous avons fait recours à la méthodologie de sondages auprès des consommateurs ou enquêtes. Pour ce fait, on a réalisé durant les mois de mai à juillet de 2009 des entretiens avec un échantillon représentatif des habitants de l'Andalousie choisis d'une manière aléatoire et stratifiés selon le genre, les groupes d'âge et le lieu de résidence pour un niveau d'erreur de 3,10%. L'échantillon enquêté regroupe 439 consommateurs d'huile d'olive. Le questionnaire se compose de deux grandes parties. La première est le test de préférence ou la preuve d'Analyse Conjointe (AC), où les personnes interrogées évaluent les profils d'huile d'olive par rapport à leurs préférences. La seconde présente des données sur les caractéristiques sociales et démographiques et les modes de vie des personnes interrogées (genre, âge, niveau d'études, revenus, etc.).

Pour traiter les données obtenues à travers le questionnaire et estimer l'importance relative de «l'origine territoriale » par rapport à d'autres attributs d'huile d'olive, nous avons fais recours à la méthode de l'Analyse Conjointe (AC). Cette méthode est devenue un outil de grande importance pour l'évaluation des préférences exprimées par un consommateur aux différents attributs qui composent un bien (Ruiz de Maya et Munuera, 1993). Elle est très utilisée dans le champ de la psychologie commerciale et le marketing (Luce et Tukey, 1964 ; Green et Rao, 1971 ; Cunhal-Sendim et al., 1999 ; Van der Lans et al., 2001 ; Brugarolas et al., 2003 ; Krystallis et Ness, 2005 ; Rodríguez et Sayadi, 2008 ; Mesías et al., 2009) et plus récemment dans l'économie environnementale (Sánchez et Pérez, 2000 ; Sayadi et al., 2005 y 2009).

La principale hypothèse de cette méthodologie stipule que la demande d'un produit répond à la perception et à l'évaluation de ses attributs, plus qu'à sa conception intégrale (Múgica, 1989), étant donné que, d'après Lancaster (1971), l'utilité reçue par un individu, le consommateur dans notre cas, dépend de l'utilité fournie par les différents attributs.

Dans le processus de prise de décision, l'individu évalue l'utilité de chaque combinaison, et son choix manifeste sa préférence relative aux différentes combinaisons des attributs. On accepte que l'utilité totale, résultat du choix du produit, soit déterminée par les différentes utilités partielles (part-worths) de chaque niveau d'attribut (Steekamp, 1987). On assume ici, la règle de la décomposition additive que d'après Hair et al., (1992 y 1999) ce modèle explique, dans presque tous les cas, un pourcentage très élevé (entre le 80% et le 90%) de la variance de la préférence des individus.

Pour appliquer la méthode AC, il est fondamental de bien choisir les attributs les plus explicatifs du comportement d'achat des produits ainsi que leurs niveaux respectifs. Dans notre cas, ce choix a été fait sur la base de la consultation de la bibliographie existante, des entretiens avec des experts, d'un focus-group et d'un sondage préliminaire avec des mères au foyer des consommateurs, etc. A travers les informations préalables et en considérant les objectifs du cette étude, les attributs et leurs niveaux respectifs sont : «Origine » (certifiée avec AOP ; mentionnée sans AOP et non mentionnée), le « Prix » (3€ ; 4,5€ et 6€), la « Couleur de l'huile » (Jaune-verdâtre et jaune-doré) et finalement «Emballage » (Plastique ; verre normal ; verre de design). Avec ces quatre attributs et leurs niveaux on a obtenu 54 profils possibles ou stimulus (combinaison d'attributs - niveaux). Etant donné le grand nombre de produits à évaluer par les consommateurs on a utilisé un modèle orthogonal qui nous a permis de réduire les combinaisons à seulement 9 produits finaux (Tableau 1).

Tableau 1: Conception fractionnée incomplète orthogonale.

Huiles	Couleur	Prix (€/l)	Type d'emballage	Origine
Huile 1	Jaune-verdâtre	3	Plastique	Certifiée avec AOP
Huile 2	Jaune-doré	3	Plastique	Mentionnée sans AOP
Huile 3	Jaune-doré	4,5	Verre de design	Mentionnée sans AOP
Huile 4	Jaune-doré	4,5	Verre normal	Certifiée avec AOO ?
Huile 5	Jaune-verdâtre	4,5	Plastique	Non mentionnée
Huile 6	Jaune-doré	6	Verre de design	Non mentionnée
Huile 7	Jaune-doré	6	Verre de design	Certifiée avec AOP
Huile 8	Jaune-doré	3	Verre normal	Non mentionnée
Huile 9	Jaune-verdâtre	6	Verre normal	Mentionnée sans AOP

Source: Elaboration personnelle.

Concernant la quantification des préférences relatives aux huiles d'olive, on a utilisé la technique d'évaluation en échelle "Rating", où les consommateurs donnent une ponctuation aux huiles d'olive de 0 (ils n'aiment pas du tout cette huile) à 10 (ils aiment beaucoup cette huile). La présentation du stimulus aux personnes interrogées a été réalisée à partir de l'élaboration de neuf cartes à base de la conception orthogonale qui se montre dans le Tableau 1. Le choix d'une conception orthogonale par rapport à la présentation de toutes les combinaisons possibles d'huile limite l'obtention d'informations aux principales effets des attributs, en ignorant les interactions entre eux mais au même temps elle présente l'avantage d'offrir seulement neuf huiles à chaque personne interrogée, en estimant que cet avantage est supérieur aux inconvénients mentionnés (Kirk, 1982 ; Braña et al., 1995 ; Sayadi et al., 2005).

3. Résultats

D'après, les résultats de l'estimation du modèle de l'Analyse Conjointe (Tableau 2), on peut observer l'importance relative accordée aux différents attributs par le groupe de personnes interrogées ainsi que les utilités partielles (Part-Worths) de leurs niveaux correspondants.

Tableau 2: Résultats agrégés de l'importance des attributs et les utilités partielles de leurs niveaux respectifs.

Attributs d'huile	Importance Relative (%)	Niveaux	Utilités partielles (Part-Worth)
Prix	36,66	6€	-1,167
		4,5€	-0,050
		3€	**1,217**
Couleur	16,57	Jaune-doré	-0,157
		Jaune-verdâtre	**0,157**
Emballage	18,67	Verre de design	-0,050
		Verre normal	-0,257
		Plastique	**0,307**
Origine	28,10	Certifiée avec AOP	**0,971**
		Mentionnée sans certificat AOP	-0,188
		Non mentionnée	-0,784
Constante : 4,516 R de Pearson : 0,923*** Tau de Kendall : 0,995**			

***: Niveau de signification ($p \leq 0,001$)
Source: Elaboration personnelle. Sondage sur les préférences des consommateurs par rapport à l'huile d'olive, (2009).

