

**S08**
**OLIVETRENDS: FROM THE OLIVE TREE TO OLIVE OIL, NEW TRENDS AND FUTURE CHALLENGES**
**S08.001**
**Environmental Stress and Sustainable Olive Growing**
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The responses of *Olea europaea* L. to environmental stresses include a number of interesting features. Olive trees are very resistant to soil water deficit, high irradiance, high temperature and vapour pressure deficit. Olive trees are also more resistant to salinity than most perennial crops. On the other hand, olive trees are sensitive to low oxygen conditions in the soil and low temperature. Cultivar differences in morphology and physiology have also been associated with stress resistance. Traditional orchard management relies upon adaptation of the species and its cultivars to the environment. Innovations in production systems and the growing concern for environmental conservation issues impose revisiting and updating cultural practices in the field. Moreover, expansion of olive growing to new areas outside the Mediterranean region where climates and soils are often not optimal for olive growing may require changes in management protocols. Understanding the stress physiology of the olive tree may be beneficial to optimize orchard design and develop sustainable strategies in water, soil, fertilizer and canopy management. The mechanisms of adaptation of olive trees to main environmental stresses (drought, temperature, salinity) are here reviewed as well as the implications of olive stress physiology on sustainable management of olive growing.

**S08.002**
**The Use of the Evapotranspiration Stress Index (ETSI) to Guide Irrigation Management in Young Olives**
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Early detection of plant water stress in any crop planted for commercial reasons is an advantage when trying to match limited water resources with optimum crop yield and health. Daily water use of young olive trees (var. Frantoio and Barnea) was measured on a near-continuous basis (15 minute intervals) for a full year using Sentek® capacitance sensors. Basic growth indicators were also taken on selected trees. A new measure called the Evapotranspiration Stress Index (ETSI) to aid in the early detection of plant water stress is proposed. Using site-specific calibrated soil pore water values, it was possible to derive accurate estimates for the Volumetric Water Content (VWC) independent of soil texture. Independently collected and calculated potential evapotranspiration (ET<sub>p</sub>) values were obtained from a weather station. The ratio of the observed evapotranspiration and the Daily Water Use as recorded on the Sentek sensors gave a numerical value of ETSI. This was aligned graphically with established concepts of stress indicators developed by Sentek Pty Ltd to more clearly indicate where plants were experiencing significant water stress. It was also possible to set Plant Water Stress thresholds using this index, giving clear indications as to where intervention by the irrigation operator was required. The increasing use of weather stations and soil water monitoring equipment by horticultural operators has driven the need for simpler data integration and clearer indicators for when irrigation is required. The development of ETSI as an additional grower tool, fulfils this need.

**S08.003**
**Biennial Bearing in Olive - Physiological Background and Control**
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Biennial bearing is a widespread phenomenon in olive orchards which brings about severe instability in management input (labor, harvesting machinery, mills), oil quality and marketing. The current work explores basic as well as practical aspects related to biennial bearing in olive. The critical time in which flowering and subsequent fruiting in the following year by fruit removal are affected, occur 120 days after full bloom, later than previously suggested. Monitoring mineral concentrations in different types of plant tissue has revealed significant differences between 'Off' and the adjacent 'On' trees. We observed a dramatic decrease in K levels in leaves and roots of trees along the 'On' year in comparison with the 'Off' year. However, no such differences occurred in relation to N and P contents in the different types of plant tissues. Nevertheless, supplying different levels of K to olive trees in controlled conditions had only a minor effect on their productivity compared with a much greater effect by N and P. These results indicate that K level responded to fruit load but it's not the cause for potential bud differentiation for the following crop. From the practical aspect, we evaluated the dosage response of 'Barnea' olive trees to NAA post bloom spray in the 'On' year and its effect on yield return in the following 'Off' year. The highest tested level- 320 pp'm NAA resulted in a reduction of the fruit number from 40,000 to 15,000 in the 'On' year and followed by a balanced oil yield of 10 kg per tree during both the 'On' and 'Off' years. In contrast, in the non-treated trees the oil yield was 18 kg in the 'On' year compared to only 2 kg in the 'Off' year.

**S08.004**
**Effect of Nitrogen Status on Frost Tolerance of Olive Trees**
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Trees with different nitrogen status: adequate (1.4-1.6%), low (1.1-1.3%) or high (1.7-1.8%) leaf N concentration of July samples, were selected from a long-term experiment in which mature 'Picual' olive trees were subjected for 13 consecutive years to different nitrogen fertilization regimens. Leaves were sampled from each group of trees from October to April. Discs of 7 mm diameter were collected from these leaves, placed in test tubes with an aqueous solution, and subjected them to controlled freezing (0 to -26 °C) in a glycol bath. After freezing, the electrolytic conductivity was measured, the leaf discs were then autoclaved to kill the tissues completely, and the conductivity was measured again. The relative conductivity was calculated as the percentage of the final reading. Lethal frost temperature (LT<sub>50</sub>) was determined by plotting conductivity data against temperature, using a logistic regression model. In the autumn, at the onset of dormancy, LT<sub>50</sub> decrease as leaf N concentration increase, showing more frost tolerance those trees with an excess of nitrogen. The contrary occurs in spring, where deficient trees were more tolerant than trees with an excess of nitrogen, since LT<sub>50</sub> increase with leaf N concentration. No differences were observed during the winter, probably because the rest mechanism is more potent than nitrogen status of the tree.

## S08.005

### Gene Expression Analysis of Olive Tree (*Olea europaea* L.) in Response to Salt Stress

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The Olive (*Olea europaea* L.) is one of the most significant crops in the Mediterranean region. The expansion in olive cultivation to areas irrigated with low quality water, mostly saline, limits growth and productivity. Although, a number of studies have been conducted on the effects of salinity on olive growth, the molecular basis of salt tolerance has not been investigated. A five month salt stress experiment was set up with one year-old plants of three different cultivars (cvs Kalamon, Manaki, Arbequina) which exhibit differential tolerance to salinity. A cDNA library from 25-day old olive seedlings (cv Koroneiki) was constructed and 1211 unique ESTs were identified. We were able to identify numerous salt stress-related cDNA homologues through nucleotide blast and blastX analysis against NCBI and KEGG database. Custom DNA microarrays were constructed from the unique ESTs and used for comparative transcriptome analysis of untreated and salt-treated roots and leaves. In addition, the expression profiles of selected cDNAs were further investigated.

## S08.006

### High Temperatures Before Budbreak Inhibits Olive Tree Flower Differentiation but Not Development

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The expansion of the olive crop to new areas of cultivation, especially subtropical regions, represents a challenge to researchers and growers to evaluate and achieve crop adaptation to these new environments. Determining the effects of the temperature during the budbreak process provides critical information regarding suitability of new areas and appropriate olive cultivars. We used both woody explants from field-grown trees (cv. Manzanilla and cv. Arbequina) and whole trees (cv. Arbequina) in containers to study the effect of spring temperatures on reproductive budbreak and development. We tested 20 °C and 30 °C budbreak temperatures imposed at different times following winter chilling. Bud morphogenetic response was observed in sequential histological preparations and reproductive budbreak was evaluated after 3 weeks. Following sufficient winter chilling, 20 °C was optimal for normal reproductive budburst. Results showed, however, that temperatures as high as 30 °C any time before budburst has commenced will inhibit flowering, even when the amount of winter chilling was sufficient for promoting reproductive development at 20 °C. After budburst 30 °C promote inflorescence growth.

## S08.007

### Quantifying Indicators for Short-Term Water Stress in Juvenile 'Barnea' Olives Using Large Weighing-Drainage Lysimeters

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Water status can be measured along the soil-plant atmosphere continuum through direct measurement of soil and plant water status or by measuring the indirect

effects of the stress. We used 2.5 m<sup>3</sup> drainage-weighing lysimeters to determine actual tree-scale evapotranspiration (ET) of juvenile olives (*Olea europaea* cv. 'Barnea'). Twice during the summer of 2009, irrigation was discontinued for five (of 15 total) trees. Irrigation was reinstated as available soil water was estimated to approach zero. As the trees entered into and then recovered from water stress, continuous monitoring was conducted for meteorological data, actual ET from lysimeter water balance, and soil water content from TDR. Additional measurements of water status and water stress were conducted daily between 12:00-14:00 and included stem water potential from Scholander pressure chamber, stomatal resistance from porometer, canopy temperature using thermal imaging, and effective quantum yield of photosynthesis yield using MINI-PAM fluorometer. Vegetative growth rate (stem elongation) was measured for the periods of stress to recovery. At approximately 6-7 days after cessation of irrigation, actual ET was minimized and visual symptoms of drought stress including curled leaves became obvious. Good agreement was found between each of the parameters in both the daily measurements and in diurnal measurements made on days of maximum stress. Leaf level transpiration and photosynthesis, stomatal conductance, and stem water potential all reflected water status beginning at moderate stress levels and fully recovered to the levels of non-stressed trees quickly after water application was renewed. Whole tree ET was reduced by as much as 20%, indicating reduced canopy growth and size due to the stress period. The potential use of non-destructive, continuous methods of monitoring primary (turgor pressure) and secondary (canopy temperature) effects of water stress is particularly promising providing that the technologies become reliable and affordable.

## S08.008

### Transcript Analysis and Metabolome Profiling of Developing Olive Fruit in Relation to Different Water Regimes

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Although drought conditions are tremendously increasing in areas where olive is cultivated, little is known about their effects on gene expression and the overall metabolism in developing fruit. In this work we present a first metabolome profiling of olive fruit in plants grown in field and pots under different water regimes. Olive fruit were sampled at different developmental stages including veraison and full ripe stage. High-throughput metabolomics studies were performed using GC-MS TOF that analyzes more than 400 metabolites. Of these, 48 and 42 were the metabolites that significantly changed their amounts in the field and in pot conditions, respectively. Considering fruit from field trials, part of these metabolites (22) were identified including several phenolic compounds such as 2-4-hydroxyphenylethanol, 4-hydroxy-3-methoxybenzoic acid, shikimic acid, phenyl-beta-glucopyranoside, squalene, 4-vinyl-phenol. In the pot study significant changes in the amount were observed for 15 identified compounds involved in secondary metabolism. Other metabolites differently accumulating in samples from control and water stressed plants in the field (26) and in the pot (27) were functionally categorized using the BinBase database. To complement the metabolomics analysis, we studied the expression of some genes involved in key metabolic pathways (flavonoids, fatty acids, terpenoids, ethylene) during fruit development, some of them previously identified through a genomics approach (Galla *et al.*, BMC Plant Biology, 2009). One  $\beta$ -amyrin synthase gene was up-regulated under water stress conditions before pit-hardening while cycloartenol synthase was not affected. Considering phenol compounds, differential expressions between watered and water-stressed samples for phenyl-ammonia-lyase (PAL), chalcone synthase (CHS), and di-hydro-flavonol reductase (DFR). This study, a novelty for olive, demonstrates the possibility to integrate metabolomics with transcriptomic studies to better decipher and elucidate the regulation of fruit metabolism throughout development and in relation to different environmental conditions.

### S08.009

#### Monitoring of Atmospheric Temperatures in Emilia Romagna Region, a New Approach to Estimate the Jaén Index

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Jaén Index is currently considered one of the most indicative methods to determine the ripening stage of olives, nevertheless it can still represent some troubles for olive growers in terms of practical applicability and adaptability in different environments. The present study was performed obtaining data of atmospheric hourly temperatures during three years collected in three experimental fields localized in different areas of Emilia-Romagna region. Data so achieved were related with data about ripening, expressed with the Jaén Index, of olives belonging to the most widely diffused cultivars in the monitored area: Leccino, Correggiolo and Nostrana di Brisighella. Through statistical analysis was determined the correspondence between DDA (Degree-Day Accumulation) and increase of Jaén Index of each cultivar. Thus it is possible to provide an immediate and non-destructive approach which can be used to supply real-time indications about the ripening phase of a specific cultivar.

### S08.010

#### Effects of Saline Stress on Two Varieties of *Olea europaea* cv. Arbequina and Barnea

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Soil salinization increases progressively around the world, drought conditions and soil salinity are the major causes of stress in crops and cause economic losses in world agriculture. The olive tree is a species moderately tolerant to salinity, which has allowed the establishment of new plantations in arid and marginal areas. The aim of this study was to assess the effects of salinity on growth of two cultivars of *Olea europaea* Barnea and Arbequina, and identify some of the underlying physiological mechanisms. We used 60 plants of each cultivar which were kept under greenhouse conditions throughout the experiment. They were placed in pots using as substrate a mixture of sand, perlite, peat (1:1:1) and irrigated with NaCl solutions of 4 dS/m, 6 dS/m and 8 dS/m on a nutrient solution basis of 2 dS/m. Measurements of morphometric parameters (height, number of leaves), physiological parameters (water potential, osmotic and water content) and chemical parameters (ionic content) were carried out periodically. The results indicated that the growth of *Olea europaea* decreases with exposure to increasing levels of salinity, being cv Barnea the most affected. Both cultivars updated their water potentials making them more negative in response to stress, Arbequina adjusted their potential more and in less time. The osmotic potential was modified becoming more negative with increasing salt concentrations. While the relative water content (RWC) did not change with treatments, these results suggest that both varieties perform osmotic adjustment. With regard to ion analysis we noticed that the cv Barnea accumulated significantly greater amounts of Cl<sup>-</sup> in the leaf than Arbequina did, which coincided with lower growth of stems and leaves and a greater percentage of leaf damage on cv Barnea.

### S08.011

#### Olive Fruit and Root Growth with Different Irrigation Regimes in Central Italy

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Climate of Central Italy is characterised by 600-800 mm of rain per year, almost uniformly distributed throughout the year. Only in short periods of the summer olive trees may experience water stress that can compromise fruit production or quality. The aim of the present research work was to investigate olive fruit and root growth under different irrigation regimes during the summer drought period. Three different irrigation levels, 0% (control), 35% and 70% of ET<sub>c</sub>, were ap-

plied to 8-years-old olive trees. The drip irrigation started on July 1<sup>st</sup> and ended on August 31<sup>st</sup> 2009, when the drought period finished. Five fruits per plant were sampled every 20 days in order to estimate fruit growth rate. Fruit production per plant was measured at harvest (24 October 2009). Four rhizotrones (1x0.8 m) installed in October 2007, 0.5 m apart from the trunk, allowed to monitor root growth every 15 days. Results showed that 35% and 70% of ET<sub>c</sub> irrigation similarly increased fruit fresh and dry weight in comparison to control, but no significant differences were found in total fruit production per tree. Both irrigation treatments increased root growth in terms of growing roots number and total lengthening when compared to control. In conclusion irrigation was effective on preserving the fruit quality and avoiding shrinkage and also caused higher root growth without increasing the total fruit production per plant. No significant differences were found between the two irrigation levels for the measured parameters, confirming that 75% ET<sub>c</sub> irrigation level induces a luxury consumption for olive tree in Central Italy.

### S08.012

#### New Challenges for Pest and Disease Management in Olive Orchards and Nurseries

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Olive growers face a variety of challenges in effectively managing pests and diseases. The mechanisms for management can be broadly divided into three categories: exclusion, protection and environmental modification. Exclusion, or keeping the olives and the pest or disease separated, involves the use of measures such as border quarantine, on-farm biosecurity and certification schemes to ensure pathogen free planting material. With protection, the pest or disease is managed by preventing or eradicating infection or attack using pesticides, biological agents, resistant cultivars and cultural controls. The effectiveness of protection can be impacted by variable efficacy of biological agents, lack of suitable resistant cultivars and restricted pesticide use through resistance to the chemicals, legislative requirements or food safety concerns. Environmental modification creates conditions unfavourable for the pathogen or pest to invade and/or infect, using pruning practices, soil improvements and amendments. All management mechanisms can be influenced by the challenges facing all modern agricultural industries: climate variability, globalisation, changing community perception, requirement for sustainability, diminishing resources, increasing input costs and technological advances. In response to some of these issues, farm practices are evolving, which can result in new or changing pest and disease pressures. Currently there are many significant advances in the more traditional areas of pest and disease management, including soil health improvements, improved certification schemes, breeding resistant cultivars both traditionally and using genetic modification, precision agriculture and pesticide development and application techniques. However the future may lie with advances in technology, for example nanobiology, robotics and automatic sensors are being investigated as new tools for managing pests and diseases. This paper will discuss current and future mechanisms for pest and disease management and how they can address the challenges faced by the olive industry today.

### S08.013

#### Screening Olive Progenies for Resistance to *Verticillium dahliae*

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*Verticillium* wilt of olive (*Olea europaea*), caused by the fungus *Verticillium dahliae*, is the most serious disease in the Mediterranean countries. The use of resistant cultivars or rootstocks is one of the most efficient measures for the control of this disease. Olive shows a wide range of genetic variability useful for finding resistance to the disease. Therefore, screening a great number of olive genotypes for resistance to *V. dahliae* is the first step to get this objective. More than 3,000 olive seedlings from crosses and open pollination of different genitors were evaluated

for resistance to *V. dahliae* under controlled conditions. One month-old seedlings were inoculated by dipping their root system in a conidial suspension of the highly virulent isolate V117. Disease symptoms (defoliation, wilt, chlorosis and necrosis) were assessed by using a 0-4 scale. Results demonstrated that young olive seedlings can be successfully infected by *V. dahliae*, showing consistent *Verticillium* wilt symptoms. A wide range of disease resistance levels were found among olive progenies obtained from crosses of susceptible and resistant cultivars. Results have also provided information about inheritance of resistance, as well as suitable genitors to breed for *Verticillium* wilt resistance in olive. Many olive genotypes were selected for resistance to *Verticillium* wilt and they will be tested in field conditions. Screening of new genotypes will continue in order to develop resistant cultivars or rootstocks. Additional trials are currently searching resistance in a great number of seedlings from wild olives.

### S08.014

#### Investigation on Frost Damaging and Selection of Tolerance Olive (*Olea europaea* L.) Cultivars and Genotype

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The evaluation of frost damage and selection of tolerant olive cultivars for cold were researched in 2008 year at Tarom Olive Research Station. When the weather temperature fell down to 5 °C below zero, the frost damaged olive trees in Tarom area were recorded, during winter period. In this investigation, the external frost damage signs like leaf wilt, necroses and fall down leaf, whiter shoots and longitudinal cracks of shoots cortex were evaluated. The young and old leaves of susceptible and tolerant to cold olive cultivars sampled for measurement of nutrients, during summer period. For determination of amount frost damage on olive cultivars recorded fruit and yield traits. Results showed that "Zard" local olive cvs. was more resistance to cold in comparison with other local olive cvs. and has higher yield performance (18.1kg per tree). The Greek cvs. Konservolia and French cvs., Grossane have completely resistance to cold, and higher yield performances (34.9 and 20.7 kg per tree respectively). These two cultivars are suitable for colder weathers which have frost risks. Although the other cvs. such as Leccino, Mastoidis, Baladi, Koroneiki, Kalamata, Kailietier, Hamed and Oblonga were observed susceptible to cold and less yield performances (less than 3 kg per tree) in Tarom region.

### S08.015

#### Diseases and Disorders Associated with Environmental Stress on Sustainable Olive Orchards in Australia

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The main aim of our research is to contribute to the knowledge on diseases and disorders, of olives in Australia. Based on our diagnostic and field research work associated with projects we report on several new records of fruit rots and of fungi associated with foliage, trunk and root diseases. As well as being affected by a range of pathogenic diseases, olives are also subject to non-pathogenic disorders, such as damage by heat and sun and other weather conditions, irregular watering or nutrient imbalances, all of which interfere with the normal physiological processes in trees. As a result, reduced crop set and fruit size, and a range of other symptoms, can be directly caused by environmental conditions which may, in turn, in a greater tendency to become diseased. The sunburn can occur, particularly in young olive trees. In some cases sunburn, together with water stress, can be expressed as a slow tree decline and dieback. High temperatures coinciding with excess soil moisture between early fruit ripening and harvesting may predispose the fruit to pit burn

disorder. Some symptoms are consistent with those caused by water stress which produces partial dehydration of the fruit at the apical end or whole fruits. The internal flesh and pip may be blackened. The number of different symptoms, which are associated with shriveling of the apical end of the fruit commonly described as 'soft nose'. A condition of hard brown end of the green immature fruits of olives also occurs in some olive groves. The discoloration usually does not extend deep into the flesh; sometimes, it only affects the skin. A number of Australian olive production areas also experience hailstorms. The causes of some disorders are, as yet, unknown and further investigation is needed on their economic importance and sustainable production of olives in Australia.

### S08.016

#### Dropping Irrigation versus Antracnose Disease Development in a Reconverted Traditional Olive Grove

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The olive Anthracnose is caused by a fungal pathogen of the genus *Colletotrichum*. Under favorable environmental conditions the disease can devastate a great part of field production. In rainy years the application of chemical treatments is difficult. So, the disease is not controlled. The aim of this study is to know how the dropping irrigation installed in a traditional adult olive grove (more than 50 years) interact with anthracnose disease. With this purpose three different volumes of water and a dry situation were used in field. All the four modalities were done with and without chemical treatment. The results showed that irrigation affects significantly the disease intensity, mainly when chemical treatment is applied. Effect of volume of dropping irrigation in olive production and level of Anthracnose infection is discussed.