Pour vérifier l'adéquation de l'ajustement, on a utilisé des indicateurs qui mesurent le coefficient de corrélation existant entre les valeurs manifestées par les consommateurs et les valeurs pré-estimées par le modèle (le taux de Kendall et le coefficient de corrélation de Pearson). Dans ce cas, les deux coefficients sont très significatifs ($p \leq 0,001$).

A partir des résultats obtenus sur l'importance relative des attributs, on peut affirmer que le « Prix » est le critère le plus important dans la formation des préférences des consommateurs avec une importance relative du 36,66%, en second rang on trouve l'attribut « Origine » (28,10%), suivi par l'attribut « Emballage » (18,67%) et en dernier rang figure l'attribut « Couleur » (avec une importance relative du 16,57%).

Pour l'attribut « Prix », le niveau le plus bas (3€) est le plus déterminant dans la formation des préférences d'huile. La valeur de son utilité moyenne relative est positive (1,217). Les deux niveaux de prix moyen (4,5€) et élevé (6€) ont une importance secondaire dans la formation des préférences vis à vis l'huile et leurs utilités moyennes relatives sont négatives (respectivement : -0,050 y -1,167). Plus le prix est élevé pour les consommateurs, moindres sont leurs utilités et par conséquent les préférences d'une alternative déterminé. En somme, l'utilité marginale d'une alternative diminue lorsque les niveaux de prix augmentent.

En ce qui concerne « l'Origine » territoriale de la production d'huile d'olive, la « Certification avec une AOP » est le niveau majeur dans la formation des préférences des consommateurs, suivi par « l'Origine mentionnée » et finalement par « l'Origine non mentionnée ». Les utilités négatives de ces derniers niveaux sont très inférieures à l'utilité de la « Certification avec AOP ». Alors, lorsque l'origine territoriale d'huile d'olive est à la fois connue, et garantie avec une certification, l'utilité et la satisfaction sont majeures pour les consommateurs d'huile d'olive. L'importance de la certification relative au territoire et son utilité pour les consommateurs est aussi confirmée par l'application de l'Analyse Conjointe en huiles (Krystallis et Ness, 2005 ; Van der Lans et al., 2001) et dans le cas d'autres produits agroalimentaires comme la pomme avec AOP (Fotopoulos et Krystallis, 2003), le fromage avec AOP (Mojardino et Ventura, 2001), le vin avec AOP (Bernabéu et al., 2001) et la viande bovine avec IGP (Ulloa et Gil, 2007).

En ce qui concerne « l'Emballage», l'huile emballée en « Plastique » est plus appréciée que celles en « Verre de design » et en « Verre normal » étant donné qu'elle a une utilité partielle positive (0,307). Les huiles emballées en bouteilles de verre normal ou de design ont été les moins valorisées, ainsi que leurs utilités étaient négatives de l'ordre de -0,257 y -0,050 respectivement. Ce fait paraît très logique vue que les consommateurs associent mentalement ces deux types d'emballage avec des produits de haute gamme et de prix élevés.

Finalement, on a pu authentifier que l'attribut qui a la moindre influence dans la formation des préférences des consommateurs est la « Couleur d'huile », et que le niveau « Jaune-verdâtre » est plus valorisé que le niveau « Jaune-doré » avec une utilité partielle positive (0,157).

A travers les résultats ci-dessus, on peut souligner que « l'huile d'olive la plus préférée » pour les consommateurs est celle de couleur jaune-verdâtre, vendu à un prix de 3€/l, avec un emballage en plastique et avec certification AOP. Par contre, « l'huile d'olive la moins préférée » est celle de couleur jaune-doré, sans information sur son origine territoriale de production, commercialisée avec un emballage de verre de design et à un prix élevé (6€/l).

4. Conclusions

Cette étude tente à analyser l'importance relative de l'origine géographique dans la formation des préférences des consommateurs d'huile d'olive en Andalousie à travers une Analyse Conjointe entre neuf alternatives qui combinent quatre attributs considérés les plus relevants lors de l'achat du produit: Prix, Origine, Emballage et Couleur d'huile.

Une des premières conclusions dérivées de l'analyse de la structure des préférences des consommateurs d'huile en Andalousie, est la grande importance accordée au « Prix » et à « l'Origine » territoriale d'huile, étant les deux attributs ayant la majeure importance relative dans la formation des préférences des consommateurs. On doit souligner que les attributs « Couleur » et « Emballage » d'huile ne semblent pas avoir trop d'importance dans la fonction d'utilité des consommateurs interrogés.

En ce qui concerne « l'Origine » territoriale de la production d'huile, la « Certification AOP » est le niveau le plus relevant dans la formation des préférences des consommateurs, suivi de « l'Origine mentionnée » et finalement par « l'Origine non mentionnée ». Partant de ce constat on peut déduire que plus l'origine territoriale d'huile d'olive est connue, et encore plus elle est garantie par une certification, majeure sont son utilité et sa satisfaction pour les consommateurs.

Par rapport aux conclusions mentionnées ci-dessus on peut conclure que la différentiation des huiles d'olive espagnoles à travers l'emploi des alternatives de certification liées avec le territoire, plus concrètement les Appellations d'Origine Protégée, peuvent être un exemple d'outil efficace pour acquérir des avantages compétitifs sur les marchés, en profitant des caractéristiques différentielles du milieu naturel et la méthode d'élaboration propre à chaque territoire.

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S14-C1

Les politiques de l'huile d'olive, la Pauvreté Rurale et le Développement Economique en Méditerranée

Elabed Ghada, Travis Lybbert

Résumé

Plusieurs pays de la méditerranée favorisent la production d'huile d'olive comme facteur de développement. Ces politiques sont la conséquence de l'essor de la demande grâce à l'adoption du régime méditerranéen à travers le monde et grâce à l'émergence de marché de produits à haute valeur. En outre, ces politiques s'inscrivent dans le cadre du renforcement des politiques de développement rural ainsi que de l'émergence des préoccupations environnementales. Nous décrivons les dernières tendances de ces marchés et les nouvelles orientations des politiques gouvernementales de l'huile d'olive comme outil de développement. Nous documentons et comparons ces politiques en Tunisie, au Maroc, en Syrie et au Liban. Nous évaluons par ailleurs leurs impacts potentiels sur la pauvreté ainsi que les décalages entre ces politiques et la réalité du marché international.

Mots clés: développement économique, pauvreté rurale, MENA, marché de l'huile d'olive, politiques de l'huile d'olive.

Olive oil policies, Rural Poverty & Economics Development in the Mediterranean

Abstract

Many Mediterranean countries are actively promoting olive oil as a promising vector of rural development and poverty reduction .These policies are a response to a booming olive oil market characterized by the spread of the Mediterranean diet and the emergence of high value markets, reinvigorated agricultural and rural development policies, and concerns about water availability, soil preservation and natural resources. We describe the recent market and policy trends responsible for casting olive oil as a rural development tool. We then document and compare olive oil policies in Tunisia, Morocco, Lebanon and Syria. We assess their likely poverty impacts, including limitations such as credit constraints among smallholders and inconsistencies between these policies and the realities of the current international olive oil market.