### S08.017

#### Evaluation of Olive Cultivars for Resistance to *Colletotrichum* spp.

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Anthracoze of olive, caused by the fungi *Colletotrichum acutatum* and *C. gloeosporioides*, is the most destructive disease of olive (*Olea europaea*) fruit and is widely distributed in many olive-growing regions of the world. Information on the resistance to anthracnose in olive cultivars is based on field observations and farmer experience rather than on systematic studies. An inoculation method using immature green fruit and high *inoculum* densities (105 to 106 conidia/ml) sprayed on the fruit has been proposed to evaluate olive cultivars for anthracnose resistance under controlled conditions. Moreover, a rating scale (0-10) to assess fruit rot incidence in naturally infected trees was validated by comparing ratings with direct counts of affected fruit. Under field conditions, fruit-rot incidence varied greatly among tested cultivars and was well correlated with the severity of branch dieback symptoms that developed after fruit-rot epidemics. The best correlation between fruit-rot incidence in the field and disease severity on inoculated fruit was obtained using a disease susceptibility index that integrated the maximum disease progress rate and the estimated time to reach 50% disease severity. Based on field observations and laboratory data on susceptibility to anthracnose, 318 cultivars were classified into three groups: 258 highly susceptible (e.g. Cobrançosa, Cornicabra, Galega Vulgar, Hojiblanca, Lechín de Sevilla, Manzanilla de Sevilla, Morona, Ocal, Picudo, Sevillena, Verdial de Huévar); 24 moderately susceptible (e.g. Arbequina, Arbosana, Morrut, Pajarero, and Villalonga); and 18 resistant (e.g. Blanqueta, Empeltre, Frantoio, Koroneiki, Leccino, Picual, and Razzola). The knowledge of the olive resistance to Anthracnose is essential for a correct choice of the cultivars and for the current breeding programs.

## S08.018

### Premature Death Disease in Olive (*Olea europaea*) in Argentina

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Olive plants of new orchards may dry prematurely, causing considerable losses of plants in the orchards, with major economic consequences for the growers. This pathology begins with dry branches located in any sector of the plant. Flowers or fruits of these branches also dry and finally fruits mummified. The disease has been noted mainly in La Rioja province since 1997. In 1999, affected orchards (90%) averaged 5% of diseased plants. Chilecito, Arauco, and Capital counties reached 10, 6, and 0.3%, respectively. At that time, olive growers pruned and removed dead branches and plants. The disease has been recorded in other olive-producing provinces with low incidence such as Mendoza and San Juan. *Pythium* and *Phytophthora* root rot were recorded in San Juan associated to premature death of plants while *Phytophthora* crown rot was noted in Catamarca. In both sites, the plantations had drip irrigation. However, it was also noted in orchards with furrow irrigation in the central valley of Catamarca. *Fusarium* root rot was recorded in Atuel, Lavalle, and Maipu (Mendoza), Pocito (San Juan), Viedma Valley (Rio Negro), and La Rioja (Arauco, Chilecito, and Capital counties). Nematode genera *Meloidogyne* and *Xiphinema* interacted with *Fusarium*, as observed in La Rioja, Mendoza, and San Juan. *Verticillium dahliae* was isolated from olive roots in Capital county (La Rioja). Symptoms can be confused with frost damage. Other organisms associated with dead branch disease were *Armillaria mellea*, *Cylindrocarpus* sp., *Rhizoctonia solani*, and *Rosellinia necatrix*. In 2009, a close follow up of this pathology was started in a plantation of Picual located in Chilecito (La Rioja) and other provinces to know the microorganisms responsible of the premature death of young and 15-20 year old plants.

## S08.019

### Sanitary Status of Olive (*Olea europaea*) in Argentina

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Disease surveys were conducted in new and old olive plantations during 2009. Olive knot disease was recorded in south Buenos Aires, Mendoza, and San Juan provinces with high incidence in both new and old plants of Arauco. Also, in an orchard located south of La Rioja (Chilecito), and Cordoba. Olive trunks, branches, leaves, and twigs were affected. Flowers and fruits showed no damage. There were differences among cultivars. Arbequina, Gordal, and Manzanilla showed disease resistance. This pathology was associated to wounds caused by hail and frost. Rio Negro remains free of olive knot. *Verticillium* wilt was observed in Catamarca, La Rioja, and Mendoza in new and old olive trees with low incidence in Arauco. Phomopsis twig blight was only detected in Carolea and Coratina in the central valley of Catamarca. The teleomorph stage of this fungus was not observed. *Fusarium* root rot was present in young and old plantations in south Buenos Aires, Catamarca, La Rioja, Mendoza, San Juan, and Rio Negro. Foliar and fruit diseases such as anthracnose, peacock spot, Cercospora leaf spot, and sooty mould were noted in olive trees in south Buenos Aires (Dorrego, Patagones), Catamarca, Cordoba, La

Rioja, Mendoza, Rio Negro (Viedma valley), and San Juan. Leaf and fruit anthracnose was common in wet areas in new and old orchards. Weeping was observed in Arbequina, Carolea, Picual, and old orchards of Arauco in Aimogasta (La Rioja) and Tinogasta (Catamarca). Leaf and fruit deformations associated to mites *Eriophyoidea* and ash whitefly *Siphoninus phillyrae* were usually recorded in untreated orchards. Symptoms of unknown etiology such as sickle leaf, bell-shaped leaf, trunk grid, trunk lumps were also noted throughout the olive planting area.

## S08.020

### Olive Production Systems and Mechanization

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The productivity of the traditional olive orchards (80 to 100 trees/ha) is relatively low and the crop costs are very high (harvesting, pruning, etc.). Since the 70s, the increase of the olive surface has been associated to an intensification of the orchards and several authors recommend to use higher densities (HD), about 200-500 trees/ha, with drip irrigation, designed for harvesting with trunk shakers, and with higher yields and low-medium production costs. Finally, at the beginning of the 90s, a new type of olive orchards (super-high density hedgerow, SHD) appeared in Catalonia (NE of Spain), with densities ranging between 1,500 and 2,500 trees/ha. Later they were introduced into other Spanish regions and other countries. This system facilitates the use of continuous straddle mechanical harvesters and the achievement of higher yields within a few years after planting. To improve the efficiency of the harvester, vigour must be managed to limit tree size while maintaining high productivity. However, there are few cultivars adapted to this system and currently 'Arbequina IRTA-i•18', 'Arbosana i•43' and 'Koroneiki i•38' are the most used in this type of super-high density orchards. A comparative study on the economic viability of high and super-high density olive orchards in Spain, indicate that HD economic ratios are more profitable than the SHD ratios. However, the latter could be the most sustainable option in large farms with short term investments, mainly for the full harvest mechanization and reduced labor requirements. In the last years some R+D works began to improve the viability of these model orchards (HD and SHD), standing out some breeding programs, rootstocks selection, planting layout, and crop management. This review shows the most current trends towards olive production systems and mechanical harvesting.

## S08.021

### Vigor Control of the Olive Tree in High Density Planting System: Two Experimental Approaches

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In France, high density planting of olive orchards is progressively increasing. This new training system necessitate to control tree vigor. The French research and experimental organizations aim to answer to this request, by improving the management of existing orchards (better tree management, optimization of pruning, reduction of inputs) and searching for low vigor plant material adapted to high-density training system (identification of low vigor genotypes, use of dwarfing rootstocks). This work relies on a network of producers who are involved in the definition of experimental protocols and in orchard measurements. This network, established in 2002, is managed by INRA Montpellier. A first trial was carried out since 2004 at Ctifl on the tree management in palmette rather than in central axis in order to try to distribute tree vigor on several axis. Three varieties ('Picholine du Languedoc', 'Aglандаu' and 'Arbequina') were planted in hedgerows, on the basis of 30 trees per variety and management system. First results show a non-significant decrease in vigor when the trees are managed in palmette rather than axis, but a better suitability of this form for harvesting machine. A second trial was established in 2005 by AFIDOL-CTO on the adaptability to high-density training system of new French genotypes, noticed for their low vigor. Eight genotypes were planted in hedgerow on the basis of 20 trees per genotype. Some genotypes were also planted

in four other locations in 2009 to evaluate the Genotype x Environment interaction. The first results obtained in the orchard planted in 2005 show that two genotypes are more interesting than the others in terms of low vigor and oil quality. These results are discussed in comparison with those obtained in other countries where similar trials have been carried out and in terms of technical solutions they can offer to French producers.

### S08.022

#### Soil-Management Systems in the Olive Orchard and Influence on the Organic Carbon and Nutrient Contents

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The management systems in olive orchards alter the physical-chemical properties of the soil. Currently, cover systems are being used, both of diverse types of plants as well as shredded pruning remains, both in traditional as well as ecological olive orchards. The plant remains (from the covers or the olive trees) are managed with herbicides or mowers and shredders. Sometimes, the material is left on the surface and sometimes buried. The olive orchard, with appropriate soil management could be considered as a sink for atmospheric carbon. To ascertain the potentiality, it is necessary to ascertain the contents in organic carbon and other nutrients for different types of soils and managements. In this way, various management recommendations could be established for the entire diversity of the olive orchards in Andalusia (S Spain) as well as for other areas of the Mediterranean. The aim of the present work was to determine the contents in organic carbon (SOC), nitrogen (N), and potassium (K) in olive-orchard soils with 8 different soil-management systems and management of the different plant remains. For this, 5 orchards of conventional and 3 or organic management were selected. The results show that the SOC, N, and K contents are directly related to the soil-management system and the handling of the remains. The SOC content declined in all cases in comparison with those registered in the forest floors studied near the olive orchards. However, the management of the olive-orchard soils with plant covers of all types and the addition of pruning remains augmented the SOC, N, and K contents in the soils studied with respect to those of conventional tillage.

### S08.023

#### Olive Fruit Production in Central Italy in Response to Different Pruning Systems

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In Central Italy, the olive trees are traditionally pruned removing a high amount of foliage in open vase training systems at the end of the winter, to avoid the risk of freeze damages. This approach tends to maintain a regular tree shape, without internal shaded branches, but it reduces the overall flowering potentiality and insufficiently counteracts the olive alternate bearing. The objective was to investigate in Marche region (Central Italy) the possibility to extend the pruning period up to the beginning of the summer to better control the tree vigour and productivity. The pruning was performed on 5-years-old productive trees of three interesting Italian olive varieties (Raggia, Maurino and Leccino), trained as free polyconic open vase, in April (at early vegetative growth) and in June (during the fruit set), compared with no pruned trees (control). Two pruning intensities were applied in April: low (3.9 kg of pruning material per plant) and high (7.9 kg per plant), and one in June: high intensity (9.7 kg per plant). The results showed that the intense pruning at early vegetative growth strongly reduced the fruit production and caused heavy vegetative growth; while the low intensity pruning in April and the high intensity pruning at fruit set did not reduce the fruit production in comparison to control. Moreover, the pruning in

June stimulated a much less vegetative growth in comparison with other pruning systems, which is a very useful result in intensive orchards. This study suggests that olive pruning in Central Italy must be minimal, if it is performed soon after the winter, to avoid a decrease of fruit production. In alternative, the pruning can be heavier after the fruit set to better exploit the whole plant pollination potential, maintain olive production, reduce watershoot and avoid excess vigor, and likely control alternate bearing.

### S08.024

#### Mechanical Pruning of Adult Olive Trees and Influence on Yield and on Efficiency on Mechanical Harvesting

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Mechanical pruning was applied using a bar with four rotating toothed disks, each cm 50 in diameter, on adult olive trees trained on vase shape. The 20 – years old trees of Frantoio, Leccino and Moraiolo cultivars were planted at m 5 x 5 and olive orchard was located in Umbria region in Central Italy. The pruning methods were: hand pruning with traditional mechanical tools; wholly mechanically pruning by topping to control the tree height; mechanical topping completed by removing of the internal suckers with traditional mechanical tools; mechanical topping plus bilateral hedging supplemented by sucker removal with traditional mechanical tools. The work efficiency and the tiredness of the different pruning operations were determined and, in the following two years after pruning, the yield and the adaptability of the pruned olive trees to mechanical harvesting by trunk shaker with interceptor were evaluated. The results showed that mechanical pruning lets have a considerable reduction in manpower. The highest reduction of canopy volume, over 60%, was observed in topping plus hedging completed with hand pruning. The mechanical pruning did not affect the yield and the mechanical harvest percentage, achieving good yield efficiency and getting crown volume fit to trunk shaker.

### S08.025

#### Evaluation of Portuguese Olive Tree Cultivars in High Density Planting System – An Account of Five Years

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In Portugal the implementation of very high density planting system started in the year 2000. Since then, large areas have been planted in arrays of 3.5 to 4 m between rows by 1.35m between trees in the row. Only Arbequina cultivar has been used. With the aim to study the adequacy of Portuguese olive tree cultivars to this kind of planting system, a trial was established in Herdade do Lameirões – Safara-Moura (38° 04'N 7° 16'W) in March 2002. In this trial, based on a randomised complete block design with three replications, a factorial experiment with two densities and six cultivars are being compared leading to 36 plots with 3 lines of 20 trees per plot. The densities used are 1850 trees per hectare (4m x 1.35m) and 1250 trees per hectare (4m x 2m). Cultivars being tested are: Azeitira, Cobrançosa, Cordovil de Serpa, Galega, Redondil and Arbequina. Trees are trained according to the central leader design with no pruning intervention so far. Each year, before the growing season, tree height, tree width, trunk diameter was measured. Yield was also evaluated. After five harvest campaigns, the results reveal that Arbequina cultivar is better adapted to this kind of olive orchard in terms of production and biometric parameters. Galega and Cobrançosa cultivars revealed a cumulative yield near to the values of Arbequina, but with a growing habit that promotes large canopy, jeopardizing the adequacy for this kind of orchard.

### S08.026

#### Influence of Planting Density on Yield of cv. Arbequina Hedgerow Olive Orchards

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High-density hedgerows olive orchards have spread out in the last years due to the advantage of harvesting by vineyard type straddle-harvesters. The lack of specific low-vigour olive cultivars or dwarfing rootstocks adapted to this system make highly important the selection of optimum planting density in order to maximize the yield without causing serious competition problems between plants. In this work, ten density treatments, ranging from 780 to 2.581 trees ha<sup>-1</sup>, were evaluated in cv. Arbequina hedgerow olive orchard located in Cordoba (South of Spain). Accumulated oil yield ha<sup>-1</sup> increased linearly with densities in the nine harvests after planting, with average values of 750 and 2.000 kg oil ha<sup>-1</sup> for the lower and higher densities, respectively.

### S08.027

#### Vegetative and Productive Behaviour of Four Olive Italian Cultivars and Arbequina According to Superintensive Olive Training System in Central Italy

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In the last years, superintensive olive groves are being planted worldwide to increase yield and reduce cost, plus to eliminate labour availability problem by using over – the row modified grape harvester. All over the world the superintensive oliveculture method is based, essentially, on cultivars Arbequina and Arbosana. The benefit of this new training system mainly depends on the availability of cultivars characterised by compact habit, early bearing and low vigour. Since the Italian germplasm of olive is characterised by many well - known cultivars such as Frantoio and Leccino ones, and it doesn't include Arbequina and Arbosana cvs., a trial to evaluate the adaptability of four Italian cultivars to the superintensive olive training system and to mechanical harvesting by over – the row modified grape harvester was set up in Umbria region, in Central Italy, place characterized by cold winter and short dry summer, assuming as reference Arbequina cultivar. The parameters determined were: tree characteristics (tree height, crown width, trunk diameter), early bearing, yield per tree and per hectare, harvester efficiency and fruit losses, olive characteristics during ripening and at harvesting time. Arbequina showed the most precocious reproductive stage, followed by Maurino, on the contrary the latest bearing was Frantoio with only 18% of productive trees at the second leaf. In 2009 Maurino and Leccino achieved the highest yield (around 3 kg of olive / tree), followed by Arbequina (2.3 kg of olive / tree), and Frantoio and Moraiolo the lowest with 1.7 and 1.3 kg of olive / tree respectively. The harvester efficiency was very good all over. At third leaf Arbequina, Moraiolo and Maurino resulted to be less vigorous than Leccino and Frantoio. Up to now, among the four Italian cultivars, Maurino seemed to be suitable for superintensive oliveculture in terms of vegetative growth and reproductive aptitude.

### S08.028

#### Genetic Resources, from Conservation to New Cultivars

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The need for new olive cultivars is an on going process due to the continuous developments of new cultivation techniques. The olive is an old traditional crop domesticated from the wild at various locations around the Mediterranean basin resulting in a vast number of different cultivars in the traditional olive regions. The preference of olives to cross pollination increased the diversity within local populations.

Geological and climatic changes as well as natural and directed dispersal created geographic isolated populations. The phenotypic diversity was the basis for selection and thereafter breeding of new cultivars for the developing cultivation systems. Thus, conservation of the diverse genetic resources became a major issue in the breeding effort of cultivars suitable for the new developing intensive olive industry. Methodologies to shorten the juvenile period, and the accumulation of genetic information on character dominance and character linkage enabled enhancing the efficiency of breeding yielded a number of new olive varieties but remained rather arbitrary and slow. Most of the characters aimed for in modern breeding such as response to fertigation, oil quantity, oil quality, tree form and various environmental resistances are of multi gene nature. Thus, for modern targeted breeding more accurate markers for the different required traits are needed in addition to the phenotypic ones. Such markers are being presently developed using molecular methods such as SSR and SNPs based on analogous gene expression in model plants and previously selected series of crossings. The application of recent techniques such as 454 for sequencing the functional genome of some olive cultivars will enable a better choice of the parental plants in olive breeding. In some breeding programs the use DArT technology is applied for identifying markers for specific traits. A rapid advancement in olive breeding could be expected in the near future.

### S08.029

#### Strategy for the Conservation of French Olive Genetic Resources

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In France, olive growing covers the 13 departments around the Mediterranean Sea. The genetic diversity is important since about 150 varieties are grown, but only a dozen are grown on large areas. The rest is made of many local varieties, specific to each department, with a shrinkage of their areas. To preserve and study these local genetic resources, we established a network including local people. In this communication, we describe this network and propose a conservation strategy at national and local levels. At national level, a varietal collection of reference was established in 1980 by INRA Montpellier and CBNM Porquerolles. This collection is located on Porquerolles Island, out of frost, thereby ensuring the long-term preservation of varieties. It currently includes about a hundred of French varieties. This collection presents the advantages to gather in one site the main varieties of the different production areas. But it presents two disadvantages: the poor adaptability of some varieties to the location, which prevents any work of agronomical characterization, and the distance from some production areas. Therefore, varietal collections were established in each department. At local level, varietal collections have been established to preserve not only the main varieties but also local and minor ones. To date, 13 collections, each gathering only the varieties of the department, have been established with a minimum of 3 trees per variety. These collections are managed by local people involved in «the national network for the management of olive tree genetic resources». Local collections, at the opposite of national collections, allow the *in situ* conservation of the varieties cultivated in the department and the agronomic observation of the varieties in their natural environment. They also facilitate the recognition of varieties by local growers and can be used as mother-plants for varieties propagation and dissemination by nurserymen.

### S08.030

#### Contribution of Conventional and Unconventional Techniques in Genetic Improvement of Olive

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In olive, classical methods of genetic improvement have given rather scarce results so far. At the same time, the need for new suitable cultivars for intensive cultivation

require innovative strategies through the support of molecular and biotechnological techniques to speed up the novel hybridisation method, named “gene pools”. This method requires wide germplasm collection availability of both cultivated and wild olive genotypes, and the knowledge of the genetic structure of both germplasm pools. Here, first of all, we summarize the state of art of the results obtained in olive by conventional methods (clonal selection, induced mutation and recurrent selection) and by unconventional techniques (*in vitro* pollination, embryo rescue, dihaploids constitution, spontaneous and under selective pressure somaclonal variation, somatic hybridisation, genetic transformation and germplasm conservation). The methodologies available for plant manipulation has been incredibly expanded in number and type, which allow to adopt fine strategies to reach precise goals. In addition, technologies derived from the knowledge of genetic, phytopathology, entomology, biochemistry, cell biology, molecular and system biology, can be combined to develop suitable strategies. All the aforementioned strategies can also be supported by the research on gene isolation and molecular marker used to characterize the genetic structure of the germplasm in order to improve the plasticity of cultivar behavior and quality of production. Suggestion concerning the use of both new molecular technologies and biotechnologies will be discussed.

### S08.031

#### Advances in the Joint UCO-IFAPA Olive Breeding Program (JOBP)

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A joint olive breeding program (JOBP) including crosses carried out from 1991 to 2006 is on progress in Córdoba (Spain). This program was previously reported at the ASHS Annual Meeting in 2007. The initial protocol of forcing growth to shorten the Juvenile Phase (JP) has been improved. We have also advanced on the establishment of early and simplified criteria of selection for earliness of bearing and oil yield. Three phases on the breeding process has been developed: 1) Evaluation of single seedlings for early bearing, size of fruit, high oil yield and plant architecture for adaptation to mechanical harvesting, 2) Agronomic evaluation of preselections from phase 1 for yield, vigor, oil composition, time of flowering and ripening, susceptibility to *Spilocaea oleagina* and other defoliating diseases, and other traits in one field experiment with few random replications, and 3) Agronomic and oleotechnic evaluation of advanced selections within field trials in different environments. More than 10000 seedlings from 83 crosses are under evaluation in some of the above mentioned phases among which more than 300 in phase 2 and 33 in phase 3. The new cultivar ‘Sikitita’ (‘Chiquitita’ in USA) has been released and protected in 2008 and planted for the first time in commercial orchards in 2009-2010. The most outstanding results are summarized.