Key words: economic development, rural poverty, MENA, olive oil policies & markets

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S14-C2

L'intégration de la demande sociale dans l'évaluation et la conception des pratiques d'innovation dans le secteur agro-alimentaire : le cas d'huile d'olive en Espagne

Samir Sayadi, Yamna Erraach , Carlos Perra-Lopez & Ascensión Hinojosa-Rodríguez

Résumé

La compétitivité et la durabilité du système oléicole sont fortement liées à la satisfaction de la demande sociale des consommateurs ainsi que leurs préférences vis-à-vis la qualité, la sécurité et les issues environnementaux. L'adoption des 'bonnes pratiques agroalimentaires' innovatrices, doit engendrer une maximisation du bien être social et une plus grande diversification, compétitivité et rentabilité financière pour les agents producteurs. Dans cette recherche, la relation entrez la production oléicole, la transformation, les pratiques de gestion et d'organisation et la demande sociale du consommateur est explorée à travers l'application de la méthode 'Quality Function Deployment' (QFD) dans le cas de l'Espagne. Cette méthodologie permet d'identifier les 'bonnes pratiques agro-alimentaires' innovatrices, qui optimisent mieux la demande sociale du consommateur et les bénéfices des agents producteurs. Les résultats seront de grande utilité pour la conception des poitiques publiques et des stratégies économiques permettant le durabilité et la compétitivité du système oléicole Espagnol.

Mots clés : Les pratiques agro-alimentaires durables, compétitivité, politiques publiques et économiques, système oléicole, QFD.

Integrating social demands in the evaluation and design of innovative practices in the agro-food system: The case of Spanish olive oil sector

Abstract

The competitiveness and sustainability of the olive agro-food system are strongly related to the satisfaction of social and consumers' demands and preferences for quality, security and environmental issues. The adoption of innovative 'good agro-food practices' would entail the maximization of social welfare and a greater differentiation, competitiveness and financial profit for productive agents. In this research, the relationships among olive farming, processing, management and organisation practices and the social and consumers' demands are explored by using the Quality Function Deployment (QFD) method in the case of Spain. It allows identifying innovative 'good agro-food practices' that optimally satisfy social demands, and consumers' and productive agents' benefits. These results are aimed to be useful for the design of public policies and business strategies towards the sustainability and competitiveness of the Spanish olive system.

Key words: Agro-food practices for sustainability, competitiveness, public and business policies, olive system, QFD.

1. Introduction

La croissance de la concurrence dans le secteur agro-alimentaire, accentuée par la mondialisation et la globalisation économique, oblige les entreprises à promouvoir et à mettre en œuvre des politiques et des stratégies innovantes de mise en valeur de leurs productions, en les différentiant selon des signes distinctifs de qualité, durabilité, territorialité, modes de productions alternatifs, etc. L'innovation paraît être une excellente opportunité de différentiation, diversification et de positionnement des produits dans un marché de plus en plus dynamique et exigent. Aussi, la croissante préoccupation et l'intérêt des consommateurs envers les produits qu'ils consomment, la sécurité et la salubrité des aliments et le respect de l'environnement dans les processus de production, sont à l'origine de

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l'imposition des nouvelles demandes pour l'industrie alimentaire (Van der Valk et Wynstra, 2005). Un des facteurs clés de réussite dans le marché actuel, consiste à comprendre le comportement du consommateur, d'identifier ses besoins et exigences, et les intégrer dans la planification stratégique de toutes les étapes du processus de production, transformation et commercialisation des produits et/ou services.

Cela présente un grand défi pour un nombre considérable d'industries agro-alimentaires, en général, et pour l'industrie oléicole, en particulier. Plus concrètement, en Andalousie, le Système Oléicole (SO) est de grande importance économique, socioculturelle et territoriale. Le choix d'huile d'olive comme objet de cette étude revient au fait qu'il est considéré par les consommateurs comme un produit peu différentié et ainsi les résultats dégagés peuvent être une opportunité pour le développement d'un produit plus orienté au consommateur et pour des futures segmentations du marché.

L'objectif de cette étude est de développer et de mettre en œuvre un cadre méthodologique qui intègre les exigences du consommateur vis-à-vis les attributs de qualité d'huile (organoleptiques, socioculturelles, environnementales, etc.) et les pratiques agricoles potentielles pour les satisfaire. En définitive, il s'agit de développer une approximation complète du concept de la qualité d'huile d'olive, qui inclue les demandes du consommateur et les techniques susceptibles d'être appliquées dans la production d'olives.

2. Méthodologie

Le processus d'intégration de la « Voix du consommateur » avec les possibilités technologiques du secteur ou le « Déploiement Fonctionnel de la Qualité » a été mis en œuvre selon quatre étapes chronologiques que se détaillent ci-dessous.

2.1. Identification, definition et quantification des exigencies de qualité des consommateurs d'huile d'olive (QUOI – WHATS)

La première étape du travail consiste à identifier les caractéristiques perçues para le consommateur (*Voix du consommateur*) d'huile d'olive de qualité. A cet effet, on a fais recours à des techniques qualitatives basées sur des entretiens informelles et de discussions en groupe pour la collecte des informations relatives aux aspects, besoins ou exigences des personnes interrogées par rapport à l'huile d'olive, en obtenant une large liste des demandes des clients (QUOI). Postérieurement, on a effectué une *purification* de l'information, en évitant les répétitions et les manifestations ambiguës qui ne peuvent pas être mesurées et par conséquent classées comme besoins. Finalement, on a élaboré une liste définitive de 8 besoins ou demandes (Tableau 1).

A partir des résultats extraits dans l'étape qualitative, on a développé une étude quantitative approfondie basée sur l'emploi d'une enquête à 439 consommateurs pour quantifier et pondérer les demandes et les exigences des consommateurs (W_{di}) et qui seront utilisés postérieurement dans la matrice du QFD (Tableau 2). La pondération des besoins a été effectuée selon une échelle allant de 1 (peu important) à 5 (très important).

2.2. Identification et définition des pratiques dans le secteur de la production (COMMENT – HOWs)

Au-delà de la révision documentaire (Junta de Andalucía, 2006 ; Parra-López, 2006 ; Jiménez et Carpio, 2008 ; Alba et al., 2009 ; Humanes et Humanes, 2009 ; Uceda, 2009 ; Vega et al., 2009, entre autres), on a réalisé plusieurs discussions en groupe pendant le mois de septembre 2009 avec des experts du secteur (agriculteurs, chercheurs, techniciens et responsables des huileries, laboratoires de qualité, etc.) pour identifier et définir les pratiques potentielles ou les « Attributs de Conception » potentiellement utilisables par les agriculteurs pour satisfaire les nécessités sociales préalablement établies, étant certaines pratiques très innovantes et d'autres plus étendues. Ce processus n'a pas été seulement qualitatif mais également quantitatif pour pouvoir sélectionner uniquement les pratiques qui peuvent techniquement avoir des relations potentielles avec les demandes identifiées ultérieurement. Finalement on a choisi 47 pratiques agronomiques qui ont été classées en sous-niveaux (Tableau 3).

2.3. Déploiement des demandes du consommateur (QFD): Corrélation entre QUOI - COMMENT

Le principe de base de la méthodologie QFD consiste dans la construction de la matrice de la qualité ou «la maison de qualité » (Rudolph, 1995 ; Bech et al., 1997 ; Viaene et Januszewka, 1999 ; Benner et al., 2003) qui est essentiellement, une matrice qui met en rapport la voix du client (QUOI), avec les exigences techniques qui la satisfont (COMMENT). Dans ce cas, pour déterminer la contribution des pratiques (exigences techniques) dans la satisfaction de la demande du consommateur envers la qualité d'huile d'olive, on a construit la matrice de relations entre les « QUOI » et les « COMMENT » au niveau des pratiques individuelles et au niveau de pratiques agrégées. La *matrice stratégique ou matrice de production* ($W_{pj,di}$) est obtenue en pondérant les relations au niveau désagrégé (par exemple : rapport entre « saveur fruitée » et « variété picual » du Tableau I) avec le rapport au niveau agrégé (par exemple : dans ce cas rapport entre « saveur » et « variété »). Pour ce fait, on a interrogé 10 experts dans l'oléiculture (oléiculteurs, techniciens des huileries, chercheurs, distributeurs, etc.) pour décrire et quantifier les relations, en obtenant une matrice pour chacun d'entre eux. L'échelle de quantification employée a été de 0 (il n'existe pas une relation) à 9 (relation très forte) (voir Ramanathan et Ganesh, 1994 ; Parra-López et al., 2008). Ainsi on obtient une pondération pour chaque expert ou agent décideur ($W_{pj,di\ (e)}$). La contribution relative moyenne correspondante de chaque pratique (j) à chaque demande (i) est calculée comme la moyenne arithmétique des pondérations de tous les experts :

$$W_{pj,di(groupexp)} = \sum_{e=1}^{E} W_{pj,di\ (e)} / E$$

Étant E le nombre d'expert.

2.4. Pondération de la contribution des pratiques pour satisfaire l'ensemble des demandes

La contribution totale d'une pratique (W_{pj}) est déterminée par la somme des contributions relatives moyennes de cette pratique pour chaque demande (i) ($W_{pj,di(groupexp)}$) multipliées par l'importance relative de chaque demande selon les consommateurs (W_{di}):

$$W_{pj} = \sum_{i=1}^{n} W_{pj,di(groupexp)} * W_{di}$$

Dans notre cas: n = 8 demandes ; j = 47 pratiques

3. Résultats

Le consommateur évalue une série de caractéristiques de qualité dans l'huile d'olive qui peuvent être identifiées comme *qualité totale* perçue. On remarque que cette qualité inclue, non seulement des caractéristiques organoleptiques d'huile (saveur, couleur, acidité, etc.), mais aussi d'autre du genre socioculturelles (maintenir la population locale, créer des postes du travail, etc.), et y compris territoriales et environnementales (compatibilité avec l'environnement, etc.) (Tableau 1). Parmi ces caractéristiques se distinguent : la saveur fruitée, le faible degré d'acidité d'huile, la couleur jaune-verdâtre, le prix raisonnable et la production selon les normes de l'agriculture écologique, comme étant les demandes les plus prioritaires pour le consommateur d'huile d'olive (Tableau 3).

En ce qui concerne les pratiques agronomiques considérées dans cette étude, plus du 60% s'avèrent significatives pour mieux satisfaire les exigences et les désirs d'une huile d'olive de qualité. Ces alternatives constituent les pratiques critiques qu'il faut prendre en considération lors de la production selon les indiquent la demande du consommateur.

Selon les résultats obtenus, on peut souligner que les pratiques agronomiques les plus significatives et déterminantes de la qualité d'huile d'olive selon la demande sociale sont: La séparation des olives du sol et du vol ; les critères pour la récolte (selon le taux de maturation) ; le mode de récolte de sol (non récolte du sol) ; le mode de récolte de vol (gauler) ; le traitement des infections et des maladies ; le mode de transport depuis l'exploitation aux huileries (en caisses) ; les substances utilisées pour fertiliser (engrais organiques qui incluent les restes de la taille, grignon d'olive, etc.) ; le contrôle des infections-Mouche (lutte biologique avec pulvérisation terrestre) ; la variété (Picual) ; le contrôle des infections-Mites (lutte biologique *Bacillus thuringiensis* en floraison) ; la gestion du sol (sol couvert) ; la fertilisation (non fertilisation) ; le moment de l'irrigation (selon les recommandations des techniciens).

Dans le Tableau 1, on détaille la contribution relative de chacune des pratiques agronomiques considérées. Par exemple, « la séparation de l'olive du sol et du vol » est la pratique qui satisfait plus « le bas degré d'acidité » et l'ensemble des exigences du consommateur vers la qualité exigée d'huile d'olive.

4. Conclusions

Le travail présente une approche méthodologique d'intégration des demandes des consommateurs et des citoyens vis-à-vis la qualité d'huile d'olive et les externalités de son processus de production avec les pratiques potentielles les plus adéquates pour satisfaire ces demandes.

La qualité d'huile d'olive la plus demandée par les consommateurs incorpore des aspects liés aux caractéristiques organoleptiques (l'acidité, la saveur, la couleur, etc.) ainsi que socioculturelles (la création des postes du travail, le maintien de la population, etc.) et environnementales (le respect environnemental, etc.).

La séparation des olives du sol et du vol ; les critères pour la récolte ; le mode de récolte-sol ; le mode de récolte-vol ; le traitement des infections et des maladies ; le mode de transport depuis l'exploitation aux huileries ; les substances utilisées pour fertiliser ; le contrôle des infections**-**Mouche ; la variété ; le contrôle des infections**-**Mites ; la gestion du sol ; la fertilisation et le moment d'irrigation, sont les pratiques agronomiques optimales selon la perspective sociale (optimum technico**-**social).

Toute politique agricole dans le secteur oléicole qui vise être efficace et prendre en compte les préférences de la société devrait être orientée à favoriser la mise en œuvre de ces pratiques au niveau des exploitations oléicoles. Ceci permettrait d'améliorer la légitimité et l'appui social aux aides de la PAC pour le secteur agricole en général et le secteur oléicole en particulier.