### S08.032

#### The Portuguese Olive (*Olea europaea* subsp. *europaea*) Germplasm

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Portugal has a reach phenotypic and ecotypic diversity of cultivated olives. Some of the cultivars are synonyms of cultivars from other countries, but others seem to be autochthonous. We have been using nuclear SSRs to discriminate between the different Portuguese cultivars and to try to understand the genetic relationship among them and among the cultivated olives and their wild related variety oleaster. The use of numerical analysis to evaluate the relationships among cultivated and wild Portuguese olives, among cultivars, among ecotypes collected in an oil producing region in the north of Portugal (Trás-os-Montes) and within two of the most relevant cultivars (Galega and Cobrançosa) will be presented and discussed. Also

the analysis of the accessions of three Portuguese germplasm collections will be discussed. The conclusion of our analysis is that Portugal has a reach olive genetic diversity, which is still poorly known and poorly utilized. The investment in better knowing and preserving the Portuguese germplasm and to use it for breeding programs, being a challenge, is also an opportunity to improve olive characteristics and to develop new olive cultivars.

### S08.033

#### A Gene Required for a Non-Delayed Juvenile-to-Adult Transition in Cultivated Olive Trees

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The juvenile-to-adult transition is a complex and poorly understood process in plant development required to reach reproduction competence. In woody plants, knowledge of this transition is even scantier and no genes have been definitively identified as involved in this transition. To search for genes involved in the juvenile-to-adult transition in olive, we constructed juvenile and adult subtractive cDNA gene libraries and identified genes that were differentially expressed in the juvenile and adult phases. In the analysis of these libraries we found a gene designated as Juvenile to Adult Transition (JAT) that was of special interest because it was highly expressed at the mRNA level in the early developmental phases and repressed in the adult phase. The analysis of mutant trees altered in the juvenile-to-adult transition, as well as a segregating progeny of 31 trees from a ‘Picual’ × ‘Jabaluna’ cross, support the contention that its activity might be required for a non-delayed transition. JAT is expressed in different parts of the plant, showing an unexpectedly high level of mRNA in the roots. However, the JAT expression level is not determined by the distance to the roots, but depends on the developmental stage of the branch meristems. JAT is a widely represented gene in plants that seems to be involved in the control of the juvenile-to-adult transition in olive. The deduced amino-acid sequence was compared by blast on NCBI databases, with the available sequences. A preliminary phylogenetic analysis of the amino-acid sequences showed a quite high similarity with *V. vinifera*, *P. trichocarpa*, *A. thaliana*, *G. max* and *R. communis* peptides that seem to be coded by JAT orthologues, as well as a high similarity with the monocots *Oriza sativa*, *Sorghum bicolor* and *Zea mays* peptides, which are also likely to be coded by orthologues of *O. europaea* JAT gene.

### S08.034

#### Olive Tree Seedling Architecture and Juvenility Period for Early Selection

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A critical step for olive breeding is the early elimination of seedlings with a long juvenile period. In olive, as in other fruit trees, seedling height is strongly correlated with juvenile period duration. Furthermore, in apple and pear, more detailed growth-habit parameters as well as height have been associated with the transition to flowering. For young olive seedlings we defined eight architectural models, based on the number of main axes, apical growth continuity and the height at which branching occurred, in order to examine the relationship of growth habit with the duration of the juvenile period. We evaluated a population of 860 seedlings from six different crosses of seven parental cultivars and open pollination. The seedlings were grown in a greenhouse under optimum conditions and without any pruning, and were assigned to the appropriate models at 11 months, just prior to transplanting to the field. All of the models were present in varying degrees in the different crosses, but the “mono-axis” model was clearly dominant. Two years after transplanting, only the “mono-axis” and “high primary axis” plants produced flowers. The following year, although other models also flowered, those two models showed



the highest percentage of flowering plants. In addition to their application to juvenile period longevity, we will be using these olive seedling architecture models to study the growth habit of the developing trees.

### S08.035

#### Studying the Genetic Determinism of Olive Tree Architecture in a F1 Progeny Olivière x Arbequina

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Tree architecture is of major importance for the agronomic performance of fruit tree orchards. It influences tree adaptability to cultivation systems, but also yield and fruit quality. In the present study, we aim at investigating the genetic determinism of architectural traits in the olive tree. A F1 hybrid population was created between two genotypes with contrasted architectures, 'Olivière' and 'Arbequina'. A phenotyping methodology was applied to analyze traits related to growth, branching and fruiting behavior, that were measured during the first 4 years of tree growth. Taking into account the influence of tree ontogeny, the phenotypic variability was decomposed into genotypic and residual effects in order to identify the most heritable characters. Topological and geometrical variables showed relatively low to medium broad sense heritability values. The highest heritability values were found for the number of internodes per growth unit, the number of short growth units and the mean internodes length with H<sup>2</sup> value greater than 0.5. Flowering variables showed high heritability values exceeding 0.6. In parallel, we initiate QTLs mapping related to architectural traits, on the basis of a framework genetic map constructed with 44 SSR loci and 494 AFLP markers.

### S08.036

#### Localization of Olive S-Adenosyl Methionine Decarboxylase and Spermidine Synthase mRNA Transcripts in Flower Opening and during Early Fruit Development

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Polyamines (PAs) are required for cell growth and cell division in eukaryotic and prokaryotic organisms. The biosynthesis of the PA spermidine (Spd) from putrescine (Put) is catalysed by the action of an aminopropyltransferase, spermidine synthase (SPDS EC: 2.5.1.16) and S-adenosyl methionine synthase (SAMDC EC: EC 4.1.50). Two full-length cDNA clones coding for SPDS and SAMDC homologues in the stone-fruit of olive (*Olea europaea* L.) were identified and described the spatial and temporal organization of Spd biosynthesis genes in flower opening and early stone-fruit development. In *O. europaea* flowers, transition to flower opening phase coincided with a increase in ADC and SAMDC activity and a 2.8- and a 3.6-fold increase, respectively, in the Put and Spd levels. The level of OeSAMDC gene transcripts were highly expressed in the ovary wall, the placenta and the ovules, while the OeSPDS transcript is confined to the ovules of ovary at anthesis stage. This result suggests a putative role for OeSAMDC during ovarian development. OeSAMDC and OeSPDS transcripts are expressed in olive fruit mesocarp and exocarp at all developmental stages analyzed as well as in nucellus, integuments and inner epidermis tissues of fertilized ovule. OeSAMDC and OeSPDS were differentially expressed in flowers and fruits, and vary with olive cultivars. The presented results supply novel data about localization of PA biosynthesis gene transcripts, and indicate that transcript levels of PA biosynthesis genes are all highly regulated during flower opening and early cell division in the stone-fruit development.

### S08.037

#### Influence of Different Olive Rootstocks on Growth and Yield of 'Arbequina IRTA-i•18' Clone

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An olive rootstock selection has been established, in order to modify tree vigour on grafted scions. Also their tolerance to specific soil diseases and their adaptability to different soil conditions were considered. The current problem for the new super high density (SHD) system is the lack of both low vigour varieties and dwarfing rootstocks. 'Arbequina' cultivar is the most widely used variety in the hedgerow system. However, reduction in vigour would contribute to better orchard management. 'Arbequina IRTA-i•18' clone was grown on two root systems, own-rooted and grafted onto eleven clonal rootstocks ('Arbosana', 'Corbella', 'Frantoio', 'Fs-17', 'Joaneca', 'Limoncillo', 'Llorón de Castuera', 'Llumet', 'Menya', 'Picual de Estepa' and 'Royal de Cazorla'). This irrigated trial was planted in 2000 (bark grafting on the field in 2001), trained to vase and it was spaced 4 x 5 m (500 trees/ha). The design was a randomized complete block with five replications and one tree per replication and treatment. Preliminary results have shown that some rootstocks have a strong influence on scion vigour and yield. The lowest tree vigour were observed in 'Arbosana', 'Corbella' and 'Limoncillo' rootstocks, while 'Menya', 'Fs-17' and 'Joaneca' were the most vigorous scions. The vigorous rootstocks in the first years of planting normally show the highest cumulated production, mainly for 'Menya' (169 kg/tree), 'Joaneca' (143 kg/tree) and 'Arbequina-i•18' (control, 139 kg/tree). On the other hand, the dwarfing rootstocks as 'Picual de Estepa' and 'Llorón de Castuera' showed the lowest potential yield (about 68 kg/tree). Concerning fruit characteristics and oil content, the rootstocks had little influence on the scion so far. Thus, at present 'Arbosana' and 'Limoncillo' seem to be the preferred rootstocks to reduce the vigour of 'Arbequina-i•18' cultivar and also show higher productivity (kg/cm<sup>2</sup>) for this clone. These rootstocks are a potential choice to design a super-high density orchard.

### S08.038

#### From the Orchard to the Virgin Olive Oil Quality: a Critical Overview

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A new approach to the definition of virgin olive oil (VOO) quality should include instrumental parameters explaining its health and sensory properties. Nowadays in fact, the VOO marketable quality corresponding to analytical parameters indicates its alteration state and its genuineness while other criteria for defining VOO health and sensory qualities are not included. As regard the health aspect VOO fatty acids and polyphenols, are high biological value compounds whereas VOO sensory characteristics are determined by phenolic and volatile substances which can be considered impact components of the typical VOO sensory notes. Polyphenols affecting VOO shelf-life, health and sensory aspects, are phenolic alcohols, lignans and, in particular, secoiridoids which is exclusive phenolic class of the olive family. Volatile compounds generating the typical VOO flavour are C5 and C6 saturated and unsaturated aldehydes, alcohols and esters which have been already correlated to VOO "cut grass" and "floral" sensory notes. Concentrations of phenolic and volatile compounds in VOO, are strongly affected by the agronomic and technological conditions of production. Cultivar, fruit ripening stage and several agronomic practices such as irrigation, as well as crushing, malaxation and extraction system are the critical points which strongly influence phenolic and volatile composition with a corresponding significant impact on VOO sensory and health qualities. Several researches have already demonstrated that the final phenolic and volatile composition of VOO mainly depend on the oxidoreductases enzymatic activity. Indeed, polyphenoloxidases and peroxidases oxidize polyphenols while lipoxygenase pathway produces C6 and C5 aldehydes, alcohols and esters. Different crushers and time, temperature and oxygen concentration of pastes during malaxation can

influence these enzymes, thus, the regulation of their activities allows to strongly influence the final concentration of VOO phenolic and volatile substances. This review focuses on agronomic and technological practices for VOO production having as target the improvement of the VOO quality.

### S08.039

#### New Trends in the Extravirgin Olive Oil Extraction Technologies

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The olive-oil sector includes a number of operators that intervenes independently in the olive oil production: for this it results important today to define a new strategy of the sector where also the olive mill must have a well precise role. So the technological innovations become essential for a competitive olive-oil sector. The extraction technologies have changed considerably and the traditional extraction plants have been slowly replaced by the modern continuous system through the introduction of the centrifugation. Today the continuous system is a combination of machines coordinated into a rational, and technologically advanced line. The most diffused extraction technology in Italy is the "3 phases" system where the olive paste is diluted and centrifuged to separate oil, husk and waste water. This last is polluting and phytotoxic for which difficult to manage and to dispose. Environmental and economic demands brought, during the first 90th, to the introduction of a new centrifugal extraction system called "2 phases" where there isn't dilution of the olive paste in the decanter. Without water's addition to the process it is possible to obtain a greater quantity of oil with a best quality, less environmental effects for the absence of waste waters and lower water and energetic consumption. The actual technological innovations in the extraction of olive oil, with particular reference to the 2 phases system, are the evolution of a process dated 2.000 years; an evolution that born to answer to environmental, economic and qualitative necessities. The last innovation in the oil extraction from olives is represented by a centrifuge of last generation denominated DMF, projected and patented by Pieralisi. The DMF is still in phase of study but until today has given good results of production, of quality, of water and energetic saving and of exploitation of the secondary products.

### S08.040

#### Effect of Different Environmental Stresses on the Expression of Genes Involved in the Fatty Acid Composition and Aroma Biogenesis of Virgin Olive Oil

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High-quality virgin olive oil is today demanded by consumers not only because of its health benefits but also due to its excellent organoleptic properties, including aroma, color and flavor. We have initiated a program focused on the identification and characterization of gene/enzymes of olive fruit involved in the biosynthesis of the compounds responsible for these exceptional properties, principally fatty acids, volatiles and antioxidants, with the aim of improving olive oil quality using a molecular approach. In this way, we have previously isolated and characterized genes responsible for the linoleic acid content such as oleate desaturases (FAD2 and FAD6), and for the aroma biogenesis such as lipoxygenases (LOX1 and LOX2) and hydroperoxide lyase (HPL). In order to identify the environmental factors that can alter the composition and quality of virgin olive oil, the effect of different abiotic stresses on the expression levels of the above mentioned genes has been studied in olive fruit using qRT-PCR, to elucidate the molecular mechanisms responsible for their regulation. In particular, we have investigated the effect of water regime, low and high temperature, dark conditions and wounding in Picual and Arbequina varieties. The results obtained will allow the establishment of optimal culture conditions, and also the development of molecular markers for the selection of new varieties with enhanced resistance to those stresses and improved olive oil quality.

### S08.041

#### Virgin Olive Phenolic Profile as a Result of the Anabolic and Catabolic Enzymes Status in the Olive Fruit

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The demand for high quality virgin olive oil (VOO) may be attributed to its potential health benefits related to protection against cancer and cardiovascular diseases and also to its extraordinary organoleptic properties. It is well established that phenolic compounds have a direct influence on both factors, as antioxidants they provide important nutritional benefits, and additionally some of them are associated with the bitter and pungent sensory notes of the oil. VOO phenolic profile may be affected by multiple preharvest and postharvest factors. In this sense, though processing technology may severely affect VOO quality, it is clear that most quality attributes of the oil are determined by the chemical composition and biochemical status of the olive fruit. Thus, the content of the main hydrophilic phenols found in VOO is closely related to the activity of enzymes hydrolyzing the main phenolic glycosides initially present in the olive tissue and the pool of enzymes, associated to the oxidative catabolism, acting both on those glycosides and on the derived hydrophilic phenols. Manage of VOO phenolic profile requires a deeper knowledge on the anabolic and catabolic enzymes features in the olive fruit and the activity pattern of these enzymes during the industrial process to obtain this product.

### S08.042

#### Fatty Acids and Sterol Composition of 'Empeltre' Virgin Oil in Ebro Valley and Balearic Islands

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'Empeltre' is a native olive cultivar from Spanish NE. It grows in 79.000 ha in Castellon, LaRioja, Mallorca, Navarra, Tarragona and Teruel provinces. During four years (1998-2001) 69 olive trees were studied *in situ* to compare virgin oil characteristics for 'Empeltre' cultivar within its original growing area, analyzing the environmental effects on fruit and oil composition. Results show significant differences related to original area for fatty acid composition. Mean values for every area are reported. Significant area $\times$ year interaction was pointed out for fruit characteristics and sterols composition. Discriminant analysis show certain specificity for fatty acids, sterols and fruit characteristics, related to growing area. Cluster analysis show that Teruel, Castellon and Navarra have a similar fatty acid composition, while LaRioja, Mallorca and Tarragona belong to different clusters. Concerning sterols composition, Teruel and Castellon belong to the same cluster, Navarra and LaRioja belong to a second one, and Mallorca and Tarragona perform a third group. Canonical correlation analysis shows some interesting relations between fruit characteristics and fatty acids and sterols composition. In fact, larger fruits and those with a high pulp to stone ratio are usually richer in linoleic acid and poorer in oleic acid; in addition, fruits with a lower ripening index are usually richer in palmitic acid. Estigmasterol and total sterols seem higher when good growing conditions increase fruit size; by the other hand, fruits with higher water content are usually richer in campesterol, while small fruits with a low pulp to stone ratio could be richer in delta-7-stigmasterol. Finally, oils poorer in total sterols use to be richer in campesterol and monounsaturated fatty acids, while oils from green fruits are usually higher in palmitic acid and lower in delta-7-stigmasterol and delta-7-avenasterol.

### S08.043

#### Antioxidant Profile of VOO as Affected by Genotype and Fruit Ripening in Advanced Olive Selections

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Virgin Olive Oil (VOO) is the natural juice of olive fruits being the only vegetable oil, together with sesame oil, that can be consumed as it is —freshly pressed from the fruit. It conserves the taste, aroma, vitamins and nutraceutical properties of the olive fruit. These nutraceutical benefits result from its chemical composition containing a wide family of antioxidant compounds. The main antioxidants are carotenes and phenolic compounds, which can be lipophilic or hydrophilic phenols. Most hydrophilic phenols found in olive oil are not common to other oils or fats. Interest in hydrophilic phenols has increased in recent decades and has stimulated multidisciplinary research on the main aspects affecting the composition of olive biophenols. In this work, the influence of two relevant aspects such as genetic variability and maturation index on the phenolic profile of selected varieties of olive trees according to the Olive Breeding Program (Córdoba, Spain) is studied. Olives generated by different cultivars — namely, 'Arbequina', 'Picual' and 'Frantoio' and the corresponding crosses 'Arbequina' × 'Picual', 'Picual' × 'Arbequina' and 'Frantoio' × 'Picual' — were collected at five maturation stages from 1<sup>st</sup> October to 26<sup>th</sup> November 2009. Extra virgin olive oil was extracted with an Abencor system at 28 °C following the protocol recommended by the manufacturer. Then, characterization of the phenolic antioxidant profile was performed by liquid–liquid extraction with 60:40 (v/v) methanol–water and subsequent chromatographic analysis with diode array and fluorescence detection in a sequential configuration. Data treatment showed a dual effect on the phenolic antioxidant profile by combination of the maturation index and the genetic variability using the genitors as control.

### S08.044

#### Effect of Temperature and Storage Period of Olive Fruits on Oil Quality

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Black-ripe olive store conditions have direct effects on quality characters of olive oil. So in this experiment the effect of temperature and storage period of olive fruits on olive oil quality were investigated. Black-ripe olive fruits (*Olea europaea* cv. Zard) were harvested from Tarom olive orchards (Zanjan province) and stored in 5 °C, 8 °C and 20 °C with 90-95% RH for 5, 10, 15, 20, 25 and 30 days. This investigation was conducted as a factorial experiment in a complete randomized block design with three replications. Some quality characters of stored fruit and extracted oil respectively (firmness, weight loss, chilling injury and fungal development) and (acidity, peroxide value, total polyphenol content and sensorial quality) were evaluated. The results of analysis of variance indicated that stored olives at 5 °C and their oil had high quality after 30 day, but 8 °C protected high quality until 20 days. Stored olives at room temperature after 5 days were decayed and unsuitable for oil extraction.

### S08.045

#### Effects of Geographical Origin and Variety on Phytosterol and Tocopherol Components in Olive (*Olea europaea* L.) Varieties Cultivated in Turkey

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Phytosterols and tocopherols play significant roles on human health. Olive oil has been receiving special attention from consumers due to health beneficial effects. The aims of this research were to investigate the effects of geographical origin and variety on phytosterol and tocopherol components of twelve different varieties of olive cultivated in Turkey. The data were collected from North Aegean, South Aegean, Marmara, East Mediterranean and Southeast Anatolia regions of Turkey in 2004-2005 and 2005-2006 growing seasons. The separation, identification and quantification of free phytosterols and tocopherols were successfully achieved using the capillary

column gas chromatographic (GC) and high performance liquid chromatographic (HPLC) methods, respectively. The analytical results showed significant differences among surveyed olive varieties. According to the obtained results, the total phytosterol content ranged between 0.91-4.64 g/kg in olive varieties. Sitosterol was the major phytosterol with high contribution to total phytosterol content followed by avenasterol in olive oil. Sitosterol content changed between 0.85-3.82 g/kg, followed by avenasterol ranged from 0.06 to 0.81 g/kg. Regarding for tocopherols, the total tocopherol content ranged between 0.04-0.347 g/kg in olive oils. Although  $\alpha$ -tocopherol was the most abundant tocopherol fraction in olive varieties, there was also  $\gamma$ -tocopherol in some varieties.  $\alpha$ -tocopherol content changed between 0.03-0.275 g/kg followed by  $\gamma$ -tocopherol ranged from 0.00 to 0.06 g/kg. Geographical region differences significantly affected the phytosterol and tocopherol contents. The total phytosterol and tocopherol contents of North Aegean region samples were higher than the other regions. This study revealed that an important variability exist for phytosterol and tocopherol contents in olive varieties and geographical origin affected the amounts and components of the both organic compounds.

### S08.046

#### Influence of Genetic Matrix on Chemical and Sensory Profiles of Italian Monovarietal Olive Oils

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Commercial virgin olive oils belonging to some cultivars most represented at the Italian National Review of Monovarietal olive oils were considered. The nutritional properties, expressed as fatty acids and total phenols, and the sensory profiles were determined. The evaluation of the influence of the cultivar on oil composition was also statistically analyzed by a complete factorial design and by Principal Components Analysis. In phenolic content as well as in fatty acids composition, the effect of the cultivar was highly significant. Considering organoleptic analysis, the statistical analysis showed that the sensory attributes: olive fruity, grassy, fresh almonds, artichoke, tomato, aromatic herbs, bitter and pungent were strongly influenced by the cultivar. The construction of a data-bank based on a large number of samples, which is available at URL <http://www.olimonovarietali.it>, has contributed to the reductions of the variable effects involved in the oil production process.

### S08.047

#### Authentication of the Varietal Origin of Olive Oil Using PCR-RFLP and Single Nucleotide Primer Extension

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The beneficial effects of olive oil in human health and its significance in the Mediterranean diet, along with the price differential with other plant oils, makes it vulnerable to fraud. Therefore, authenticity of olive oil is of great commercial and nutritional importance. Some types of adulteration of olive oil involve the mixing of extra virgin grade with lower grades or/and other vegetable oils. However, there is also a need for authenticating the varietal origin of olive oil due to relevant European regulations on Protected Designation of Origin (P.D.O.) products. The development of cost-efficient, DNA-based analytical assays to detect varietal adulteration of olive oil will provide to the olive industry the means to protect the misdescription of their products. Towards this direction, a SNP database of Greek olive oil varieties was developed. The ten major Greek olive varieties have been screened through sequencing for SNP discovery. After sequencing alignment and further analysis of their nucleotide sequences, a significant number of SNPs have been identified. Moreover, extra virgin olive oils from the above varieties were used to extract DNA, which was successfully subjected to PCR amplification, single nucleotide primer extension and PCR-RFLP analysis. The results of this study indicate the usefulness of SNPs as molecular markers towards the varietal authentication of olive oil.

### S08.048

## Identification of the Appropriate Harvest Time for 'Arbequina' and 'Changlot Real' Olive (*Olea europaea* L.) in the Province of San Juan, Argentina

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Arbequina occupies the largest area planted with olive tree and Changlot Real arises like a good alternative in San Juan. To plan the logistics of collection it is important to identify the appropriate harvest time which is when the fruits has highest oil content and commercial quality within the purity criteria of IOC. To determine the appropriate harvest times for 'Arbequina' and 'Changlot Real' to obtain Extra Virgen oil in this region using the Maturity Index (MI) as indicator, samples of olives were taken on 5 different dates, every 20 days from pit hardening, from three replicate plots of 10 trees each. MI, oil content on dry matter, acidity, peroxide index, composition of fatty acids, oxidative stability and total polyphenols levels were determined. The relations between the observed variables were obtained by means of linear correlation of Pearson. For the cv 'Arbequina' the maximum oil content (43.63%) was obtained in the second fortnight of April with MI 4.03 and adjusting to the majority of the fatty acid composition from IOC purity criteria, except the Oleic acid (54%). However, in the second fortnight of March the value of Oleic acid was over 58%, with MI 2.28 and 33.89% oil content on dry matter. For the cv. 'Changlot Real' the appropriate harvest time was in the last days of April with MI 3.49, this moment coincide with the maximum oil content (48.99%) and comply with IOC rules. It was observed high correlations for the two varieties between the dates of sampling and the MI (0.94 Arbequina, 0.96 Changlot Real) and, between the MI and the maximum oil content (0.96 Arbequina, 0.83 Changlot Real), which notices the usefulness of these tools for the identification of the opportune moment of harvest.

### S08.200

## Effect of the Nitrogen, Transplanting Time and Growing Medium on Photosynthesis, Growth and Macronutrients Concentration of Saplings of Two Olive Cultivars

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The effects of N supply, growing medium and transplanting time on the photosynthesis, growth and macronutrients level of two olive (*Olea europaea* L.) cultivars ('Arbequina' and 'Roughani') were studied. In two propagation time (spring and autumn), the rooted plants were grown in plastic pots containing perlite : sand (1:1) or soil : sand : organic matter (1:1:1) mixtures and were irrigated with different amounts and forms of nitrogen for 9 month. The result of this study indicated that olive could use both the nitrate and ammonium forms of N. However, high level of Nitrogen, especially ammonium form, is deleterious to the photosynthesis and growth of olive nursery plants, therefore, depended on olive cultivars, addition of small amounts of  $\text{NH}_4$  to  $\text{NO}_3$  solution (12 meq/lit), increases of the photosynthesis and growth (4,6 meq/lit  $\text{NH}_4$ ) for 'Arbequina' and 'Roughani', respectively. Addition of  $\text{NH}_4$  to nutrient solutions influences not only the rate of growth but also the absorption of other ions. Absorption of  $\text{NH}_4$  by plants, as compared to  $\text{NO}_3$ , decreases K and increases N and P uptake. The mean effect of the cultivar on the organs concentration of nutrients was significant and high N & P and high K use efficiency was seeing in 'Arbequina' and 'Roughani' cultivars, respectively. On the other hand, olive growth and photosynthesis affected by growing media and transplanting time, so that, the soil media and springtime was the best for these purpose.

### S08.201

## The Measurement of Nitrate Reductase Activity in Olive (*Olea europea*) Zard and Koroneiki Cultivars

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In the all pathways of nitrate metabolism, nitrate reductase is the key enzyme that catalyzes nitrate to nitrite. In this experiment, two cultivars of olive, (zard and koroneiki) were used to measure variation on nitrate reductase activity (NRA) of plant organs. In one year olive grown in perlite : sand (1:1) and soil : sand : organic matter (1:1:1), the effect of nitrogen forms and levels, cultivar differences and sampling time, on nitrate reductase activity (NRA), were studied. The result of this study indicated that increasing nitrate supply, increased NRA of all organs, significantly. NRA appeared to be different among the tested cultivars. The highest level of activity of the enzyme was found in Zard cultivar. Irrespective of cultivar, results showed that NRA in the leaf was significantly highest, compared with that in other organs such as roots and stems. Significant seasonal variation in nitrate reductase activity was observed too. So that, NRA was high during growth period and declined during warm months. Leaf NRA was the highest when the plants received both forms of nitrogen. But increasing proportion of ammonium to more than 4 meq/litre reduced the NRA in the leaves and nitrate concentration in the roots. This decreasing effect was low in perlite : sand medium.

### S08.202

## Assessment of the Frost Tolerance in Olive Cultivars Using Visual Method and Chlorophyll Fluorescence

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Application of olive (*Olea europaea*) tree due to salt and drought tolerance and ever greening, increasingly is considered in landscape in recent years. The low resistance to cold has been a major problem in using this tree in temperate and cold zones. So, determination of frost resistance cultivars is one of the important measures for use of this tree in urban landscape. To evaluate frost resistance of 15 olive cultivars and to compare the visual assessment and chlorophyll fluorescence methods, a factorial experiment was carried out using 7- year- old trees which were planted in randomized complete block design with 3 replications. In visual method, frost damage of winters 2007, 2008 to plants were measured. Results showed that 'Amphis' was hardy and 'Kroneiki' and 'Rashid' were sensitive cultivars to low temperatures. In method of chlorophyll fluorescence, leave samples of each cultivar was gradually incubated in 0, -5, -10, -15 and -20 °C for one hour at least. Then Fv/Fm value of each sample was measured with fluorescence spectrometer. Results indicated that 0 and -5 °C had no damage on samples and all of the cultivars tolerated these temperatures. When temperature reduced to -10 and -15 °C the stress on plants was increased and 'Rashid' showed the lowest Fv/Fm and was the most frost sensitive. Decreasing temperature to -20 °C had no further significant effect to decrease Fv/Fm index and showed no difference between cultivars. Based on this method, 'Shengeh', 'Gorgan' and 'Amphis' were hardy cultivars and 'Rashid', 'Spain', 'Manzanilla' and 'Kroneiki' were sensitive cultivars to low temperatures which confirmed the results of visual assessment.

### S08.203

## Effect of Soil Solarization on Growth and Length of the Juvenile Period in Olive Seedlings

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The effect of soil solarization on growth and on the length of the juvenile period (JP) of olive seedlings has been studied. A Split plot with and without solarization on the main plots and crosses in the subplots was designed. Solarization treatment consisted on covering the soil of the trees by a black plastic film. Seedlings obtained from free pollination of 'Manzanilla de Sevilla' and from crosses between 'Arbequina' × 'Arbosana' and 'Picual' × 'Koroneiki' were tested within each main plot. 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 respect to not solarized. Preliminary data showed that solarization may advance flowering thus shorten the JP.

### S08.204

#### Seasonal Changes in Mineral Nutrients of Olive Leaves in an Arid Region of Argentina (San Juan): Nitrogen, Phosphorus, and Potassium

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The objective of this study was to determine the time of year when nitrogen (N), phosphorus (P), and potassium (K) are most stable in leaves from current season shoots in var. 'Arbequina' olive trees in San Juan, Argentina (31°58'06" S – 69°35'37" O). The concentration of foliar N, P and K was evaluated monthly over 4 years, ON 8-year-old trees in a commercial orchard with a density of 357 trees per hectare. Samples were taken from three replicate plots of 10 trees each. Leaf nitrogen concentration was highest in March (late summer in the Southern Hemisphere) and minimum values were found in the spring months of September, October, and November. For P, maximum and minimum concentrations were found in August-September (late winter) and December-January (early summer), respectively. Lastly, the minimum concentrations of K were detected in October (spring) similar to nitrogen, while maximum values occurred in November. Statistical analyses indicated minimum values of standard deviation and of the coefficient of variation for both N and K in the spring (October) and for P during the winter. In that N and K are the nutrients most likely to be deficient in this region, the period prior to flowering in the spring appears to be the most appropriate for foliar nutrition sampling given the stability of these nutrients at that time of year.

### S08.205

#### Benefits of the Mixed Ammonium / Nitrate Nutrition in Olive Trees: Use of 3,4 Dimethylpirazole Phosphate Plant Nutrition Products

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More than 12% of Spain surface has been declared vulnerable area to ground-water nitrate pollution according to the 91/676/CEE legislation. Fertigation and the control of N chemical cycle in the soil with the nitrification inhibitors are complementary tools that can reduce this problem. This paper present three trials performed in fertigated olive trees that compares the use of nitrogen conventional fertilisers with fertilisers that includes the nitrification inhibitor 3,4 dimethylpirazole phosphate (NOVATEC®). Trial 1 is performed by the Polytechnic University of Valencia (Responsible: Dr.Salazar). Trial two is performed in an intensive orchard by the University of La Rioja (Dr. Peña). Trial 3 is performed in greenhouse conditions by the Cordoba University (Dr. Fernandez Escobar). In trials 1 and 2 the use of ammonium fertilisers + nitrification inhibitor (NOVATEC®) increases the growth of the lateral branches and the number of buds (an average of 29-33%). This result probably is related with the energy saving associated with ammonium nutrition and with the effects over the phytohormones equilibrium. The final yield is improved with this technology by an increase of the total yield per ha and by a higher oil content of the olives (and average of 5-8%). Results of trial 3 shows that with a conventional fertilization the global N-losses were a 48% of the N applied, and with the inclusion of the DMPP (NOVATEC®) losses were reduced to 32%. Nitrogen extractions by the trees were similar between treatments, but the inclusion of the nitrification inhibitor produce a significant increase of N retention in the soil, that finally improves the efficiency of the nitrogen fertilization. Conclusions shows that the olive crop has a positive response to the increase of the N-ammonium nutrition obtained with this technology, in terms of growth and yields and in the reduction of N-pollution.

### S08.206

#### Effect of Zinc Sulfate and Potassium Nitrate on Fruit Growth and Oil Yield of Olive (*Olea europea* L.) cv. *Amygdalifolia*

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This research was carried out in a factorial experiment 4×4 in a randomized complete block design with 4 replications on 11-year-old olive trees cv. 'Amygdalifolia' at Kazerun Olive Research Station during 2007 and 2008 years. Treatments were 4 levels of zinc sulfate (0, 0.25, 0.5 and 0.75%) and 4 levels of potassium nitrate (0, 0.5, 1 and 1.5%) which were applied as foliar spray in the end of pit hardening and in the beginning of fruit coloring stages. The results showed that 0.5% zinc sulfate in combination with 0.5% potassium nitrate produced the highest fruit and flesh weight (9.53 and 8.07g respectively) which were significantly higher than control (6.87 and 1.57 g respectively). The greatest rate of fruit oil was gained by using of 0.5% zinc sulfate (50.75%) which was significantly higher than control (35.78%). In relation to polyphenols (natural antioxidants) which protect the oil from oxidation during storage, 0.5% zinc sulfate and 1.5% potassium nitrate provided the highest amount (1.83 mg/kg) which was significantly higher than 1% KNO<sub>3</sub> and 0.75% ZnSO<sub>4</sub> (1.12 mg/kg fruit).

### S08.207

#### Effects of Fertilization with Phosphorus on Iron Chlorosis

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Iron (Fe) deficiency chlorosis is an important nutritional problem in sensitive plant species cultivated in calcareous soils, its main symptoms being lack of chlorophyll in the younger leaves and reduced growth. Iron chlorosis has been related to the content and reactivity of carbonates and iron oxides in soil. The effect of other nutrients, especially phosphorus (P), is, however, a matter of debate. In this work we examined whether fertilization with P affected the availability of Fe to sensitive plants growing in two different Fe chlorosis-inducing calcareous soils. Phosphate at rates of 0, 25, 50, 100 and 200 µg P/g soil was applied to pots in which where six-months-old olive trees cv. Arbequina were grown. Chlorophyll concentration in the young leaves was estimated from the SPAD value recorded with a Minolta apparatus. In the period of maximum growth—June and July—, the SPAD value in the leaves of the control trees was higher than in those of the trees fertilized with P; this effect, however, was not observed in September and October.

### S08.208

#### Effectiveness of Iron Salts to Correct Iron Chlorosis in Olive Trees

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Iron (Fe) deficiency chlorosis is a common problem in olive trees (*Olea europaea* L.) growing in calcareous soils, its typical symptom being the interveinal yellowing of the youngest leaves. In Mediterranean countries, plants affected by iron chlorosis normally require fertilization with Fe; otherwise vigour and yield are reduced. The objective of this work was to determine the effectiveness and persistence of synthetic siderite (FeCO<sub>3</sub>) and Fe sulphate injected in the form of suspension/solution into the soil for preventing iron chlorosis in olive trees in southern Spain. Experiments were established in 2009 in orchards with cv. Ocal (14 years old), cv. Arbequina (3 years old), and cv. Manzanilla (3 years old) in a randomized block design with three treatments ("control" with no Fe fertilizer, "siderite", and "Fe sulphate"). In each or-

chard, the rate of Fe was the same for the “siderite” “Fe sulphate” treatments (0.17 kg/tree in the 3-years-old trees and 0.45 kg/tree in the 14-years-old trees). The suspension of siderite was prepared in the field by dissolving  $K_2CO_3$  and  $FeSO_4 \cdot 7H_2O$  in a tank with water and injected into the soil at 10–20 points around the tree at the depth of maximum root density (25–35 cm). The Fe sulphate dissolution was injected into the soil in the same way as siderite. The leaf chlorophyll concentration was estimated with a Minolta apparatus (SPAD units). Differences in the SPAD value of young leaves between fertilized and control trees were significant during the active growth period. However, no significant differences in SPAD value were observed between the trees fertilized with siderite and Fe sulphate.

### S08.209

#### Effect of Leaf-to-Fruit Ratio and Girdling on Gas Exchanges, Fruit Growth and Carbohydrate Contents at Different Stages of Fruit-Development of *Olea europaea* L. cv. Picholine

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The effects of different source-sink ratios on fruit quality were studied for the table olive cultivar ‘Picholine’. This cultivar represents 15% of the production of table olives in Tunisia. Fruit sizes are an important quality parameter for table olives as small fruits have a lower economical value. Three tertiary branches/tree were selected on 27-years-old olive trees (10 trees in total) at the start of the growing season 2009. The experimental plot was located in Enfidha, Tunisia (36°08’N, 10°22’ E, 23m). After fruit set early May for each branch four fruit loads were imposed on current season shoots (no fruit load, 1 fruit:1 leaf, 1 fruit:2 leaves and 1 fruit:3 leaves), also apexes of all shoots were removed in order to reduce the vegetative sinks. On the 13<sup>th</sup> of July five trees were randomly selected and all branches were girdled. For three dates (mid-July, mid-August and beginning of October) gas exchanges, carbohydrate contents and fruit characteristics were measured. Net photosynthesis was reduced if no fruit load was present on the shoots, girdling also decreased net photosynthesis. Absence of fruit load was associated with a higher carbohydrate content in the leaves. Girdling also enhanced the carbohydrate content of the leaves. Girdling promoted both the diameter and the length of the olive fruits, while the effect of leaf-to-fruit ratio was less pronounced. Olive dry weight however was strongly influenced by both girdling and leaf-to-fruit ratio.

### S08.210

#### Possible Role of Mannitol as an Oxygen Radical Scavenger in Olive

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Along with mannose and sucrose, olive produces large amounts of mannitol in photosynthesizing leaves. Leaf mannitol shows large variation among olive genotypes and Sicilian cultivars with different leaf mannitol content were used in our trials to see whether mannitol may function as an additional non-enzymatic system to protect cells from oxygen radicals and photosystem damage. In one experiment, entire shoots of ‘Passulunara’, ‘Castriciana’, ‘Nocellara del Belice’, and ‘Moresca’ olive trees were taken to a greenhouse, and mature leaves were treated with paraquat solution, which generates oxygen radicals in presence of light. After 48 hours leaves were sampled, photographed and percent of necrosis was quantified by digital image analysis. Mannitol content was determined in control non-treated leaves positioned in the same node as the paraquat-treated ones. The same experiment was repeated using field-grown trees under full sun light. In both cases, a significant inverse relation was found between leaf necrosis and mannitol content suggesting

that mannitol may provide some scavenging action on paraquat-generated oxygen radicals. In another experiment, leaf gas exchange and chlorophyll fluorescence were measured on field-grown ‘Castriciana’ (higher mannitol) and ‘Nocellara del Belice’ (lower mannitol) trees in the morning, at noon, and in the afternoon to quantify partitioning of absorbed energy among net photosynthesis (JCO<sub>2</sub>), photoprotection by heat dissipation (non-photochemical quenching, JNPQ) and by alternative electron transport and photorespiration (JNC), formation of reactive oxygen species (ROS) and heat re-emission inactive photosystem (PSII). ‘Nocellara’ reported higher JCO<sub>2</sub> and JNC than ‘Castriciana’, whereas JNPQ was similar in the two cultivars. Despite a greater amount of energy for ROS formation, ‘Castriciana’ yielded a smaller percentage of inactive photosystems compared to ‘Nocellara’. Also in this case mannitol may act as an additional oxygen radical scavenger and explain the differences in photosystem inactivation between the two olive cultivars.

### S08.211

#### Vigor Control in Olive Hedgerow Orchard Trough Deficit Irrigation Strategies

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In recent years, a new olive-growing system, called “hedgerow” and based on a high density of planting, has gained great interest. Because the total mechanization of cultivation and the rapid entry into production, this system has spread rapidly in many olive growing countries. However, current varieties are so vigorous for this system, forcing severe pruning. This paper presents preliminary results of the first year of work in a hedge planting olive orchard of “Arbequina” variety where is evaluated the control of vigour and the maintenance of productivity through deficit irrigation strategies. The orchard is located in Las Vegas del Guadiana (Badajoz, Spain) at the Finca la Orden in the second year of planting. Deficit irrigation treatments were established according to levels of stem water potential and differentiating stages of the crop. Phase I (from budding to pit hardening) were marked levels of -1.0, -1.4 and -2.0 MPa for T1, T2 and T3 respectively, phase II (from pit hardening to veraison) of -1.4, -2.0 and -3.0 MPa; while in stage III (veraison to harvest) were -1.2, -1.6 and -1.6 MPa. Preliminary results indicate that after the first year of testing, deficit irrigation strategies managed to save water and effectively reduce the vigour of trees, more intense when the deficit was more severe implemented. However severe deficits directly affect production, especially in the initial and final stage of the crop and directly influences the number of fruits and its final size.

### S08.212

#### Organization and Mobilization of Storage Components in the Olive Seed

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Endosperm and cotyledon constitute seed storage tissues. Proteins and lipids are their main storage components in the olive. The most abundant proteins in the mature olive seeds belong to the 11S protein family of seed storage proteins (SSPs). Because of this protein content, a potential use of olive seeds for animal feeding has been proposed. Additionally, the abundant presence of storage lipids makes olive seeds putative raw material for manufacturing oil which can be used by food, pharmacological and cosmetic industries. In spite of this interest, no studies devoted to analyze olive seed components and tissue ultrastructure. We have studied the structural organization and the developmental processes underwent by the olive cotyledon cells during *in vitro* germination and seedling growth. At the macroscopic level, the mature olive seed consisted of a brown seed coat and a relatively thick layer of white endosperm surrounding the embryo. In the mature embryo, two cotyledons and a radicle were distinguishable. Light and transmission electron mi-

crosscopy analysis of endosperm and cotyledon showed the presence of large dense proteins bodies (PBs), surrounded by numerous oil bodies (OBs) filling up the cytoplasm. Water intake by the seed during imbibition produced PBs swelling. The most noteworthy feature after 3 days of *in vitro* germination was the presence of large PBs originated by fusion of smaller PBs. Close spatial proximity between PBs and OBs was also observed. Between 3-12 days germination, PBs experimented important changes leading to the formation of a large vacuolar compartment and a decrease in the number of PBs and OBs. These changes were accompanied by plastids biogenesis. After 26 days germination, the cellular organization became typical for a mesoderm leaf cell, with well differentiated chloroplasts surrounding a large central vacuole. All these cellular changes are the result of a gradual mobilization of SSPs and lipids.