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Tableau 1: Matrice de Déploiement de la conception des pratiques agricoles et des demandes du consommateur d'huile d'olive

Pratiques agronomiques / Demande du consommateur	Importance relative (W_{dj})	Variétés		Gestion des sols				Irrigation		Systèmes d'irrigation		Moment irrigation		Analyse de la qualité de l'eau	
		Picual	Hojiblanca	Sol nu avec labour	Sol nu avec labour (contrôle des mauvaises herbes avec herbicides)	Sol nu avec labour réduit	Sol couvert	Oui	Non	Irrigation au goutte-à-goutte	Autres systèmes (aspersion, a mante, etc.)	Calendrier fixe	Selon les recommandations des techniciens	Oui	Non
Saveur fruitée	4,3	54,7	58,0	0,0	0,0	0,0	0,0	28,6	11,1	0,0	0,0	0,0	0,0	0,0	0,0
Couleur jaune-verdâtre	3,3	48,0	25,3	0,0	0,0	0,0	0,0	1,3	1,1	0,0	0,0	0,0	0,0	0,0	0,0
Bas degré d'acidité	4,1	4,9	5,8	0,0	0,0	0,0	0,0	3,8	2,3	0,0	0,0	3,9	6,0	0,0	0,0
Prix	3,8	0,0	0,0	0,0	0,0	0,0	0,0	5,0	6,8	0,0	0,0	0,0	0,0	3,7	1,3
Système de Production Ecologique	2,6	0,0	0,0	34,1	17,5	17,5	49,8	0,0	0,0	0,0	0,0	23,0	30,8	25,9	6,2
Production respectueuse de l'environnement	2,4	0,0	0,0	13,8	19,1	32,9	62,7	28,8	35,2	66,9	34,0	22,9	61,0	30,5	12,2
Création d'emploi en milieu rural	1,8	0,0	0,0	0,0	0,0	0,0	0,0	13,1	8,2	0,0	0,0	0,0	0,0	0,0	0,0
Maintient de la population rurale	1,8	0,0	0,0	0,0	0,0	0,0	0,0	9,4	6,7	0,0	0,0	0,0	0,0	0,0	0,0
Contribution totale (W_{pj})		413,6	356,7	121,9	91,5	124,6	280	271,1	197,7	160,7	81,6	130,5	251,1	154,6	50,4

Pratiques agronomiques / Demande du consommateur	Importance relative (W_{di})	Fertilisation		Méthode de fertilisation			Substances pour la fertilisation		Analyses préalables à la fertilisation	
		Oui	Non	Application directe sur le sol	Application foliaire	Autre méthodes	Engrais organiques (restes élagage, l'alpeorujo, etc.)	Engrais NPK	Analyse foliaire ou du sol	Analyse foliaire ou du sol
Saveur fruitée	4,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Couleur jaune-verdâtre	3,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Bas degré d'acidité	4,1	1,3	3,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Prix	3,8	3,5	4,5	0,0	0,0	0,0	0,0	5,9	2,3	2,3
Système de Production Ecologique	2,6	29,0	39,0	25,3	28,7	16,9	71,0	11,0	40,7	9,7
Production respectueuse de l'environnement	2,4	22,4	46,7	24,0	49,8	36,0	69,0	17,0	55,8	21,8
Création d'emploi en milieu rural	1,8	13,6	5,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Maintient de la population rurale	1,8	5,6	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Contribution totale (W_{pj})		181,9	257,2	123,4	194,1	130,2	350,2	91,9	248,5	86,3

Tableau 2: Matrice de Déploiement de la conception des pratiques agricoles et des demandes du consommateur d'huile d'olive (suite)

Pratiques agronomiques / Demande du consommateur	Importance relative (W_{di})	Traitement des infections et des maladies		Contrôle des infections et maladies - Mouches			Contrôle des infections et des maladies - Mites		Moment du traitement		Localisation des traitements phytosanitaires	
		Oui	Non	Trempage massif	Lutte biologique avec pulvérisation terrestre	Lutte biologique avec pulvérisation terrestre	Contrôle biologique (Bacillus thuringiensis en floraison)	Traitement chimique	Calendrier fixe	Quand il dépasse un seuil déterminé de population	Sur toute la parcelle	Sur le foyer d'infection
Saveur fruitée	4,3	20,3	5,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Couleur jaune-verdâtre	3,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Bas degré d'acidité	4,1	37,5	3,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Prix	3,8	3,8	4,4	0,0	0,0	0,0	0,0	0,0	1,9	1,4	1,7	1,8
Système de Production Ecologique	2,6	43,2	13,6	63,8	74,4	11,3	67,0	11,3	11,1	39,7	16,4	28,1
Production respectueuse de l'environnement	2,4	29,0	36,0	41,0	62,0	26,7	64,7	17,8	16,3	46,4	23,3	61,0
Création d'emploi en milieu rural	1,8	11,8	2,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Maintient de la population rurale	1,8	7,2	1,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Contribution totale (W_{pj})		471,4	184,8	264,2	342,2	93,3	329,5	72,2	75,1	220,0	104,9	226,6

Tableau 3: Matrice de Déploiement de la conception des pratiques agricoles et des demandes du consommateur d'huile d'olive (suite)

Pratiques agronomiques / Demande du consommateur	Importance relative (W_{di})	Critères de récolte		Mode récolte - Sol			Mode de récolte - Vol			Séparation des olives du sol et du vol		Mode de transport de l'exploitation au moulin d'huile		
		Selon degré de maturité	Calendrier fixe	Récolte à main	Récolte mécanique	Non récolte du sol	Gaulage	Vibreurs (de branches ou de troncs)	Le peigne manuel	Séparation	Non séparation	Sacs	Caisses	Remorque du tracteur ou camion
Saveur fruitée	4,3	48,6	33,4	13,4	7,6	33,4	12,3	4,4	14,0	65,8	11,9	15,1	26,8	24,3
Couleur jaune-verdâtre	3,3	53,5	36,0	7,2	7,2	27,1	3,3	3,3	3,3	45,3	7,0	9,9	6,7	7,3
Bas degré d'acidité	4,1	55,7	16,5	39,1	18,1	50,5	34,9	39,7	51,3	75,4	8,5	14,1	52,3	34,4
Prix	3,8	6,7	12,8	15,3	14,3	19,6	12,2	15,4	12,2	8,3	17,5	2,8	4,1	4,1
Système de Production Ecologique	2,6	28,1	27,3	18,9	22,8	39,3	26,0	16,5	21,8	53,1	18,8	22,5	29,6	18,0
Production respectueuse de l'environnement	2,4	2,9	1,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Création d'emploi en milieu rural	1,8	0,0	0,0	37,2	18,9	12,2	60,1	37,4	54,2	0,0	0,0	0,0	0,0	0,0
Maintient de la population rurale	1,8	0,0	0,0	29,3	13,5	10,7	51,7	31,7	44,4	0,0	0,0	0,0	0,0	0,0
Contribution totale (W_{pj})		719,6	452,0	468,1	302,6	658,1	521,4	418,6	562,1	911,5	224,4	224,2	444,4	331,6

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S14-C3

Le secteur de l'huile d'olive biologique en Tunisie et les différentes structures de production: une analyse comparative

Saida Elfkih, **Boubaker Karray**

Résumé

En Tunisie l'agriculture biologique remonte aux années 90 avec une domination majeure de l'activité oléicole représentant actuellement environ 85% de la superficie totale des cultures biologiques. Ce secteur est régi par une gamme assez diversifiée de structures de production où les plus importantes sont: Sociétés d'agriculteur, agro-combinats, et agriculteurs privés. Ces structures se distinguent par leurs différentes logiques de fonctionnement, d'organisation et de décision, dégageant ainsi des différences au niveau de leurs performances économiques, techniques et sociales. Ce travail consiste alors en une analyse comparative des fonctionnements et des performances de ces structures, synthétisant leurs points forts et leurs points faibles. Les données qui ont servi à cette étude ont été collectées à travers des enquêtes et des entretiens auprès des producteurs de la zone de Sfax; la plus importante en termes de superficie, de production de l'huile d'olive biologique du pays et en termes de nombre d'opérateurs.