### S08.213

#### Assessment of the Allergenic Content of Olive Pollen from Portuguese Cultivars

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Olive pollen contains allergenic proteins able to elicit respiratory allergy. Up to date, 10 allergens have been characterized in this material, referred as Ole e 1 to Ole e 10. The presence of variability in their level of expression and in the number and molecular characteristics of the expressed forms has been reported. These differences are intrinsic to the genetics of each cultivar and have been analyzed in a number of Spanish cultivars. In the present study, we identify and quantify the presence of four allergens (Ole e 1, Ole e 2, Ole e 5 and Ole e 9) in the pollen of twelve Portuguese olive cultivars. Ole e 1 allergen is considered the olive pollen major allergen, playing an important role in olive pollen hydration and germination. Ole e 2 is a pollen profilin which binds to actin and is also involved in signal transduction. Ole e 5 is a protein exercising Cu, Zn superoxide dismutase activity, whereas Ole e 9 corresponds to a 1,3 beta-glucanase. In order to determine the level of expression of these proteins, we carried out SDS-PAGE by using pollen protein extracts. Protein profiles were analyzed by Coomassie staining. Equivalent gels were transferred to membranes and probed with antibodies to the four allergens studied. Different isoforms of these allergens were detected, with differential distribution in the pollen cultivars, both in number and in the level of expression. Immunoblots were also probed with sera from allergic patients. The reported differences are likely involved not only in the allergenic properties of this pollen, but also in the physiology of the olive reproductive system, including pollen dynamics, ability to adapt to different conditions, capacity to fertilize etc.

### S08.214

#### A Histological Evaluation of Adventitious Root Formation in Olive (*Olea europaea* L. cv. Galega Vulgar) Microshoots Cultured *in vitro*

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The main Portuguese olive cultivar, 'Galega vulgar', presents a recalcitrant behaviour regarding its propagation by semi-hardwood cuttings, with average rooting rates between 5-20%. The *in vitro* culture techniques recently allowed increasing these rooting rates to 60-75% creating for the first time conditions to perform anatomical studies on adventitious root origin on this olive cultivar. After a 15s quick-deep treatment in a 14.700µM IBA sterile solution, microshoots of 'Galega vulgar' were *in vitro* rooted using semi-solid OM culture media devoid of growth

regulators. Samples from the basal portion of the *in vitro* cultured microshoots were taken at established times. The samples were fixed in FAA, impregnated in liquefied paraffin and, after being sectioned in a rotary microtome, stained with Safranin O + Aniline Blue and observed in an optical microscope. After 5-7 days in culture, some cells from the cortex and also from the sub-epidermal tissue revealed a dense cytoplasm and presented high mitosis rates. The first morphogenetic root fields were observed in the former tissues and also in the parenchymatic calli after 12-16 days in culture. Root primordial, already presenting a connection to the plant vascular system, became visible at epidermal surface after 20 days. Parenchymatic calli vascularisation as revealed by the presence of xylem traqueids was also observed. No root morphogenesis arising from the cambial region was reported contrasting to what is common in stem cuttings from easy to root plant species.

### S08.215

#### Interaction of Crop Load and Water Status on Growth of Olive Fruit Tissues and Mesocarp Cells

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Field-grown olive trees (*Olea europaea* L. cv. Leccino) were hand-thinned at 5-6 or 2-3 weeks after full bloom (AFB) over two consecutive growing seasons to establish heavy or low crop loads, and then were subjected to three irrigation regimes: a) FI, fully-irrigated with pre-dawn leaf water potential (LPWP) greater than -1.1 MPa; b) DI, deficit irrigated (LPWP between -1 and -3.3 MPa); c) SI, water stressed (LPWP higher than -4.2 MPa). Fruits were sampled at pit hardening and harvest, the mesocarp separated from the endocarp and their fresh weight, dry weight determined. Additional fruits were fixed in FAE for anatomical studies, their mesocarp and endocarp area measured on transverse slices with an image analysis system connected to a stereo microscope. Mesocarp cell size and number were then determined by microscope after standard paraffin processing. The effect of crop load on growth of fruit tissues depended on the irrigation regime. Within the FI or DI treatments the mesocarp dry weight was higher at low than at high crop load. In the SI treatment, mesocarp dry and fresh weights were 180 and 200% respectively those for trees bearing high crop loads. The pulp-pit ratio was decreased by high cropping for both DI and SI treatments, but was unaffected for the fully-irrigated trees. High crop load significantly decreased fruit oil content (% dw) of FI trees. Comparing trees with high crop loads, DI showed higher fruit oil content than FI. Overall, there was a notable interaction of crop load and water status on fruit growth processes at multiple levels, including the absolute and relative growth of pulp and pit, mesocarp cell division and enlargement, and oil accumulation.

### S08.216

#### Vegetative Growth and Ecophysiological Aspects in Young Olive Plants Inoculated with Olive Leaf Yellowing Associated Virus (OLYaV)

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An investigation started some years ago in the Sicilian olive culture, to ascertain the sanitary status of trees, revealed that large part of the plants were affected by Olive Leaf Yellowing associated Virus (OLYaV), although the symptoms of the above mentioned diseases were not exhibit. In order to organize and manage an olive nursery under the European rules (Conformitas Agricola Communitatis) it is important to ascertain if an asymptomatic diseases can affects morphological and eco-physiological traits of young, potted, olive trees. Researches were carried out using two years old virus free plants of cv. Frantoio grafted on seedling, grown in pots (32 l) and compared with the same plants previously inoculated with OLYaV by chip budding. Data on vegetative growth, within tree dry matter partitioning, gas exchange and stem water potential at the end of the second year were collected in both, virus free and virus affected trees. Data related to trunk diameter, shoots

number and length, leaf area, resulted significantly higher ( $P \leq 0,05$ ) on virus free plants. An higher amount of dry mater was diverted in the roots and the stem, respectively for OLYaV infected and virus free trees. This different behaviour in dry matter partitioning affected the canopy/root ratio, higher in healthy plants. OLYaV did not affect gas exchange and stem water potential.

### 508.217

#### Olive Growth and Photosynthesis under Drought and Application Efficacy of Alleviating Products with Different Mode of Action

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Two years old self-rooted Chondrolia Chalkidikis olive trees (*Olea europaea* L.) were subjected to two irrigation regimes, i.e. the fully irrigated and the severely water stressed trees, previously treated with three alleviating products of different mode of action. The alleviating products were the osmolyte glycine betaine, the antioxidant Ambiol and the heat and irradiance reflecting kaolin clay particles. The effects of product application and water regime on shoot growth and trunk expansion, photosynthesis and leaf carbohydrates concentration were evaluated. Irrigated trees presented greater trunk expansion than drought stressed ones, while carbon assimilation rate, stomatal conductance and intrinsic water use efficiency were significantly reduced under drought stress. The opposite stood for intercellular CO<sub>2</sub> which was greatly increased under drought stress conditions. Drought stress resulted in elevated mannitol leaf concentration, while the application of kaolin clay particles resulted in sucrose concentration increase. Among the alleviating products tested in this experiment kaolin clay particles and glycine betaine had a slight positive effect on photosynthesis under drought stress conditions.

### 508.218

#### The Effect of IBA, NAA and Carbohydrates on Rooting Capacity of Leaf Cuttings in Three Olive Cultivars (*Olea europea* L.)

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Rooting experiments were performed in two seasons in 2008 (summer and autumn). Twenty thousand cuttings of three olive cultivars ('Arbequina', 'Kalamon' and 'Mastoidis') were used for each experimental season. Cuttings were treated with IBA and NAA at four various concentrations each (500, 1000, 2000 and 4000 ppm) and the combination of these two hormones at three concentrations (500, 1000 and 2000 ppm). Two different rooting media (one organic and another a mixture of peat:perlite, 1:1) were also used. Starch and sugar analyses of the cuttings were performed at 0, 3 and 7 days after they were put under mist propagation. The results showed that all cuttings of the three cultivars rooted better in the organic substrate. 'Arbequina' gave the highest percentage of rooted cuttings (up to 96%), followed by 'Mastoidis' (up to 75%) and 'Kalamon' (2-5%). Hormone treatments affected the rooting ability of cultivars differently. Thus, 'Arbequina' gave the highest rooting percentage with IBA (2000 ppm) in summer and IBA+NAA (1000 ppm) in autumn. The treatment of NAA (1000 ppm) gave the best results for both seasons in 'Mastoidis' cv. while IBA (500 ppm) treatment gave the highest rooting percentage in 'Kalamon' (5% in summer). The main sugars found in all cuttings were glucose, sucrose, stachyose, raffinose, fructose and the sugar-alcohol mannitol. Initially, 'Arbequina' had higher stachyose, mannitol and total soluble sugar concentrations than the other cultivars, while 'Kalamon' had the highest starch concentration. The pattern of changes in sugar and starch concentrations at 3 and 7 days after planting was different for each cultivar. However, the individual and total sugar concentrations continuously decreased in 'Arbequina', while those of 'Kalamon' decreased at three days but then remain unchanged or increased. Initial sugar and starch concentrations and sugar metabolism might be important during the early period of the rooting process.

### 508.219

#### Photosynthesis and Vegetative-Productive Activities of the Olive Cultivars Arbequina, Leccino and Maurino in a Highly Intensive Olive Grove in Central Italy

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The trial was carried out in a three-year-old highly intensive olive grove in central Italy to compare the photosynthesis and vegetative and productive activities of the Spanish cultivar Arbequina, currently considered to be the best cultivar for this type of productive system and of the Leccino and Maurino cultivars, typical of central Italy. Arbequina had low vigor even though its crown volume was not significantly less than that of Maurino, which was taller and had a more compact crown than Arbequina. The total leaf surface area of Maurino was less than that of Arbequina and Leccino. Arbequina appears to put more resources into the formation of leaf surface area rather than into skeleton. Leccino had high vigor. The productivity of Maurino was greater than that of Arbequina and similar to that of Leccino. The productive efficiency, calculated as the quantity of product per unit of leaf surface area or trunk section area, was higher in Maurino than in the other two cultivars. The greater productive efficiency of Maurino could be the result of greater net photosynthesis per unit of leaf surface area and better distribution of light in the crown, due to less empty space in the crown compared to Arbequina and even less in comparison to Leccino, but, above all, to the orientation of the leaves that tend to be more vertical. Although Maurino has a LAI that is greater than that of Leccino, it has better illumination within the crown that is attributable, only in part, to the smaller crown size. The satisfactory response of Maurino to the wall-training system will be of great interest if these initial findings are confirmed in the next years. The identification of some regional cultivars that can adapt to highly intensive planting will reduce the risk of the standardization of oil.

### 508.220

#### Water Requirement of Young Olive (*Olea europaea* L.) Plants under the Arid Environment of Kuwait

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In Kuwait, plants are frequently exposed to high temperatures, low relative humidity and drought. As water resources available for agriculture are limited, an efficient irrigation strategy is vital for sustainable olive production. In view of these facts, an irrigation study comprising five cultivars (Arbequina, Barnea, Coratina, Koroneiki and UC13A6) and three levels of irrigation (50, 75 or 100% of ET<sub>c</sub>) was conducted using brackish water with 5.0 dS. m<sup>-1</sup> EC<sub>e</sub>. All five cultivars showed excellent adaptation to harsh weather conditions of Kuwait and brackish water irrigation. Vegetative growth in these cultivars was not significantly affected when the quantity of the irrigation water was reduced to 50% of ET<sub>c</sub>, indicating that these cultivars were able to tolerate severe and prolonged drought conditions. However, cultivar differences in respect of adaptation to harsh weather were significant at  $p \leq 0.01$ . Overall, cultivars Barnea and Arbequina exhibited better adaptability than other cultivars to harsh environmental conditions of Kuwait and produced growth rates in excess of 75% during the initial 24 mo. after planting even when the quantity of irrigation water was reduced to 50% of actual ET<sub>c</sub>. UC13A6 was affected the most by the harsh environmental conditions and reduction in the quantity of water applied.

### 508.221

#### Influence of Potassium Fertigation in Olive Production and Fruit Ripening in a cv. Manzanilla Table Olive Orchard in Andalusia, Spain

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The response of an olive grove var. 'Manzanilla de Sevilla' to the application of po-



tassium fertilizers by fertigation has been studied during four years (2.006-2.009). The experiment has been conducted in a table olive orchard located on the farm “El Hecho” (Córdoba, Spain). The field trial is situated in an adult, intensive olive orchard (7x7 m tree spacing) in a sandy loam soil with very shallow and with low exchangeable potassium content. Three doses of potassium applied in fertigation (K / 2, K, 2K) compared to a control treatment (0K) have been tested. Annual amounts of Potassium for the olive trees (K) have been calculated according to the schedule proposed by Pastor *et al.* (2005). Olive production, oil yield, sizes and maturity index of the fruits have been controlled. All fertigation K treatments show a tendency to increase the olives production regarding treatment 0K, being this difference significant between 2K and 0K. In addition the fruit size in the K treatments has increased significantly for the doses K and 2K. The evolution of the maturity index of fruit has shown an advance of the fruit ripening about 20 days in all the treatments in which potassium was applied.

### S08.222

#### Olive Grove in Biological Production Mode

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The main objective of this work was to observe the different stages of development of an olive tree (*Olea europaea* L.); to accompany the development of the insular life of the branches of the year; studying the influence of space factors and the conduction system, focusing productivity (kg ha<sup>-1</sup>); olive quality (fatness and olive oil acidity in “%” percentage so as the humid grade of the olive oil in percentage as well); accompanying the execution of some cultural techniques and identifying the main infesters on olive grove in Biological Production Mode. The results obtained led to the following conclusions: i. good adaptation when Galega vulgar cultivar having in mind the particular characteristics of Beja area; ii. when analyzing space factors and the conduction system results, only the space factors showed some significant results considering the productivity of the olive grove. A space with the dimension 5x5 m reached a productivity of 6075 kg ha<sup>-1</sup>, which is a great result having in mind that the olive grove was a parched one biological olive grove; iii. one can achieve great amounts of olive oil without changing the good quality level, using the Galega vulgar cultivar with the Biological Production Mode; iv. cultural techniques used such as (pruning; organic fertilization and controlling infesters between lines) assured a great productivity of the olive grove and a better result of his physical characteristics so as the chemical and biological conditions of the ground; v. the main identified infesters were: *Calendula arvensis* L.; *Chamaemelum fuscum* (Brot.); *Foeniculum vulgare* Mill.; *Geranium molle* L.; *Oxalis pes-caprae* L.; *Raphanus raphanistrum* L. e *Sinapis arvensis* L., all these being fundamental in the preservation of the auxiliary fauna; vi. *Euphyllura olivina* had been verified on the olive grove. This plague has been extinguished thanks to special treatment using “óleo de Verão”.

### S08.223

#### Thrips in *Olea europaea* L: Organic Versus Conventional Production

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Olive grove canopy contains a wide range of arthropods. Few attention has been given to thrips (*Insecta: Thysanoptera*) so far, in spite of the damages they cause, since attention has been focused on the main pests. Thrips are considered secondary pests, with occasional severe damages caused, for example, by *Liothrips oleae* L., the olive thrips, and *Frankliniella occidentalis* (Pergande), the western flower thrips. However, thrips fauna is composed not only by phytophagous species, but also by predators and fungiphagous species. Thrips have long been known as useful predators of mites and *Lepidoptera*, and they can also prey on psocids, scale insects and

other thrips. Their role as pollinators must also be emphasized. In the study here presented, a total of 1361 thrips were captured in four olive groves in the Alentejo region, south Portugal, by the beating technique, weekly in the spring, summer and autumn, during two years. Two olive groves were conducted under organic farming system and the other two under conventional agriculture system. The impact of both productions systems on thrips species presence and on their population dynamics is analyzed, and thrips functional role in the ecosystems is discussed.

### S08.224

#### Determination of Organophosphorus Pesticide Residues in Olives Grown in Bursa, Turkey

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The use of pesticides in agriculture, which has been continuously increasing in recent years, has led to an increase in world food production. Organophosphorus are among the most widely used pesticides in the world. Because of their long half-life time, they contaminate soils as well as surface and ground water. In addition, these residues in food constitute a significant health risk. Olive and their products are extensively consumed foods in the Mediterranean countries. However, olive trees are attacked by several pests and diseases make it necessary to apply pesticides to ensure crop protection, which can leave residues on the fruit. Because pesticide residues in food constitute a significant health risk, and olive oil has a high consumption rate among people of the producing countries, the continuous control of pesticide residues in olive oil is of great importance. In this study, the occurrence of nine organophosphorus pesticide residues in olives (*Olea europaea* L. cv. Gemlik) in Mudanya and Gemlik towns (Bursa, Turkey; latitude: 29° 04' E, longitude: 40° 39' N) was investigated. Olive samples in black maturation stage from twenty orchards were analyzed for pesticide residues using different extraction methods by capillary gas chromatography (GC) using NPD with capillary column. Nine organophosphorus pesticides, namely azinphos-ethyl, azinphos-methyl, chlorpyrifos, chlorpyrifos-methyl, diazinon, methidathion, carbophenothion, malathion, and pirimiphos-methyl were investigated.

### S08.225

#### Olive Fly (*Bactrocera oleae* Gmelin) Population Dynamics in Terceira Olive Groves

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In the Azores Archipelago, the olive tree only grows on two islands, Terceira and Pico. According to the 2008 area production census, Terceira Island has about 58 hectares of olive tree production, limited to the Porto Martins village area. According to that survey, all olive production is for direct consumption (table olives). The main phytosanitary problems that are causing damage in Terceira olive productions are: olive fly (*Bactrocera oleae* Gmelin.), olive moth (*Prays oleae* Bern.), black scale (*Saissetia oleae* Oliv.) and a new phytosanitary problem that was detected only in 2008, the *Euphyllura olivine* Costa. The olive fly (*B. oleae*) is the key pest of this culture in Terceira Island due to fruit damage. Next in rank is the olive moth (*P. oleae*). For monitoring and risk analysis purposes, during July 2009, several traps with food attractant (diamonic phosphate) were placed in two distinct orchards.

One was treated and the other was completely without any kind of treatment. The traps were revised every 15 days and the *B. oleae* adult captures were recorded according to that time interval. According to the collected data, from 2009 July to 2009 November, the adult *B. oleae* population peak occurred during September and November. The fruit damage analysis regarding the *B. oleae* adult activity in 2009 indicated that there is 86% of fruit infestation/damage in the treated olive orchard versus 99% of fruit infestation/damage in the non treated olive orchard. The collected field data has allowed the development of charts to visually identify the time periods of the major adult population of *B. oleae*. These results will allow better decision making to reduce the high levels of infestation as well as the high number of phytosanitary treatments that are on-going during olive production season.

### 508.226

#### *Dendrothrips eremicola* Prisner, (Thysanoptera: Dendrothripidae), a New Pest Outbreak on Olive Trees and its Control in Reclaimed Desert Lands at Ismailia, Egypt

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Interference of man in the desert ecosystem through land reclamation for gaining new agro-ecosystems with monoculture characteristics has led to occurrence of new economic insect pests. The thrips species, *Dendrothrips eremicola* Prisner was recorded recently, as a new insect outbreak on olive trees in some locations in Egypt. Pest's population was under natural balance on its original wild host plants in the desert ecosystem, e.g. *Ligustrum* spp. (fam.: Oleaceae) by both biotic (natural enemies and limited host plants) and abiotic (water/dryness) factors. Ecological factors that might contributed to this outbreak in some olive groves are; removing wild plants and growing domesticated olive (*Olea europaea*) which has offered unlimited alternative food supply for the thrips, irrigation and fertilization systems providing trees nourishment year round leading to formation of juicy leaves suitable for thrips feeding and egg insertion, and training trees with low crotches favouring the reproductive cycle of thrips. Additional important factor is intensive use of chemical insecticides suppressing existing natural enemies. Managing *Dendrothrips eremicola* is not an easy task due to its hidden sites on the plant and its feeding habit using rasping-sucking mouth parts. Accordingly, population has gradually increased becoming an economic insect pest on olive, especially at Ismailia and El-Arish, as a unique case found only in Egypt. Searching for a rapid population suppression of this thrips in olive groves through commercial trials has shown successful results using the bio-pesticides Romectine 1.8% Ec (Abamectin) or Tracer 44% SC (Spinosad) or using traditional chemical pesticides; Challenger 36% SC (Chlorfenapyr) and Admire 20% SC (Imidacloprid). Bio-pesticides have been favoured by organic growers and are advocated for safe production of food.

### 508.227

#### Olive Root Hairs Are Key for the Effective Colonisation of *Pseudomonas* spp. Against *Verticillium* Wilt

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The use of natural colonisers of the rhizosphere, which have antagonist effects against to soil phyto-pathogenes, is a major strategy against diseases affecting important crops like olive (*Olea europaea* L.). Two bacteria, *Pseudomonas fluorescens* PICF7 and *Pseudomonas putida* PICP2, native bacteria of olive roots and previously demonstrated to be effective biocontrol agents (BCA) against *Verticillium* wilt, a disease caused by the fungus *Verticillium dahliae* Kleb., have been used in this work to elucidate the initial colonisation process in *in vitro* propagated olive plants. A

differential fluorescent protein tagging has been used for the *in vivo* simultaneous visualisation of *P. fluorescens* PICF7 and *P. putida* PICP2 by confocal laser scanner microscopy (CLSM) in olive root tissues of *in vitro* propagated plants. We have identified the root hairs as the preferential door for olive roots colonisation by these beneficial bacteria. We have also analysed at the single cell level the potential competition between these two strains during the colonisation process. The suitability of the *in vitro* system to investigate endophytic colonisation processes in olive roots by *Pseudomonas* spp. was assessed.

### 508.228

#### Olive Anthracnose: a Microscopic Approach

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Fungi of the genus *Colletotrichum* include *Colletotrichum acutatum*, *Colletotrichum fragariae* and *Colletotrichum gloesporioides*. One of the most pathogenic species of this genus is *Colletotrichum acutatum* J. H. Simmonds, which causes anthracnose and blight in agriculturally important host such as olive fruits. Anthracnose is the most important disease affecting olives in Portugal. The disease causes the drop of fruits or, when harvested, originates poor oil quality due to high acidity levels. Few studies are available concerning the infection and colonisation strategies adopted by *C. acutatum* when infecting susceptible and tolerant olive cultivars. The aim of this work was to elucidate key aspects of the infection process, colonisation, structures (conidial germination, germ tube and appressorium formation) and strategies adopted by *C. acutatum* on three olive cultivars ('Picual' – highly tolerant; 'Cobrançosa' – medium tolerant and 'Galega' – susceptible), which vary in terms of tolerance to the pathogen, using fluorescent microscopy. The rate of mesocarp colonisation differed among the susceptible and tolerant cultivars, and both intracellular hemibiotrophy and subcuticular intramural necrotrophy were observed.

### 508.229

#### Application of Herbicide Oxifluorfen with Boom Sprayer under the Canopy of the Olive Tree

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The olive orchard and olive oil form part of Spanish culture and heritage, and thus it is urgent to develop sustainable cultivation systems, for which herbicides are necessary. In soil-management systems, to limit weed growth under the canopy, covers crops are used and plants selection are used in the central lane of the olive orchard. Preemergent herbicides are applied under the canopy in autumn before harvest, with aim of keeping this zone weed free for the harvest. To foment the proper use of herbicides, there are rules and regulations that establish the correct application practices. The aim of the present work was to detect mechanisms of direct contamination of the fruits in the lower parts of the olive tree by the herbicide oxifluorfen (Goal Supreme®) at different application rates when applied by boom sprayers, with and without offcenter nozzles. The oils from the fruits collected exclusively from the lower parts of the tree enabled us to identify a number of risk factors. The use of spray booms with anti-drift nozzles do not completely limit the possibility of contaminating the fruits and subsequently the olive oil, even using half the herbicide dosage recommended. The combination of the boom sprayer with offcenter nozzles at the maximum application rate registered the highest number of cases of contamination. According to the assay results, we call attention to the fact that the low limbs of the trees play an important part in the pollution detected, since they bear the fruit that the eccentric and anti-drift spray nozzles reach with the herbicide, as these fruits are situated under the 50 cm of the bar height (spray booms).

**S08.230**

## Soil Management Affects Yield Components of Young Olive Trees under Deficit Irrigation

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Since erosion is a major threat in Mediterranean areas, periodic tillage is currently not the recommended practice for soil management in mature olive orchards. However, permanent grass cover increases water and fertilizer needs and, hence, may reduce growth and yield of young orchards. We compared vegetative growth, fruit growth and yield components of young olive trees (*Olea europaea* cv. Frantoio) grown under either tillage (ST) or permanent grass cover (GC) in a sandy-loam soil. The soil was tilled from the year of planting (2003) until October 2004, when both treatments were established. The ST treatment was kept weed-free by disking (about 0.2 m depth), whereas GC was obtained by letting the natural flora grow and periodic mowing. In both cases, disking or mowing was done 3-4 times a year. Trees were fully irrigated until year 3 after planting, when deficit irrigation (50% of full) was started for both soil treatments. Trunk cross sectional area (TCSA) of GC trees was 74 and 83% that of ST ones at the end of the 2006 and 2009 growing seasons, respectively. Fruit yield and oil yield of GC trees were 60% and 66% those of ST ones, respectively over the 4-year study period; however, when expressed on a TCSA basis, they resulted 85% and 94%, respectively. Yield components were differently affected by soil management. The number of fruits of GC treatment was significantly lower than that of ST treatment. The oil content in the mesocarp was similar for both treatments. Permanent GC should be used with caution in young olive orchards and should not be recommended in the first two years after planting.

**S08.231**

## Shoot Pruning and Treatment with Hexaconazole or Urea to Increase Fruit-Set in Olive

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Traditional olive industry in Mediterranean area is affected by alternate bearing. This irregular production is evidenced by spring competition between shoot growth and fruit development in the same branch. Total or partial cutting of the shoot, as well as hormonal control of shoot growth with hexaconazole reduce vegetative flush and increase fruit set. Another strategy to favourite fruit set is the somministration by leaf of nitrogen fertilizers like urea during the period between full blossom and fruit ripening. In this paper leaf fertilization with urea is compared with hexaconazole treatment and with the partial or complete cutting of the new shoot growing on the fruit bearing branch, in order to evaluate the effects on fruit set and development. The research was carried out in 2005 and 2006 in the experimental orchard of AGRIS located at Villasor in Southern Sardinia (39° 21' of latitude North). Olive trees were planted in 1990, vase shaped and submitted to standard field management. Both shoot pruning treatments were effective to increase fruit yield on the cultivar 'Bosana' (from 3.3% to 4.4 and 4.7% in 2005), 'Nera di Gonnos' (from 1.4% to 3.4 and 3.0% in 2006), and 'Tonda di Villacidro' (from 1.5% to 5.5 and 5.1% in 2006), while 'Tonda di Cagliari' and 'Semidana' cultivars showed more or less the same yield of the test in pruned branches. Hexaconazole treatment was less effective than shoot pruning and only for 'Bosana' (from 3.3 to 3.7% in 2005 and from 2.9 to 3.9% in 2006) and 'Nera di Gonnos' (from 1.4 to 2.3% in 2006). Leaf treatment with urea was effective only with the 'Bosana' cultivar, increasing the rate of fruit yield from 2.9 to 4.3% of the flowers. No positive effects of urea treatments were recorded on 'Semidana' and 'Nera di Gonnos' cultivar.

**S08.232**

## Predictive Model of Olive Grove Production in Extremadura (Spain)

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Olive grove outputs depend on various factors, both environmental and crop management, that characterize the final yield and quality of the fruit. One mathematical tool that can be used to study these relationships is regression analysis (RA). This technique answers questions about the dependence of a response variable on one or more predictors, including prediction of future values of the response, revealing which predictors are important, and estimating the impact of changing a predictor or treatment on the value of the response. The present work applies the statistical tool of multiple linear regression to correlate olive production with soil type, crop density, and rainfall in two production areas in Extremadura: Cáceres and Badajoz. Several mathematical models were studied linking production with the aforementioned agronomic variables. The set of results was compared with actual production data to estimate the accuracy of the method and choose the most appropriate model for prediction. It was necessary to establish a mathematical model to differentiate soil type and geographical area. The regression equation was constructed from crop density and rainfall data. We conclude that predictive mathematical models should include both quantitative and qualitative variables to improve the reliability of their predictions.

**S08.233**

## Olive of Iran, a Combination of Culture, Horticulture, and Sacredness

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Iran, an ancient country with a history going back more than 3000 years, has been named as one of the probable origins of olive cultivation in the world. Ancient growths of olive have been discovered in faraway reaches of the country and numerous varieties of olive have been reported from different geographical regions of Iran- from the Eastern borders of Khorasan to the heights of The Mount Zagros in the West and even some regions in the South, where a wild type olive, *Olea europaea* subsp. *Cuspidata* grow on an enclave from the hillside to an altitude of 1800 meters. They create a wonderful scene: by the time that trees on the hillside are bearing fruit trees higher up on the hilltop have not bloomed yet. Olive trees are considered sacred in some parts of Iran. They are vigilantly guarded and cutting the branches, the leaves and even fruit picking are unforgivable offenses. Today, native olive trees from different parts of the country have been identified and planted in various collections. The Iranian olive catalogue, containing detailed specification for more than 60 varieties is under development. Expansion of olive cultivation in the last fifteen years from 5 thousand hectares to more than 120 thousand hectares, which has been spurred through the initiative of the ministry of Agriculture, has lead to the production of nearly 7000 tons of olive oil annually. Customarily, olive oil was used mostly for medicinal purposes, except in the northern part of the country, where olive was a staple food; but today, consumption of olive oil is steadily increasing among Iranian people due to increased awareness of its benefits.

**S08.234**

 Determining the Best Pollinizer of Olive (*Olea europaea* L. var. *amigdalifolia*)

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In order to evaluation of the best pollinizer for olive (*Olea europaea* L. var. *amigdalifolia*), an experiment accomplished in 2008 at Kazeroun olive research station. Statistical design was randomized complete block design with 10 treatments and 3

replications. In this study, Konservolia, manzanillo, dakal, gordal sevillano, sevillano, koroneiki, amigdalifolia cultivars exclusively were used as pollinizer and pollen mixture of all cultivars, open pollination and self pollination were the other treatments for pollination of emasculated flowers. Initially, mid-summer and final fruit set, yield and components, pollen viability of different cultivars index and biology of flowers were evaluated. Results indicated that open pollination, pollen mixture and pollens of Dakal cultivar increased fruit set and yield in comparison with self pollination. Here Dakal cultivar is introduced as the best pollinizer for Amigdalifolia cultivar. Study on the pollen viability of cultivars indicated that there is a significant difference between pollen of Konservolia, dakal, amigdalifolia cultivars and pollen mixture with Manzanillo. Gordal sevillano, sevillano and koroneiki pollens. amigdalifolia cultivar is respectively self incompatible (index of self incompatibility = 0.48).

### S08.235

#### Quality Assessment of Compost Prepared with By-Product of the Olive Oil Industry. Agronomic Application in Olive Grove

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The industrial olive oil sector is very important in Mediterranean countries. The two-phase centrifugation system for olive oil extraction, from the early 1990s, has introduced a new solid waste called two phase olive mill waste (alperujo). Different solutions have been proposed for two phase olive mill waste treatment based on evaporation ponds, thermal concentration, phenolic components extraction, application to agricultural soils to sorption of herbicides and insecticides. Composting as a method for preparing organic fertilizers and amendments is economically and ecologically sound and may well represent an acceptable solution for disposing of two phase olive mill waste, at the same time increasing its value. In the present work, we study the effect of compost made from two phase olive mill waste on olive grove. The objective was to optimize the composting conditions and characterize the changes in mineral nutrients (N, P and K) concentration on plant and yield, after agronomic application of compost. The compost was prepared in a pilot-plant using the mobile-pile system. Temperature and moisture were evaluated for to optimize the composting progress. The results showed a similar evolution of the mixture of the two phase olive mill waste and olive leaf as bulking agents. To determine the quality of compost prepared were studied the parameters: N, P, K, pH, conductivity and C/N ratio. The results showed the rich in minerals elements of the compost for its use in agricultural. The application of compost on olive grove keep tree nutritional status. So, the compost of two phase olive mill waste represents an important tool for fertilization requirements.

### S08.236

#### Comparative Study on the Economic Viability of High and Super-High Density Olive Orchards in Spain

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This study makes a comparative analysis to evaluate the economic performance of two alternative production systems in the olive sector that have received a lot of attention in the last decades, both from researchers and decision olive oil groups, named high-density (HD) and super-high-density (SHD) systems. While, in average terms, HD orchards are characterized by densities between 250 and 500 trees per ha, SHD orchards can present densities over 1000 trees/ha (hedgerow system). Average full yield in high density system is around 6,000 kg/ha in rainfed farms reaching about 10,000 kg/ha in irrigated land. In the second alternative (SHD), similar yields are obtained although sooner than in the first system. However, the economic life of the SHD is shorter (around 15 years, while in the intensive system it is around 30-40 years) due to the lack of space and the competition among trees for light and ventilation inside the canopies. Data used in this study come from three alternative sources: 1) literature review; 2) a questionnaire for farmers;

and 3) personal interviews to specialists in olive growing. From such information, production costs and income have been obtained from four alternative production systems: two high-density orchards (harvested with trunk shakers or Colossus), one super-high-density and one mixed system (SHD the first 15 years passing to HD during the next 16 years). Economic assessment is evaluated through the Net Present Value (NPV) and the Internal Rate of Return (IRR, %) taking into account that the economic life of the different alternatives is not the same. Results indicate that high-density economic ratios are more profitable than the super-high-density ratios. However, the latter could be the most profitable option in large size farms with short term investments, full harvest mechanization and reduced labour requirements.

### S08.237

#### Prospectives and Limits of the Low Vigor Sicilian Cultivar Biancolilla in Superintensive Planting System

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The economical sustainability of olive industry depend on the ability of olive growers to increase productivity and reduce production costs. The agronomical solution to these aims has been to develop the superintensive planting systems, based on early cropping, low vigour, highly and constant production cultivars. Although today to the world olive varietal pool have been recognized about 500 cultivar, only three of them seems to have the horticultural traits to satisfy the requirements asked by the superintensive planting systems: Arbequina and Arbosana, from Spain; Koroneiki, from Greece. Aim of this study was to observe the horticultural behaviour of one of the less vigorous Sicilian cultivar, Biancolilla, grown in a superintensive planting system, respect to the two Spanish cultivars that worldwide have been recognized as the best performing in such planting designs. The research was carried out in Sicily, in a commercial orchard established in 2005. The above mentioned Spanish cultivars were spaced 3.5 x 1.5 m (1900/trees ha); 5 x 2.5 m Biancolilla (800 trees/ha). The lower planting density of Biancolilla was due to its higher vigour (almost twice) respect to the Spanish cultivars. The collection of data started in 2007, at the first significant blooming, and were related to aspects of tree vegetative growth, fertility (flower/cluster; ovary abortion; fruit set), crop efficiency, ripening phenology and olive oil quality. Fruits were harvested by over-head, row straddling machines. Concerning tree vegetative growth, fertility and crop efficiency, Arbequina and Arbosana performed as in all the other places they have been tested (see literature). However, regards to oil quality characteristics, significant differences were observed between Biancolilla and Spanish cultivar in terms of fatty acids and polyphenols contents. Unfortunately, Biancolilla evidenced a low adaptability to superintensive systems, due to its delay in first bearing, alternate cropping and high susceptibility to peacock leaf spot

### S08.238

#### AARENINA/FAO Olive GAP Manual for the WANA Region

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Fifteen countries in West Asia - North Africa region grow olive (*Olea europaea*) as a major economic fruit crop. Many of these countries suffered from gradual drop in olive yields as a result of extreme water scarcity, soil degradation, climate change, irrational pests control and age old inherited cultural practices enduring the least technological evolution. Sponsored by two organizations an essential guided manual for olive good agricultural practices has been published in March 2010 after taking four years to materialize, undergoing technical clearance from FAO. From the region, seven volunteered authors, one of whom is an olive grower, compiled this manual through active networking. Supported by many images, the manual provides easy to understand measures to sustain natural resources, takes a step by

step approach with practical, exhaustive information and procedures through all olive production phases and addresses growers, extensionists and researchers similarly. Preserving today in order to protect the future, the manual is expected to help olive growers with their farming activities in the best possible conditions, in terms of their own health and that of their families while protecting their groves, ecosystems and the environment at large. Traditional olive growing communities who are ready to make the change through the application of the recommendations contained in the manual and make use of innovative approaches and strategies will improve their livelihood and wellbeing. This will be the outcome of improved revenue generation from reduced production costs and sustained production and marketing as well as increasing groves' asset value. Further, the production of raw olives will meet the sophistication of today's consumer in respect of food safety and hygiene.

### S08.239

#### Olive (*Olea europaea* L.) Production in Northern Cyprus

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Cyprus is considered a secondary centre of diversification for olive. It is reported that olive has been produced in Cyprus since Bronze Age, and olive presence is witnessed by century's old trees (even groves established presumably during the Lusignans). These monumental olives together with carob trees have very important eco-touristic value for the island. Although recent development in tourism and second house construction for local and foreign residents caused tremendous damage to the most beautiful Kyrenian olive groves, olive production is still very popular in Cyprus. There are some 500 000 olive trees still being cultivated in the Northern part of Cyprus. While the most common cultivar is "Local Variety", grown both for oil and table, the Turkish cultivar "Gemlik" was introduced as an alternative in recent years. Besides propagation by cutting, grafting on wild olives is also utilized for olive propagation. "Local variety" grafted on a wild olive stock is often preferred due to its tolerance to drought stress, a common constraint together with salinization of underground water for plant production. The above conditions, together with an increasing demand of the domestic market, recently determined a shift from citrus production, by far the most important fruit crop, towards the olive. Nevertheless, there are still some 500 tons of olive oil and 1000 tons of table olive imported yearly.

### S08.240

#### Floral Quality of 'Sikitita', a New Olive Variety

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'Sikitita' (synonym 'Chiquitita') is a new olive (*Olea europaea* L.) cultivar selected for high-density plantation which was obtained from a cross between 'Picual' (female parent) and 'Arbequina' (male parent) in the olive breeding program at Cordoba, Spain (University of Cordoba and IFAPA). In this study we evaluated the floral quality of 'Sikitita' and its parent cultivars at different levels of morphological organization: inflorescence, flower and ovary. Our aims were to characterize this new cultivar and also to examine the inheritance of floral quality traits in the olive tree. The measured parameters included number of nodes and flowers per inflorescence, percentage of perfect flowers, size of the ovary and its component tissues, and number and proportion of fully developed ovules. In all parameters evaluated 'Sikitita' was found to be intermediate between its genitors or similar to 'Arbequina', the parent with better floral quality characteristics.

### S08.241

#### Characterization and Identification of the Main Algerian Olive Cultivars by Molecular Markers

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Algerian olive cultivars are very diverse; most of them are confined in their area of origin, and cultivated in different agro-climatic conditions. In the present study, SSR and SNP markers are used to characterize and identify olive cultivars from the north-eastern Algeria, an important region in terms of olive cultivation, and where most representative cultivars of the country are found. Molecular analysis was performed on 18 olive cultivars. The genetic profiles of fourteen cultivars collected in north-east Algeria were compared with a set of Tunisian (1), Moroccan (1) and Algerian (2) cultivars provided from the the World Olive Germplasm Bank of Córdoba (IFAPA "Alameda del Obispo"). Ten microsatellite markers and seven SNP markers were tested on the set of cultivars. All microsatellites were polymorphic, being 76 the total number of alleles amplified, and showing 7.6 alleles per locus on average. Mean observed heterozygosity value was 0.83 and mean expected heterozygosity value 0.81. Although less polymorphic than SSR, SNP markers were useful as well for characterization and identification purposes. The results obtained in this work confirmed the high efficiency of SSR and SNP markers for genotyping olive cultivars and highlighted the genetic richness available in the olive germplasm of olive areas as Algeria. The identification of cultivars is one of the major applications of the molecular markers in olive. SSR and SNP based fingerprinting of olive cultivars may generate a molecular database that will facilitate management of cultivar collections, trade control of plant material, and selection of genotypes for breeding programs.

### S08.242

#### Morphological and Pomological Characterization and Identification of East Mediterranean and South-East Anatolia Region Olive (*Olea europaea* L.) Cultivars with SSR's Markers

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Anatolia is the best known the home of the lots of endemic plants and animals. Olive (*Olea europaea* L.) is the one of them. Generally, natural and cultivated Olive trees are grown coastal area of the Turkey. On the other hand East Mediterranean and South East Anatolian region has a great importance for genetically olive resources. Last, genetically characterization and pomological, morphological and phenological identifications studies were carried in 2005-2008. Plant samples were collected in 12 provinces (Adana, Mersin, Hatay, Osmaniye, Gaziantep, Kilis, Sanliurfa, Mardin, Kahramanmaraş, Adıyaman, Şırnak and Diyarbakır) of East Mediterranean and South East Anatolian region of Turkey. Numerous local and some standard olive cultivars growing in South east and south Mediterranean region were studied for tree, fruit, inflorescence and stone characteristics (Rallo and Barranco 1984). The present study is aimed to identify main and local Olive cultivars from the South Mediterranean and South east Anatolian region of Turkey using SSRs markers. Ten SSR markers for selected and classified olive genotypes and standard olive cultivar, *Olea europaea* ssp. *europaea* var. *europaea*, were used to genotype about 300 accessions. SSR analyze were carried in Beckman CEQ 8800 automatic fragment analysis. The results show high diversity and a significant high level of differentiation among olive cultivars within the standard domestic and foreign olive cultivars.