Mots Clés: Oléiculture biologiques, structures de production, performances économiques techniques et sociales.

The Tunisian organic olive oil sector and the different production structures: A comparative analysis

Abstract

The Tunisian organic agriculture dates back to the end of the 90 th years where the main activity is the olive oil sector with 85% of the total area of organic crops. This sector is controlled by diversified production structures, where the more important are: Farmers associations, public farms and private farmers. These structures differed by its functional, organisational, and decision making logics. This situation leads to different levels of economics, techniques and social performances. This work aims to make a comparative analysis of the function and the performances of several production structures, focusing the synthesis on the strong and the weak issues. To reach the study objectives, we used data provided by questionnaires and interviews from the producers of Sfax region, the most important in Tunisia by its production area, its interesting number of producers and its diversified production structures.

Keywords: Organic olive oil sector; production structures; economics, social and techniques performances.

1. Introduction

L'agriculture biologique qui a surgi au début comme résultante d'une raison de dimension environnementale et spirituelle a fini pour se convertir en une alternative de valorisation des produits agricoles et une opportunité aux producteurs pour apporter une valeur ajoutée a leurs outputs. Réellement, il ne s'agit pas d'une déviation des objectifs fondamentaux de la philosophie de Rudolf Steiner[i] sur l'agriculture écologique, mais il s'agit d'une nouvelle conception de la même vision mais dans un contexte plus organisé et régi par des règlements et une législation claire. Ce n'est pas l'agriculture romantique comme pense beaucoup mais c'est l'agriculture responsable et compromis avec l'environnement et la durabilité des systèmes agricoles. L'importance environnementale de ce système est une réalité qui a été contrastée par la simple logique d'être l'alternative la plus viable pour faire face aux modes intensifs voire agressifs de production. Reste à démontrer sa viabilité économique, surtout dans un monde régi par une économie ouverte, de plus en plus globalisée et de plus en plus compétitive.

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Plusieurs études ont traité la thématique de l'agriculture biologique et sa viabilité économique. La littérature disponible est relativement abondante où les études les plus importantes pour le présent travail sont: Le Travail de Laajimi et Ben Nasr (2009) qui compare la durabilité des exploitations oléicoles biologiques et conventionnelles en Tunisie traitant le cas particulier de la région de Sfax. Le travail de Ben Salah et Hammami (2006) qui a porté sur l'étude technico-économique des exploitations oléicoles biologiques à Mahdia (Tunisie). Le travail de Alonso et Guzman (2006), où une analyse comparative des exploitations oléicoles en mode biologique et conventionnel était entamé, ceci en utilisant 17 indicateurs de durabilité (socio-économiques et environnementaux). Le travail de Parra López et Calatrava Requena (2005), où une analyse comparative de l'oléiculture biologique et conventionnelle de la région d'Andalousie (Espagne) était établie. Le travail de Parra López et Calatrava Requena (2004) où était traitée la diffusion des techniques de production biologique au niveau des exploitations oléicoles en Andalousie comme un processus de diffusion technologique. Del campo Tejedor et al. (2001) ont traité à l'agriculture biologique comme alternative de développement rural. Chinchilla fernandez (1999) a effectué une analyse du rôle des différents acteurs impliqués dans la diffusion de l'oléiculture biologique dans la zone d'Andalousie (Espagne). Lampkin et Padel (1994) ont analysé les caractéristiques, les avantages et les barrières que l'adoption de l'agriculture biologique peut représenter, ceci dés une perspective internationale. Diebel et al. (1993) ont analysé les barrières économiques à l'adoption de l'agriculture biologique.

Au niveau de la présente étude, une analyse comparative des performances techniques économiques et sociales des différentes structures de production du secteur oléicole biologique tunisien sera effectuée. Ceci afin de dégager leurs atouts et leurs contraintes, en mettant l'accent sur les liens entre elles et les différents acteurs de la filière. La présente communication est organisée de la manière suivante: i) introduction, ii) le secteur de l'oléiculture biologique en Tunisie: législation, importance et organisation, iii) Objectifs et méthodologie du travail, iv) Résultats, v) Conclusions et réflexions finales.

2. L'oléiculture biologique en Tunisie: législation, importance économique et organisation

L'agriculture biologique est une activité qui remonte à la fin des années 90 en Tunisie. Cette activité a évolué considérablement cette dernière décennie en matière législative, économique et organisationnelle.

A niveau législatif, il faut signaler que la Tunisie a sa propre législation et son propre cahier des charges en matière de production biologique (loi Nº 99-30 de 5 Avril 1999). Après huit ans de négociation, cette législation a été reconnue par un régime d'équivalence pour l'agriculture biologique par la Réglementation de la Commission Européenne (CE) Nº 537/2009, ceci en conformité à la Réglementation (CE) Nº 834/2007. Le régime d'équivalence reconnaît les normes de production et de contrôle établîtes par la législation Tunisienne en matière d'agriculture biologique. Ce régime couvre les produits végétaux non transformés et ceux transformés. Ceci facilite les opérations de commerce international en évitant les procédures d'autorisation d'importation exigées auparavant aux importateurs de la Communauté qui désirent importés des produits biologiques de la Tunisie.
Quant à son importance économique, l'oléiculture représente la composante principale de l'agriculture biologique en Tunisie. Sa superficie a augmenté de 12.666 ha en 2002 à 115.000 ha en 2008 représentant 85% de la superficie des cultures biologiques. La production de l'huile d'olive biologique a connu une évolution très importante ces dernières années, passant de 400 tonnes en 2002 à 10.000 tonnes en 2007. Les exportations ont évolué dans le même sens, de 400 tonnes en 2002 à 6061 tonnes en 2007. L'oléiculture biologique est concentrée principalement dans la région de Sfax avec 55,5% de la superficie totale, occupant le premier rang en matière de production de l'huile d'olive biologique. (CTAB, 2008)

Plusieurs acteurs contribuent à l'organisation du secteur. Ces acteurs sont principalement, les opérateurs de la filière, les organisations des Agriculteurs Biologiques, les organismes certificateurs et l'administration. Les opérateurs de la filière huile d'olive biologique ce sont principalement les agriculteurs, les transformateurs et les exportateurs. Les organismes de certification sont au nombre de 5: Ecocert SA en Tunisie, L'Institut Méditerranéen de Certification IMC,

[1] Au début des années 20 l'autrichien Rudolf Steiner (1860-1925) a travers un cours de formation dirigé aux agriculteurs, il a mis les bases de l'agriculture biodynamique qui se base sur une philosophie antimatérialiste (l'anthroposophie). Un groupe de sympathisants de Steiner a mis en pratique les techniques culturales de l'agriculture biodynamique, commercialisant en 1928 les premiers aliments sous une marque de qualité avec le nom de la décesse grecque Déméter.