### S08.243

#### Germination and Viability of Pollen in Some Olive Cultivars for Oil and Table

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The raise of olive economic importance led to new cropping techniques and research with the purpose of enhancing the crop yield. The viability and germination in olive pollen are closely related to a greater or lesser ability to produce fruit and, consequently higher or lower yields. The relationship between these parameters has been

reported by several studies and the results are very different depending on cultivar analyzed. In this work we analyzed the viability and the *in vitro* germination of pollen in olive tree from different cultivars. These trees are located in the fields of trials from the "Finca La Orden" (Badajoz, Spain). These parameters were determined in the following olive cultivars: 'Arbequina', 'Morisca', 'Pico Limon', 'Picual', 'Verdial Badajoz', 'Manzanilla Sevillana', 'Manzanilla Cacerena' and 'Gordal'. The first five are cultivars for oil production and the last three are for table. The pollen viability was determined using the Alexander's method that has been proven as an efficient test to assess pollen viability. The germination percentage is estimated using an *in vitro* germination test. The unifactorial analysis of variance revealed significant differences in the viability and germination of pollen from different cultivars used for oil production. However, the cultivars for table showed significant differences for pollen viability, but not in germination. The results show that viability is higher than the percentage of germination in all cultivars analyzed. Moreover, no correlation was found between viability and germination when the values from the different cultivars are compared.

### S08.244

#### Seed Storage Proteins of the 11S Type in the Olive. Biochemical Characterization and Potential Uses

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Olive seeds contain large amount of protein in the form of protein bodies. Precursor forms of these proteins are made up of individual proteins, which have been purified to homogeneity and further named p1-p5 (20.5, 21.5, 25.5, 27.5, and 30 kDa, respectively). N-terminal sequences of p1 and p2 proteins displayed relevant homology to the basic subunit of the 11S family of plant SSPs (legumins). Analysis of endosperm and cotyledon tissues by 2-D electrophoresis in combination with mass spectrometry indicates the basic character of p1 and p2 and the acidic character of p3, p4, and p5 proteins. In addition, the putative presence of highly similar isoforms or post-translational modifications of these polypeptides was detected. As a result, a model describing the putative association of p1- p5 proteins into subunits of alpha(acidic)/beta(basic) type has been proposed. Solubility experiments have shown that the majority of these olive seed proteins from the 11S storage protein family are extracted with aqueous alcohol and only partially with water and diluted saline solutions, therefore suggesting their similarity to prolamines. By using a rabbit antiserum raised to p1 protein, the proteins have also been immunolocalized in olive seed tissues, showing that they accumulate in conspicuous protein bodies present in both the endosperm and the cotyledon. The abundance of SSPs points out mature olive seeds as a relevant nitrogen source for animal feeding. Preliminary assays of protein digestibility have been carried out. We also show how these SSPs are also present in several byproducts of olive oil manufacturing industries. Other relevant uses may exploit a putative discriminative character of these proteins among olive cultivars for breeding purposes.

### S08.245

#### Evaluation of Four Varieties Regarding Their Suitability in High-Intensity Olive Orchards

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High-intensity olive orchards require high productive varieties with low vegetative vigor and low canopy volume. As a consequence these plants have a minimal economical life of about 15 year. The aim of this study was to test four varieties proposed by the nurseries for this training system in the Central Italian environment. In 2008 and 2009 the vegetative and productive activity of 'Arbequina', 'Arbosana', 'Don Carlo' and 'FS 17' were monitored on trees that were planted in 2006 in a high-intensity orchard in Abruzzo region (Italy). Regarding vegetative parameters the canopy growth and trunk area growth were registered. Regarding productive aspects the ripening process, the yield and the quality of the oil obtained were evaluated. The most productive varieties were 'Arbosana' and 'Arbequina', 'FS 17' and 'Don Carlo' had respectively

lower production. 'Don Carlo' and 'FS 17' had bitterer and more spicy oils than 'Arbequina' and 'Arbosana' that gave equilibrated oils. 'FS 17' and 'Don Carlo' had the highest crown volume followed by 'Arbequina' and 'Arbosana' respectively. 'Arbosana' had the highest efficiency index (yield/m<sup>3</sup> of crown) followed by 'Arbequina', 'FS 17' and 'Don Carlo'. In 2009 'FS 17' showed a particular sensibility to olive fly which was probably due to the low hardness and high dimension of the fruits. Considering the data of these two years we concluded that 'Arbosana' and 'Arbequina' are probably suitable for this kind of training system in Central Italy. 'Don Carlo' and 'FS 17' had some problems related to yield (especially 'Don Carlo') and vigor that might influence negatively their economical results in high-intensity olive orchards.

### S08.246

#### Microsatellite Markers to Fingerprinting of Extremadura (Spain) Olive Tree Varieties

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Modern societies are demanding quality control standards to ensure the origin and authenticity of the products they consume. Correct identification of varieties is crucial, since identification of olive cultivars is complicated by the large number of synonyms and homonyms, the intensive exchange of plant material, the presence of varietal clones, and problems of certification of varieties in garden center. Molecular marker techniques have been used to characterize olive cultivars and clones. Microsatellites are the most useful molecular marker used. A series of microsatellite markers already developed and its sequences have been published (Rallo *et al.*, 2000; Sefc *et al.*, 2000; Carriere *et al.*, 2002; Cipriani *et al.*, 2002). In previous work (Llerena *et al.*, 2008) a characterization of olive tree was carried out using agronomical parameters and their results show that there are several cultivars different between them. This study aims to test the efficiency of SSR markers to identify and to differentiate a set of 38 Extremadura olive varieties. The SSR dendrogram based on unweighted pair-group cluster analysis using Jaccard's index reveals that the genetic diversity is very high between some cultivars of the same variety. A trend of clustering together of accessions originating from the same or adjacent locals was also observed. Because of the confusion surrounding the origin of most olive cultivars, their molecular identification and ascertainment of origin will be extremely useful for germplasm management and breeding. This research showed that SSR is a suitable and effective tool to characterize olive varieties of Spanish region of Extremadura.

### S08.247

#### Characterization of Olive Trees of Extremadura by Cluster Analysis

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The olive (*Olea europaea* L.) is one of the oldest agricultural tree crops, and is cultivated for both oil and fruit. A characterization would help with the identification of olive tree cultivars to store plant material and gain knowledge of environmental adaptations. Molecular linkage maps based on DNA markers are widely recognized as essential tools for genetics research and breeding in many species, but often the lack of molecular markers makes their preparation a long and expensive process. The aim of the present work was to perform a cluster analysis using data obtained by two alternative methods – a genetic analysis and the morphological analysis of leaves and fruit – and to compare the degree of similarity between them. A cluster analysis seeks to identify homogeneous subgroups of cases in a population by minimizing within-group variation and maximizing between-group variation. The clustering of profiles was performed by a dendrogram following Ward's minimum variance algorithm. The same three parameters were analyzed in leaves, fruit, and

endocarps – length, width, and shape – in samples of 40 specimens/tree, as recommended by Barranco *et al.* (2005). The DNA markers used were those proposed by Cipriani *et al.* (2002). The results showed the clusters obtained by analyzing the morphological data to be very similar to those obtained from the genetic analysis. We conclude that morphological analyses are good tools with which to perform a first classification of the population.

### S08.248

#### Somatic Embryogenesis in Young and Aged Embryogenic Cultures of Olive (*Olea europaea* L.)

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The olive tree is an important crop in the Mediterranean countries and considerable efforts are being directed to the genetic improvement of this species. Application of biotechnological tools can be a valuable help for obtaining improved cultivars. In olive, the application of this technology is linked nowadays to somatic embryogenesis. However, when embryogenic cell lines are maintained through repeated subcultures, a progressive decline and sometimes a complete loss of this morphogenic potential are often observed. The development of elite plant material through biotechnology, including checking of its real value in field, requires a considerable period of time. The use of biotechnological tools from a practical point of view requires, in consequence, knowing the response of our somatic embryogenesis system to long-term maintenance of cultures under *in vitro* conditions. In the present investigation, the influence of time in maintenance of cultures on the somatic embryogenesis process has been studied. Ten embryogenic cell lines maintained under standard proliferation conditions for 2 and 8 years have been used. The results obtained revealed an increase in the proliferation rate and an alteration of the proliferation pattern with time in culture. Efficiency of the maturation phase was lower in aged cell lines. In parallel, a decline in embryos quality was observed as revealed by a decrease in the germination capacity. Both parameters resulted in a significance decrease in the regeneration potential with cultures age. Plants obtained from aged cultures were smaller than those derived from young cultures although more shoots developed per germinated somatic embryo. During the elongation phase, shoots derived from younger cultures were more vigorous, giving rise to longer axillary shoots. No significant differences were found in the rest of the parameters studied in the elongation, rooting and acclimatization phases.

### S08.249

#### Somaclonal Variation in Olive Plants Regenerated Via Somatic Embryogenesis

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Genetic variability is an important requirement in any conventional crop improvement programme. Plant biotechnology offers new opportunities for creating genetic variation. Somaclonal variation is proving to be an important source of new traits suitable for exploitation in breeding programmes. This fact is especially important for trees such as olive which is characterized by slow development, a long juvenile period and self-incompatibility. In this investigation 396 plants were regenerated by somatic embryogenesis from 10 cell lines (derived from different zygotic embryos) maintained under standard proliferation conditions for 2 and 8 years. Morphological evaluation of the plants showed different variant phenotypes which were classified into 16 different types. Variations observed mainly affected the shape of the main stem, leaf morphology and phyllotaxis. Phenotypic variants did not appear homogeneously in the cell lines studied. While some of them were present up to in 5 cell lines, others were cell line specific. An effect of the genotype was also observed on the frequency at which each variant phenotype appeared. Different variant phenotypes could be observed in a single plant. In some cell lines up to 3 abnormal characters accumulated on the same plant. The percentage of plants showing somaclonal variation was significantly influenced by the genotype but a statistical effect of time in culture could not be inferred. The results obtained revealed that the genotype can be considered the main factor determining the type

and frequency of variant phenotypes as well as the percentage of plants showing somaclonal variation. Time in maintenance of cultures appears to be a secondary factor. Among the cell lines used in this investigation, some of them appear as highly unstable and might be useful for the production of sublines showing interesting variant phenotypes. Experiments for determining the genetic stability of the changes observed are now in progress.

### S08.250

#### Performance of Clonal Gardens of Olive Tree in Successive Cuts to Propagation By Cutting in Minas Gerais State, Brazil

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The work was aiming to evaluate the performance of clonal olive tree gardens in successive cuts at its propagation by cutting. The study was conducted in the EP-AMIG of Maria da Fé, Brazil. The clonal garden was installed in March 2006, in grooves with 40 cm in depth. Were evaluated two cultivars (Ascolano 315 e Arbequina) and cuts in three years (2007, 2008 and 2009). The test was conducted in split plot in time in a randomized block design with five replications. The plots were composed of three lines, spaced between itself in one meter, three plants in each line, and the evaluations carried through in three more vigorous plants. For evaluation, three plants were cut to 20 cm height of the soil, after 12 months of cultivation, being evaluated the yield of cutting number with four knots by leaves two pairs, plants height (m), torso diameter the 20 cm height of the soil, average length of branches (m) and total green mass accumulated (kg). In most feature evaluated, better results were observed in the 'Ascolano 315'. The successive cuts may be extended for a period exceeding three years.

### S08.251

#### Morphological Evaluation of Ancient Olive Varieties Across the Zagros Mountains of Iran

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Iran with various numbers of different olive cultivars and a considerable number of old olive orchards is one of the known origins of olive among a few in the world. Therefore, evaluation and classification of existing olive genotypes in Iran is crucial. In this study we used morphological characteristics to identify ancient olive varieties collected from 5 provinces across the Zagros Mountains (West of Iran), which are situated in different altitudes and consist of various climates. 25 ancient olive samples were collected from five provinces representing the olive population of the regions. Through the use of variance analysis, the studied quantitative characters revealed significant differences between varieties and clearly separated them from each other. The genotypes were separated based on their origin of collection, which demonstrates isolation of the groups of representative genotypes based on their ecological location. Results showed new evidence of the high degree of genetic biodiversity among olive germplasm from different parts of Iran.

### S08.252

#### Olive Production Diagnosis in a Hybrid Collection Derived from Crosses with the Tunisian Oil Variety 'Chemlali Sfax'

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Chemlali Sfax is the most cultivated variety in Tunisia and its oil acid composition is not equilibrated with mainly a low oleic acid level. The genetic improvement of this variety by man-made crosses was envisioned in order to improve of the oil quality while keeping the good characteristics of the cultivar. The hybridisation program had generated 1407 hybrids planted in 1997 and 1998 at two sites in the Sfax region (Central and coastal part of Tunisia) and conducted under irrigated conditions. The average from 2007 to 2009 was used to evaluate the hybrid collection. The productive hybrids (1200) belong to 69 crosses and 55 % had an average production less than 1 kg. In the site 1 (Institute headquarters), the average production was 4.3 kg and the main crosses of Chemlali with Coratina, Sigoise and Souri had respectively 4.13, 3.16 and 5.42 kg. Less than 18 % of hybrids in this site had an average production less than 1 kg. In the site 2 (Experimental station of Taous), the average production was as low as 1.76 kg. The main 13 crosses had variable performances from 3.14 (free pollinated Chemlali) to 1.3 kg (auto-pollinated Chemlali clone K5). The crosses of Chemlali with Coratina and Sigoise had respectively 2.07 and 1.46 kg. In this site, about 64 % of hybrids were with low olive production (< 1 kg). Selection on the basis of olive production should be focused on the site 1 and especially Chemlali/Coratina cross from which 11 % had an average production higher than 10 kg.

### S08.253

#### Intra-Varietal Variability of the Greek Olive Varieties Kalamon and Koroneiki Using Molecular Markers

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The olive tree (*Olea europaea* L.) is cultivated in the Mediterranean Basin since 4000 B.C. Its socioeconomic impact is very important for the countries in the area. Greece occupies the third place in the world rank of olive oil producers and the second place in the European Union as a table olive producer. 'Koroneiki' is an olive oil variety while 'Kalamon' is a table olive variety. Both of them are the most well known worldwide Greek olive varieties. In this study, healthy, young leaves of both varieties were collected from seven different regions in Greece and Cyprus in order to study the intra-varietal variability. DNA extraction was performed according to Doyle & Doyle protocol. Markers originating from two different molecular techniques, Randomly Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR), were used for investigating the germplasm variability. In order to establish the genetic relationships among the clones of 'Kalamon' and 'Koroneiki', twenty five RAPD primers were tested and ten were used while for ISSR ten primers were tested and six of them were used. PCR products were separated in 2.5% w/v agarose gel and digitally photographed under UV light. Genetic similarities for the RAPD and ISSR data were calculated using the Jaccard similarity coefficient. Phylogenetic trees were created using the UPGMA (Unweighted Pair Group Method with Arithmetic mean) and NJ (Neighbour Joining) methods. The correlation among all genetic similarity matrices was checked using the Mantel test. The analysis was performed using the NTSYS-pc v2.11x. Based on the results from both methods, but primarily from ISSR, intra-varietal variability was present in both varieties.

### S08.254

#### Morphological and Molecular Characterization of Selected Iranian Olive Cultivars - A Comparative Analysis

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Morphological traits and SSR molecular markers were used to assess levels of polymorphism across seven Iranian olive cultivars. Twenty eight morphological characteristics including both quantitative and qualitative trait were measured. Morphological characteristics included fruit length, fruit length/width ratio, stone length and width, stone length/width ratio, and number of stone groove were highly responsible for the variation of the olive varieties. The dendrograms obtained by UPGMA method on morphological data revealed that the combination of quantitative and qualitative traits enable to discriminate olive cultivars efficiently. On the other hand, clearly separation of studied varieties was achieved based on microsatellite markers. The present study elucidates that the UPGMA cluster analysis based on morphometric data or using microsatellite markers allowed efficient identification of olive cultivars.

### S08.255

#### Clonal Selection in the Italian Olive Cultivar 'Ottobratica': Morphological and Molecular Variability

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Ottobratica is one of the main olive oil cultivar in Calabria, a region that in Italy ranks second for olive growing. Due to high productivity and ability of the tree to stand many abiotic and biotic stresses, Ottobratica widespread: its cultivation extend in territories that run from the Tyrrhenian to the Ionic sea shores. The cultivar is easy to identify due to the tree vigor and the upright habit; fruits, stretch shaped, small sized, rich in testy and flavored, high quality oil. Unfortunately fruits are extremely sensitive to leprosy (*Gleosporium olivarum*) a fungus that affect tree productivity and olive oil quality. To contribute to resolve this problems some years ago has been planned an extended territorial research activity, carried out in the above mentioned area, to select putative clones on the basis of morphological and molecular traits. Morphological characters have been evaluated using as reference the descriptor list proposed by the International Olive Oil Council (IOOC). The molecular analysis has been performed by microsatellite markers or SSR using a set of twelve SSR markers, previously tested in olive and having high polymorphism. On the whole the research contributed to clear up many cases of homonyms and synonyms. By the integration of morphological and molecular data have been detected a large variability within the cultivar Ottobratica. Eight different genotypes have been identified 8 some of which produced oval, some others round shaped fruits. Molecular analysis evidenced deep differences among the clones selected in the growing areas close to the Ionic and to the Tyrrhenian shores. One of the accession showed a certain degree of tolerance to the leprosy encourages to continue the activity of clonal selection, particularly in the Tyrrhenian side, where the above mentioned fungus causes many problems to maintain high the tree productivity and excellent the olive oil quality.

### S08.256

#### Earliness of Bearing in Olive Progenies

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An overall analysis of 739 seedlings from 12 crosses among 8 olive cultivars planted in 1998 for earliness of bearing has been carried out in the Joint Olive Breeding Program University of Córdoba - IFAPA (Andalusian Institute for Agricultural Research). An earliness of bearing scale [from 1 (no flowering after 6 year of germination) to 4 (flowering at 4 year of germination)] was used. Additionally, plant vigour [diameter (mm) of trunk and the height (cm)] was recorded. Cross effect was not studied due to illicit pollination caused by airborne pollen were detected among the seedlings by SSR paternity tests. The involvement of certain cultivars as female genitors affected the earliness bearing of their seedlings. When cv. Arbequina was used as female genitors their progeny showed a shorter JP than those which had cv. Lechín de Sevilla as female genitors. The plant vigor showing at the plantation



time was significantly correlated ( $P < 0.0001$ ) with the earliness of bearing. Thus, no plants, which showed less than 80 cm in height at this moment, flourished before five years of their germination. Besides, flowering was significantly related, according to logistic regression ( $P < 0.0001$ ), to plant height for all data ( $n = 739$ ). However, for each female genitors the inflection point (height at which the 50% seedling have flowered) of the curve took different value. When cv. Arbequina was used as female genitors the inflection point was 302 cm; on the contrary, when cv. Lechin de Sevilla was the female genitors the inflection point was 328 cm. These results reinforce the strategy of forcing growth of olive seedlings to shorten their JP.

### S08.257

#### Morphological and Molecular Characterisation of French Olive Varieties to Establish a Reference Data Base

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Although the limited area, the olive germplasm in South France is highly diversified since about 150 cultivars were described. The richness of cultivated olive and the lack of characterisation may explain the confusion of the varieties names. We propose a characterisation methodology based on morphological traits and molecular markers. Based on variation level, no redundancy information and discriminating power, we examined the efficiency of SSR locus and morphological traits to characterise 91 French olive varieties in comparison to 13 main Mediterranean varieties. To identify easily French olive varieties, we propose a key based on 8 discriminating morphological traits and 10 selected SSR locus. This study allowed to construct a reference data base of olive cultivars growing in France.

### S08.258

#### The Use of Microsatellite Markers for Germplasm Management in Spanish and Italian Olive Collections

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Olive is a Mediterranean fruit species that is cultivated mainly for oil but also for canned fruits. In addition to the international olive germplasm collection at Cordoba (Spain) preserving major olive varieties in the world, several collections exist in other Mediterranean countries. In particular, the olive germplasm collection located in the "CRA - Centro di ricerca per l'olivicoltura e l'industria olearia" (CRA-OLI, Italy) contains the major part of the Italian olive germplasm. The goal of such collections is to safeguard all cultivars, and particularly the minor ones, to avoid a loss in genetic diversity and to offer an interesting genetic basis for breeding programs. We used molecular markers to characterize all accessions and to study genetic relationships between cultivars. More than 200 olive accessions were genotyped using 14 SSR markers. This study allowed us to construct a molecular data-base for the reference collection and to analyse genetic diversity for further prospecting, and to introducing new olive accessions.

### S08.259

#### Current Results of an Olive Cultivar Trial at High Density

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The comparative behaviour of cvs. Arbequina, Arbequina IRTA-i•18, Arbosana,

Koroneiki, and Fs-17, included in a ten years old comparative hedgerow trial are presented. Cultivar Koroneiki was the most precocious of them, bearing at the second year after plantation. However, the rest of cultivars bore at the third year after plantation. After eight harvests, all the cultivars except Fs-17, showed no significant differences in accumulated fruit yield. The average fruit yield per year was around 2.000 kg oil ha<sup>-1</sup> for all cultivars except for Fs-17 which was significantly less productive than the rest. Although some significant differences were found in oil content in fresh weight, this did not affect to the accumulated oil yield that followed the same pattern than fruit yield. In the last harvest, performed ten years after planting, the global yield of the trial was not deviated from the average of the time series, indicating that the hedgerow is still fully productive.