BCS et Lacon et ICEA. La Fédération Nationale des Agriculteurs Biologiques représente l'unique organisation des agriculteurs biologiques Tunisiens. Les administrations engagées sont principalement: la Direction Générale de la Production Agricole (DGPA), le Centre Technique de l'Agriculture Biologique (CTAB) et Les Commissariats Régionales de Développement Agricole (CRDA).

Le secteur oléicole biologique dans la région de Sfax est caractérisé par la diversité de ses structures de production. En effet, il y a la présence de plusieurs structures de production qui diffèrent essentiellement par leurs statuts, leurs modes de gestions et leurs logiques de fonctionnement. Ces structures de production sont principalement: les agro-combinats issus de l'Office des Terres Domaniales, les sociétés ou groupements d'agriculteurs et les agriculteurs privés.

3. Objectifs et Méthodologie

L'objectif principal du travail est d'effectuer une analyse comparative des performances socio-économiques et techniques des différentes structures de production qui régies le secteur d'oléiculture biologique, partant de l'hypothèse que le système biologique est environnementalement durable. Cette étude était entamée dans la région de Sfax, région plus importante en termes de superficie, de production et de diversité des structures de production. Des enquêtes auprès des producteurs et des interviews auprès du reste des acteurs étaient établi. Les enquêtes comportent plusieurs rubriques: des données démographiques et de structure, Application des normes du cahier des charges, données économiques sur les productions et les charges de production, donnés techniques sur la maîtrise des opérations culturales et les opérations d'aménagement et des données sur la relation des producteurs avec les services de développement. Les interviews portent principalement sur le rôle de chaque intervenant de la filière et son interaction avec les opérateurs de la filière.

On a travaillé avec les agriculteurs (qui sont parfois des transformateurs et (ou) des exportateurs). L'objectif était d'effectuer une étude exhaustive auprès des différents opérateurs certifiés biologiques ayant les caractéristiques déjà mentionnées. Les opérateurs enquêtés sont les: quatre agro-combinats de la région de Sfax (Chaâl, Bouzouita, Essalama et Bir Ali), qui sont des fermes de l'état gérées par l'Office des Terres Domaniales. La catégorie société d'agriculteurs vient représentée par la société Ezzayatine, qui est une société d'oléiculteurs techniciens qui ont émergé suite à la restructuration des fermes de l'état. Par agriculteurs privés on désigne les agriculteurs qui travaillent ou individuellement ou en unités familiales. Des interviews étaient effectuées auprès du reste des acteurs, organisme certificateur qui est dans notre cas Ecocert SA en Tunisie, la Fédération Nationale des Producteurs Biologiques, La DGPA et le CRDA et le CTAB. A part les données quantitatives qui ont été collectées, des données qualitatives et des appréciations ont étaient obtenues à travers de tout le processus de collecte des données et de contact avec les opérateurs ainsi que le reste des acteurs.

Des indicateurs était étudiés, qui sont de nature technique et socio-économique. Les indicateurs techniques sont les suivants: date de début du projet biologique, Superficie olivier-biologique, importance de l'oléiculture biologique par rapport aux restes des spéculations de l'exploitation, densité de l'olivier biologique, pourcentage des oliviers biologiques sénescentes (>70ans), pourcentage des oliviers biologiques jeunes (<10ans), degrés d'implication dans les processus de rénovation [Utilisation de compostage, Opérations d'aménagement (Arrachage, Replantation, augmentation des densités par nouvelles plantations)] et finalement le rendement moyen des trois dernières campagnes. Les indicateurs économiques sont les coûts moyens de production par Kg d'olive à huile et par Kg d'huile d'olive, prix de vente moyen par Kg d'olive à huile et par Kg d'huile d'olive et résultat par Kg d'olive à huile et par Kg d'huile d'olive. Les aspects sociaux qui vont être étudiés portent essentiellement sur des constatations qualitatives dégagées le long du processus de travail du terrain.

4. Résultats

4.1. Indicateurs techniques

L'adoption du mode de production oléiculture biologique avait ses origines dans la région de Sfax en 1997-98 par des exploitants de la société des agriculteurs et par l'exploitant privé numéro 16. Cette initiative avait un succès ce qui se traduisait par l'augmentation du nombre des opérateurs dans ce secteur et par l'adhésion dernièrement (2004-05) des agro-combinats issus de l'office des Terres

Domaniales. Le taux de spécialisation en oléiculture biologique par les agriculteurs est élevé, en effet la superficie oléicole biologique occupe presque la totalité de la SAU chez la plupart des agriculteurs.

Les agro-combinats malgré qu'ils occupent de grandes superficies oléicoles biologiques (85% de la superficie totale oléicole biologique de la région de Sfax), sont moins spécialisés; ce sont des fermes de l'état de grandes superficies qui présentent des spéculations diversifiées. Les oléiculteurs biologiques de la région de Sfax sont tous des grands agriculteurs (des superficies en générale supérieures à 100ha). Les densités sont généralement plus élevées au niveau des exploitations des agriculteurs de la société des agriculteurs, ces derniers ont entamé des programmes d'augmentation des densités par de nouvelles plantations en intercalaire. Les agro-combinats sont les uniques producteurs qui ont entamé un programme d'arrachage d'oliviers sénescents et de replantation, ceci entre dans le cadre d'un programme national. Le compost est plus utilisé par les agriculteurs de la société d'agriculteurs. Plusieurs exploitants de la société d'agriculteurs ont introduit le mode irrigué (soit irrigation d'appoint pour les anciennes plantations, ou par de nouvelles plantation en gouttes à gouttes). Les rendements en tonnes d'olivier par hectares sont variables d'une exploitation à une autre, en moyenne, la société des agriculteurs présente la valeur la plus élevée (0,7 Tn/ha) suivi par les les agro-combinats (0,5 Tn/ha) et les privés (0,47 Tn/ha). On doit signaler qu'au niveau de la société des agriculteurs étaient enregistrés les rendements les plus élevés et les plus faibles (les faibles rendements sont dus essentiellement d'une part au phénomène de sénescence des oliviers et d'autre part à l'existence de jeunes plantations qui ne sont pas en pleine production)

4.2. Indicateurs économiques

Observant les indicateurs économiques (tableau 2), on constate que tous les exploitants dégagent des résultats moyens positifs de leurs activités oléicoles. Ces résultats sont variables dépendant des exploitations enregistrant des différences entre les différentes structures de production.

Certains agriculteurs ont optés par la vente des olives à huile, les prix de vente sont assez élevés variant entre 0.910 DT à 1.17 DT. Les bénéfices enregistrés sont variables dépendant des coûts de production. Le reste des agriculteurs et qui représentent la majorité ont opté par la vente de l'huile d'olive.

Les prix de ventes moyens de l'huile d'olive sont plus élevés, en premier lieu chez l'exploitant 15 (qui est un exportateurs) et chez la plupart des exploitants de la société des agriculteurs, les agro combinats présentent les prix de ventes les plus faibles.

Des coûts de production d'huile d'olive élevés sont enregistrés chez l'exploitant 15 ainsi que la plupart des exploitants de la société des agriculteurs. Les coûts les plus faibles sont enregistrés au niveau des agro-combinats.

Concernant les résultats par Kg d'huile d'olive, les plus élevés sont enregistrés chez l'agro-combinat 3, chez l'exploitant 15 et chez quelques exploitants de la société d'agriculteurs (exploitants 3, 4 et 10).

Ces résultats montrent d'une part, une compétitivité prix chez les agriculteurs présentant une forme d'intégration (verticale ou horizontale) et une compétitivité coût de production des agro-combinats.

4.3. Observations et implications sociales

Au niveau de la région de Sfax on a constaté que l'agriculture biologique peut être considérée comme une forme d'innovation adoptée généralement par les grands agriculteurs plus qu'une forme de développement rural. Les agriculteurs qui sont impliqués dans l'oléiculture biologique dans la région démontrent une attitude positive et réceptive ayant une relation interactive avec son environnement, surtout avec les services de développement et le reste des acteurs. Malgré les avantages qui caractérisent le secteur de l'agriculture biologique, les agriculteurs souffrent de beaucoup de problèmes qui sont liés essentiellement à la commercialisation de leurs produits. Des efforts considérables sont déployés par les producteurs dans la valorisation de l'huile d'olive biologique produite, travaillant sur la qualité et sur le conditionnement (des projets en cours).

Tableau 1: Indicateurs Techniques au niveau des différentes structures de production

	Début projet	Superficie Olivier bio	% olive bio/SAUT	Densité Olivier organique	% oliviers - bio> 70ans	% oliviers bio<10ans	Arrachage et replantation	Nouvelle plant[a] et augment[a] des densités	Utilisation du compost	Rendement – moyen en bio: tn/ha
Agriculteurs en société de service										
Exploitant 1	1997	131	92	27	60	40	0	1	1	0,38
Exploitant 2	1999	104	99	21	87	13	0	1	1	0,74
Exploitant 3	1997	120	80	33	50	50	0	1	1	1,47
Exploitant 4	1998	150	98	43	28	19	0	1	1	0,92
Exploitant 5	1999	117	91	19	100	0	0	1	1	0,38
Exploitant 6	1999	98	100	23	88	12	0	1	1	0,71
Exploitant 7	1998	109	100	21	87	13	0	1	1	0,33
Exploitant 8	1998	93,5	100	18	100	0	0	0	1	0,72
Exploitant 9	1999	104	100	19	100	0	0	0	1	0,35
Exploitant 10	2000	145	100	17	100	0	0	0	1	0,57
Agriculteurs privés										
Exploitant 11	1998	135	100	17	100	0	0	0	0	0,62
Exploitant 12	2000	235	100	17	50	0	0	0	0	0,52
Exploitant 13	2003	250	89	17	0	0	0	0	1	0,55
Exploitant 14	1998	400	84	20	100	0	0	0	0	0,22
Exploitant 15	2004	1500	100	17	10	0	0	0	0	0,40
Exploitant 16	1997	200	50	31	44,5	55,5	0	1	0	0,54
Agro-combinats										
Agro-combinat 1	2004	7209	39	23	91	9	1	0	1	0,65
Agro-combinat 2	2004	2784	69	17	50	0	0	1	0	0,32
Agro-combinat 3	2004	17125	5	17	100	0	1	0	1	0,68
Agro-combinat 4	2005	12150	81	17	100	0	1	0	0	0,36

Tableau 2: Résultats économiques[2]

Indicateurs Economiques (Valeurs moyennes des campagnes 2006/07, 07/08 et 08/09)	Prix de vente moyen / kg Olive à huile	Coût moyen de production / Kg Olive à Huile	Résultat moyen/ kg Olive à Huile	Prix e Vente Moyen / Kg Huile d'Olive	Coût moyen de production / Kg Huile d'Olive	Résultat moyen / kg Huile d'olive
Société agriculteurs						
Exploitant 1	1,008	0,584	0,424	***	***	***
Exploitant 2	0,927	0,356	0,572	***	***	***
Exploitant 3	***	0,347	***	4,756	2,296	2,460
Exploitant 4	***	0,572	***	4,849	2,536	2,313
Exploitant 5	***	0,586	***	4,141	3,034	1,107
Exploitant 6	***	0,522	***	4,533	2,783	1,750
Exploitant 7	***	0,815	***	4,318	4,128	0,189
Exploitant 8	0,910	0,858	0,052			
Exploitant 9	***	0,636	***	3,785	3,375	0,410
Exploitant 10	***	0,440	***	4,392	2,305	2,086
Agriculteurs Privés						
Exploitant 11	***	0,540	***	4,028	2,573	1,455
Exploitant 12	***	0,700	***	4,584	2,798	1,786
Exploitant 13	***	0,551	***	4,049	2,503	2,211
Exploitant 14	***	0,775	***	3,903	2,982	1,871
Exploitant 15	***	0,391	***	5,812	3,113	2,699
Exploitant 16	1,170	0,619	0,551			
Agro-combinats						
Agro-combinat 1	***	0,4207	***	4,140	2,245	1,898
Agro-combinat 3	***	0,267	***	4,129	1,240	2,889
Agro-combinat 4	***	0,386	***	3,500	2,194	1,381

[2] Pour manque d'information on n'a pas prix en considération les résultats économiques de l'Agro-combinat 2.

5. Conclusions

Cette communication a pour objectif d'étudier la rentabilité économique au niveau du secteur oléicole biologique partant de l'hypothèse qu'il est environnementalement viable. Les différentes structures de production qui régies le secteur ainsi qu'aux organismes intervenants ont été pris en considération.

A partir des résultats obtenus, on peut conclure que le secteur oléicole biologique malgré quelques difficultés observées surtout au niveau de la commercialisation des produits, c'est un secteur prépondérant. Les agriculteurs présentent des attitudes positives, d'une part une bonne relation avec les organismes d'encadrement, d'autre part une tendance innovatrice, évolutionniste (volonté de contrôle de toute la chaîne, recherche de clients à l'étranger, des projets de conditionnement en cours, etc.).

Au niveau des structures de production, une compétitivité prix était enregistrée par les agriculteurs ayant opté par une forme d'intégration et une compétitivité coût par les fermes étatiques. Cette compétitivité coût de production engendre des ventes à des prix moins élevés que le reste des producteurs, ce qui peut provoquer une situation de compétition déloyale au niveau du marché.

Finalement, à travers cette simple lecture du secteur oléicole biologique dans la région de Sfax, on peut affirmer la nécessité d'une intégration horizontale et verticale au niveau du secteur afin de pouvoir évoluer vers des structures de production plus compétitives.

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