### S08.260

#### Olive Industry in Jordan

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Jordan is one of the Middle East countries that lies between latitudes 29 °N and 33 °N and longitudes 34 °E to 39 °E which enables olive growing. Olive industry in Jordan witnessed a tremendous progress within the last 30 years (1980-2009). The progress included production of nursery olive trees using modern techniques and adoption of intensive olive growing, training and pruning systems. A landmark of olive orchards management was observed in all aspects of production starting with orchard site choice, fertilizers and fertilization, supplemental irrigation and methods of irrigation, olive cultivar choice, semi-mechanical harvesting, organic olive production, integrated pest management, and finally olive processing and olive oil extraction. For these reasons, Jordan at present is self-sufficient in olive production and is exporter. The statistics of 1987 and 2007 indicate that total number of grown trees jumped from 346,148 to 11,127,000, respectively. Olive production and area planted with olive increased in 1997 from 57145 metric tons to 146829 ton in 2008 and from 61602 ha to 64532 ha, respectively. The increased olive production is related directly to the irrigated horticulture which enabled growing olive trees in the eastern part of the country using underground water. Table olive and olive oil produced, exported, and imported changed dramatically during the period 1986 to 2006. For example, in 1986 table olive produced, exported, and imported was 31800 ton, 1200 ton, and 300 ton, respectively, while in 2006 these figures changed to 146800 ton, 2700 ton, and zero ton, respectively. For olive oil, amount exported and imported in 1986 was 1400 ton and 7400 ton, respectively, while in 2006 the exported and the imported amount of olive oil was 2500 ton and zero ton, respectively. In conclusion, future of olive industry in Jordan on the short and the long run is promising.

### S08.261

#### Olive Maturation Process and Oil Quality

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The olive oil quality results from several variables in relation to environment-genotype interactions, cultural practices, olive processing but the time of picking is the main determinants of olive quality, oil flavour and antioxidant content. Climate of northern cultivation areas results in a narrow window for optimum picking. Therefore the evaluation of the fruit maturation level is of striking relevance. A study was undertaken on maturation of some olive varieties of local origin grown in Veneto (Italy) between 45°13' and 45°59' Lat North. Phenological stages and fruit growth were determined during 2008-2009 and ripening was followed with destructive and non-destructive methods i.e. fruit weight and firmness, skin colour in the space L\*, a\*, b\*, and evolution of volatile compounds (VOC). Technological and sensorial quality of the oil was also determined between mid October and mid November. High variability of fruit maturation was observed inside the canopy as far as sunlight exposition and height were concerned. Statistical analyses of VOCs emissions together with sensory analyses, clearly demonstrated that different groups of VOCs are biosynthesised in a co-ordinated manner in coincidence with specific stages of fruit ripening, some of them being "expressed". The removal of oxygen during the olive oil extraction steps can have significant effects only on the VOCs profiles obtained from early (unripe)

and late (overripe) harvested olives. These data suggest that the determinants of olive oil aroma are highly dynamic and significantly influenced by the ripening stage of olives, and can be transiently present depending on the cultivar under investigation.

### 508.262

#### Influence of the Light on the Stability and Shelf Life of Olive Oil

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Extra virgin olive oil is obtained from olives using only mechanical processing steps under slight thermal conditions. Although this oil has a great quality, the shelf life during storage is limited due to the oxidative and hydrolytic degradations. The main factors involved in oxidation are high temperatures and light accelerating rancidity and catalyzing the process by increasing peroxide formation. The effect of light was studied in several works but in other conditions (commercial olive oils, short storage or other varieties). The aim of this work was to study the effect of light on the stability in the storage (during nine months) of Verdeña olive oil extracted in Abencor. The storage conditions used were transparent plastic containers at room temperature in the presence of direct light or in darkness. Physico-chemical and nutritional parameters of olive oil were analyzed after extraction and along storage. During the storage period an increase in the free acidity, peroxide index, absorption coefficients K232 and K270 was observed in both cases but higher in the presence of direct light. The alpha tocopherol content decreased at the same time more sharply also in the presence of light. In other side total phenols content decreased in a similar way for both cases in darkness and in the presence of light. The results obtained indicated that direct light decreases the shelf life of olive oil and it is advisable to keep olive oil at adequate temperature and in opaque containers.

### 508.263

#### Fruit Bruising in Table Olives

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Fruit bruising caused by physical impact is a major problem for mechanical harvesting of table olives, particularly in the case of green processing. We have initiated studies of the bruising process which include timing of the appearance of the surface discoloring, comparison of the degree of bruising among three different table olive cultivars and 14 new genotypes from a breeding program, and histological observations. Timing was monitored by photographs at successive times following standardized bruising by dropping from one meter high onto a floor field. To compare the degree of bruising among genotypes and cultivars a visual scale was established for evaluating fruits, which were processed in lye and saltwater according to standard commercial procedures. Significant differences have been observed among genotypes, which have been distributed in four groups according to their sensitivity to the bruising. Histological sections of bruised and unaffected fruit tissues revealed a subsurface zone of tissue discoloration but a much greater area in which cell structure was altered but not damaged. Those observations support the hypothesis that the bruising phenomenon consists of both chemical and physical processes which differ in the area which is affected and the progression of the effect.

### 508.264

#### Total Phenolic Content, Phenolic Profile and Antioxidant Activity in Leaves and Drupes of Greek Olive Cultivars

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The olive products, olive oil and table olives, play an important socioeconomic

role in the Mediterranean countries and an integral part to the Mediterranean diet, a diet with high content of bioactive substances such as vitamins, flavonoids and polyphenols. The protective effects of phenolic compounds as antioxidants, against cardiovascular diseases and certain types of cancers are well known. The olive plant phenolic profile and content depend on many factors, such as, genotype, tissue type, developmental stage, environmental conditions, and cultural practices. In the present study the phenolic profile, the total amount of phenolics and their antioxidant activity were studied in different olive cultivars and tissues. Ten olive cultivars, 'Koroneiki', 'Lianolia Kerkyras', 'Mastoidis', 'Arbequina', 'Adramytini', 'Megaritiki', 'Gaidourelia', 'Kalamon', 'Konservolia' and 'Chalkidiki', were studied in order to determine the phenolic profile, the phenolic content, and the antioxidant activity, both in leaves and drupes (green and black). Total phenolic content, in new season leaves, ranged between 1.01 mg GAE / g (Adramytini) and 2.06 mg GAE / g (Konservolia), while the antioxidant activity ranged between 157.64 ppm (Konservolia) and 285.80 ppm (Adramytini). In green drupes, total phenolic content ranged between 0.59 mg GAE / g (Megaritiki) and 1.98 mg GAE / g (Mastoidis) while the antioxidant activity ranged between 119.66 ppm (Lianolia Kerkyras) and 496.45 ppm (Megaritiki). Total phenolic content in black drupes ranged between 0.54 mg GAE / g (Gaidourelia) and 0.99 mg GAE / g (Lianolia Kerkyras), while the antioxidant activity ranged between 284.23 ppm (Lianolia Kerkyras) and 886.36 ppm (Chalkidiki). The HPLC analysis showed the presence of flavonoids, hydroxycinnamic derivatives and secoiridoids. In leaves the most abundant compounds were eleuropein, luteolin-7-o-glucoside and rutin while luteolin-4-o-glucoside, hydroxytyrosol and caffeic acid were detected in lower amounts. In drupes the most abundant compounds were verbascoside and eleuropein.

### 508.265

#### Biochemical Limiting Factors Affecting the Synthesis of Virgin Olive Oil Volatile Compounds

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Benefits of virgin olive oil (VOO) consumption are related to protection against cancer and cardiovascular diseases due to its fatty acid profile and presence of minor constituents. However, the increase in the demand for high quality VOO can be attributed not only to its potential health benefits but to its unique organoleptic properties. The aim of increasing the quality standards for VOO is continuously stimulating the study of biochemical pathways related to organoleptic properties and the development of technological procedures to improve those organoleptic properties. In this sense, the limiting factors affecting the biosynthesis of VOO aroma through the lipoxygenase (LOX) pathway were studied. Results suggest that, besides the availability of nonesterified polyunsaturated fatty acids, the biosynthesis of VOO aroma depends mainly on the enzymatic activity load of the LOX/hidroperoxide lyase system during the process to obtain the oil. This enzymatic activity load seems to be cultivar dependent, thus shaping the content of volatile compounds present in the oils from the different olive cultivars.

### 508.266

#### Influence of Cultivar, Harvesting Season and Geographical Origin on Phenolic Content in Leaves of Greek Olive Varieties

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The influence of cultivar, harvesting season and geographical origin on the concentration of phenolic compounds and their antioxidant activity of 'Kalamon' and 'Konservolia' leaves have been studied. The leaves were collected from three different locations, the Agricultural University of Athens orchard, Rovies Evoias and Poros in June and October 2009. Total phenolic content and antioxidant activity of

the samples were determined according to Folin-Ciocalteu and DPPH methods, respectively, while separation and identification of phenolic compounds was carried out by HPLC analysis. Total phenolic content for 'Kalamon' ranged between 1,03-3,42 mg GAE/gr dry tissue and for 'Konservolia' between 1,32-2,63 mg GAE/gr dry tissue, while the values for the antioxidant activity ranged between 108,24-1018,7 mg/l for 'Kalamon' and 125,47-884,48 mg/l for 'Konservolia'. The highest values for total phenolic content were detected in leaves of 'Kalamon' (3,42 mg GAE/gr dry tissue) collected from Poros in October, followed by 'Kalamon' leaves collected from Rovies Evoias in June (2,83 mg GAE/gr dry tissue). Leaves collected from Rovies in October showed the lowest total phenolic content for 'Kalamon' and 'Konservolia' (1,02 and 1,32 mg GAE/gr dry tissue, respectively). The HPLC analysis showed the presence of flavonoids, hydroxycinnamic derivatives and secoiridoids. The most abundant flavonoids were rutin, luteolin-7-o-glucoside and luteolin-4-o-glucoside while the most abundant secoiridoid and hydroxycinnamic derivative were eleuropein and p-coumaric acid, respectively.

### S08.267

#### Effect of Deficit Irrigation on Fruit and Oil Quality of 'Konservolea' Olives

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The aim of this work was to evaluate the effects of deficit irrigation on quality of olives at harvest and on the oil after processing. Olive trees (*Olea europaea* L. cv Konservolea) from Anchialos (Greece) were subjected for 3 years (2006-2008) to 2 different irrigation regimes: fully irrigated (Ctrl) and deficit irrigation (deficit). Control trees received 4000-5000 m<sup>3</sup>/ha of good water (conductivity <700 µS/cm) per year; deficit trees were irrigated as control during the growing season while deficit treatment was performed only during stone hardening and final flesh swelling when water supplied per day was the 20% of the control. The data of 2008 are presented. At harvest, respiration rate, firmness, color score (maturity index), size and shape, water and oil content were determined on drupes. On oil samples extracted at harvest, the following quality attributes were measured: color, acidity, peroxide value, extinction coefficients at 232 and 270 nm, oil stability, total polyphenols, tocopherols, carotenoids, chlorophyll, and fatty acid composition. Oil content was significantly higher in deficit treated fruits, while no other differences were observed on fruits. Yield was 9.6 MT/ha, with no difference related to irrigation treatments. Reduced water supply lead to some improvement in oil quality, as indicated by a lower acidity and higher stability, polyphenols and chlorophyll contents compared to the control. Besides, deficit treatment showed a statistically higher content in palmitic acid, whereas irrigated samples had a higher contents in palmitoleic, linoleic, linolenic and arachic acids. As a consequence, MUFA/PUFA ratio was significantly higher in oils obtained in deficit irrigation conditions. No difference between treatments were observed for sensorial attributes of oil extracted.

### S08.268

#### Aspects to Be Considered in Olive Mill Design Associated to New Olive Orchard Projects

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Over the last years many olive projects have been developed focused on large high density orchards, including the construction of a mill, in order to produce virgin oil *in situ*. Moreover, some traditional and small properties have considered to install their own mill, to achieve a total control from the production until the distribution through a top market with a higher commercial value. The aim of this work is to define the technology, quality, environmental and economic aspects which should be considered for the design of these mills. Five models are compared according to their surface and orchard design: very small (5-10 ha), small (25 ha), traditional, in-

tensive and super high density orchards (50-100 ha). Both milling and amortization costs are computed. For each one, the daily olive harvest and the total campaign duration are defined, according to the specific harvest system (hand shakers, trunk shakers with or without fruit reception systems or over-the-row harvesters) and synchronizing harvesting and milling. For small orchard models, compact mills by centrifugation are considered (nominal capacity less than 500 kg/hour), which can be installed on platforms with the auxiliary machinery, occupying reduced space and with the only need of one switching point for water and electricity. These equipments require less investment, but elaboration costs are higher. For the rest of the orchard models, complete small-medium industrial lines are considered (15-60 tm/day). Early harvesting and a rapid olive delivery to the mill are required to guarantee oil quality. In these projects the main aspects considered are related to product quality, image and business strategy, considering also agronomy and environment. The viability of these olive projects depends on the financial and commercial capacity of the manager, as the economic index has not always been favorable.

### S08.269

#### Extraction of Bioactive Compounds from Olive Leaves

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Olive leaves are an agricultural residue resulting from pruning of olive trees and from cleaning of the olive fruit in mills before the oil production. This by-product is underutilized, because it is generally used only as feed for livestock, or crushed together with minor branches from pruning and spread on the soil, or simply burned. However, the olive leaves could be an important economic resource, as they contain considerable amounts of various bioactive compounds (ubiquitous in plant kingdom or peculiar of the olive tree), which could be extracted and utilized both in foodstuffs and in pharmaceutical industries. In order to evaluate the possibility of the utilization of the olive leaves as a source of valuable compounds, various methods of extraction were investigated, taking in account the recovery and recycle of the solvents and the potential use of the extracted solid residue. In the tests carried out groups of compounds like terpenic acids, chlorophyll and polyphenols were obtained and, among phenolic compounds, oleuropein and flavonoids were further separated. The results of the experiments indicated that the separation of groups of compounds which display pharmacological effects from olive leaves could be, as well as profitable from an economic point of view, also sustainable for the soil and the environment, provided that this material, devoid of pesticides, is suitably picked, stored and processed in order to prevent it is damaged by chemical or enzymatic reactions.

### S08.270

#### Regulated Quality, Stability and Chemical Composition of the Virgin Olive Oils from Campos de Hellin (Albacete, Spain)

**Alvarez-Orti, M.; Sena, E.; Cuesta, M. A.; Alvarruiz, A.; Granell, J. D.; Zon, A.; López, E.; Pardo, J. E.**

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The olive oil is a key product from the Mediterranean Basin. Spain is the first producer of olive oil in the world, making this product important for the economy of many rural areas, and being one of the few crops capable of maintaining the population in the rural environment. In this work, the regulated physicochemical and sensory parameters, the stability parameters and the fatty acids, sterol and triterpenic dialcohol compositions of the oils from the area Campos de Hellin are analyzed. The varieties grown in this area are: Arbequina, Benizal, Cornicabra, Cuquillo, Injerta, Manzanilla de Sevilla, Manzanilla Local, Negrilla and Picual. In this work, we analyse the potential and real quality of monovarietal and mixed olive oils produced in the area of Campos de Hellin, based on physicochemical and sensory parameters. In addition, the chemical composition is evaluated, and the fatty acids and sterols patterns are analysed.

**S08.271**
**Regulated Quality, Stability and Chemical Composition of the Virgin Olive Oil from Montes de Alcaraz (Albacete, Spain)**
**Alvarez-Orti, M.; Granell, J. D.; Cuesta, M. A.; Alvarruiz, A.; Zon, A.; López, E.; Rubio, M.; Pardo, J. E.**
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The olive oil is a key product from the Mediterranean Basin. Spain is the first producer of olive oil in the world, making this product important for the economy of many rural areas, and being one of the few crops capable of maintaining the population in the rural environment. In this work, the regulated physicochemical and sensory parameters, the stability parameters and the fatty acids, sterol and triterpenic dialcohol compositions of the oils from the area Montes de Alcaraz are analyzed. The varieties grown in this area are: Cornicabra, Manzanilla de Centro, Manzanilla Local, Onil de Povedilla and Picual. The main characteristics of the oils from the area Montes de Alcaraz are the high stability due to the high proportion of the Picual variety with high total polyphenol content; a high oleic acid and low linoleic acid content; and low content in stigmaterol. As regards sensory attributes, the oils of this area showed a median-high fruity, with touches of bitter and pungent, characteristics of the varieties of this area.

**S08.272**
**The Quality Certification Like Element of Virgin Olive Oil Quality Improvement in Olive Oil Mills**
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Olive Oil Mill is a food industry. One of the problems of the sector is that often mills are not considered food industries and does not apply the correspondent regulation in terms of hygiene and design. This lack has an influence on the quality of virgin olive oil obtained. Olive Oil Mills belonging to a project of virgin olive oil quality improvement have been monitored during the olive oil campaign 2009/2010. Raw material, pomace, working parameters and the quality of the virgin olive oil obtained have been controlled. The possibility to introduce a quality certification typical of food industry has been evaluated. The introduction of ISO 22000, as well as others food international standards, could be an element to improve virgin olive oil quality produced by the mills. This because the introduction of a management quality system imply a change of mind, that is necessary to obtain better virgin olive oil quality, not just in the monitored area, but in all Mediterranean area.

**S08.273**
**Intra-Varietal Variability of the Greek Olive Varieties Kalamon and Koroneiki Using Molecular Markers**
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The olive tree (*Olea europaea* L.) is cultivated in the Mediterranean Basin since 4000 B.C. Its socioeconomic impact is very important for the countries in the area. Greece occupies the third place in the world rank of olive oil producers and the second place in the European Union as a table olive producer. 'Koroneiki' is an olive oil variety while 'Kalamon' is a table olive variety. Both of them are the most well known worldwide Greek olive varieties. In this study, healthy, young leaves of both varieties were collected from seven different regions in Greece and Cyprus in order to study the intra-varietal variability. DNA extraction was performed according to Doyle & Doyle protocol. Markers originating from two different molecular techniques, Randomly Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR), were used for investigating the germplasm variability. In order to establish the genetic relationships among the clones of 'Kalamon' and 'Koroneiki', twenty five RAPD primers were tested and ten were used while for ISSR ten primers were tested and six of them were used. PCR products were separated in 2,5% w/v agarose gel and digitally photographed under UV light. Genetic similarities for the RAPD and

ISSR data were calculated using the Jaccard similarity coefficient. Phylogenetic trees were created using the UPGMA (Unweighted Pair Group Method with Arithmetic mean) and NJ (Neighbour Joining) methods. The correlation among all genetic similarity matrices was checked using the Mantel test. The analysis was performed using the NTSYS-pc v2.11x. Based on the results from both methods, but primarily from ISSR, intra-varietal variability was present in both varieties.

**S08.274**
**Studies on Flower and Fruit Abscission in *Olea europaea* L.**
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The study of reproductive organs abscission in olive has received attention in the past decades, but only when trees were treated with fruit loosening agents. Less attention was given to the course of natural flower and fruit drop, and the anatomy of the abscission process of the same organs is still waiting for a detailed study. The scope of our work was to follow the natural abscission process to individuate any relations between fruit characteristics and its capacity to persist on the tree until completion of the natural ripening process. We also carried out preliminary anatomical investigations on the development of abscission zones. On trees of cv 'Leccino' measurements were made in 2005 and 2006 on the course of flowering, fruit set and June drop, fruit growth and final fruit drop, in the period between inflorescence emission and natural drop of the last fruits. Fruit samples were taken for anatomical observations. The first results indicate that abscission dynamics depend on both climate and peculiar fruit conditions (position on the canopy, exposure, type of shoot, position on the inflorescence). Anatomical modifications were also observed during the abscission process in the pedicel, involving the cell wall of the competent cells and other histochemical modifications in the neighbouring tissues.

**S08.275**
**Influence of Copper, Kaolin and Propolis Used in Olive Fly Management on Olive Oil Quality in Three Organic Olive Groves of Apulia, South-Eastern Italy**
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The olive fly is a key-pest in the Mediterranean. Copper allows its effective management in organic groves but its use is restricted. Therefore, alternative products such as propolis and Kaolin have been tested recently. Nevertheless, few information are available on their impact on olive oils quality. This work, carried out in 2007 in three olive groves of south-eastern Italy on Coratina and Termite di Bitetto cultivars, aimed at testing: i) copper oxichloride, kaolin (Surround® and Kaolin®) and propolis influence on oil quality, and iii) residues of trace elements (Al, Si and Cu) in olives and oils. Two applications have been performed in September/October and November using labels application rates. Quality criteria such as acidity, peroxide index, K232, K270, ΔK, and panel test were evaluated. Trace elements were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Olive oils organoleptic assessment showed that neither olive fly infestation nor treatments affected significantly oils sensorial features. Oils from the different treatments were all extra virgin. Organoleptic profiles of Coratina oils presented yellow to green coloration. Olfactory sensations in control, Surround® and Propolis were typical of olives harvested and milled during colour change stage. In Kaolin® and copper treatments, sensorial analysis showed ripen fruity sensation. Predominant direct aromatic olfactory sensations in oils were almond and grass. Concentrations of Al and Si in Kaolin-treated olives and of Cu in copper-treated olives were higher than the control but remained below the maximum residues levels (MRL). Trace elements residues in oils were very low, especially if compared with olives. Copper is probably discarded in the olive mill waste water. The major part of Al and Si, in kaolin-treated plots, could be eliminated during the olives milling as kaolinite. Therefore, trace elements concentrations in olives would not present any significant hazard for consumers.