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Ph.D. Thesis

Combined salinity and ambient ozone stress effects on olive physiology and biochemistry

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Daoud Abu safieh

**Combined salinity and ambient ozone stress effects on olive
physiology and biochemistry**

Dedication

To my wife, Aseel

To my childrens, Ismail, Sana and Rasha

To my mother, Hanan

To the memory of my father, Ismail

Abstract

The presence of high ambient ozone concentrations is common in the Mediterranean region, while saline water is often used for olive irrigation. The effects of this combined stress on physiology and biochemistry were studied in young olive trees. Two-year-old 'Konservolea' and 'Kalamata' olive plants (*Olea europaea* L.) grafted on seedling rootstock were grown in sand:perlite mixture irrigated with half strength Hoagland's solution containing or not 100 mM NaCl. In open top chambers, the plants received outside air with ambient ozone or charcoal-filtered air from April to October in 2006 and 2008. Specific leaf mass, chlorophyll content, net photosynthetic and transpiration rate, stomatal conductance, chlorophyll fluorescence, midday stem water potential and superoxide dismutase, catalase and ascorbate peroxidase activities were measured periodically from June to September. On October 15, 2006 and 2008, the plants (after sampling fresh tissues for soluble sugar and starch analysis) were divided into roots, trunk, old shoots, new shoots, old leaves and new leaves. Shoot length and total leaf area were initially measured and dry mass at each plant part was measured after complete dryness. Both studied olive cultivars showed similar behavior to salinity stress possibly due to the seedling rootstock on which both cultivars were grafted. Irrigation with 100 mM NaCl solution negatively affected most of the leaf physiological parameters evaluated resulting in reduced productivity. This was clearly evaluated by the dry mass partitioning data, where total tree dry matter decreased due to salinity as shoot length, leaf surface area and dry matter accumulated to new leaves, new shoots and roots was significantly reduced. Salinity also increased neutral sugar concentration in new shoots and leaves and decreased neutral sugar concentration in roots. The opposite was often found for starch concentration. Ambient ozone concentrations from May to September able to damage plants (daylight mean ozone concentration >60 nL L^{-1}) did not affect the olive leaf functions studied, dry matter accumulation and partitioning and stored sugar metabolism to any of the plant parts of young olive trees. This clearly shows that young olive trees are relatively resistant to ozone levels found around the Mediterranean region. The combination of salinity and ambient ozone stress did not result in any further effects to leaf physiological parameters besides the ones from the salinity stress alone.

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Chapter 1. Literature review

1.1. General introduction

The activities of humans in generating energy, manufacturing goods, and disposing of wastes result in the release of a number of pollutants into the atmosphere that may affect plant growth and productivity. Air pollution damage to plants, especially around certain types of factories, has been recognized for about a century (Emberson et al., 2003). Its extent and importance, however, have increased with continued industrialization and will, apparently, increase further with the world's increasing population and urbanization. Almost all air pollutants causing plant injury are gases, such as O_3, NO_x, and SO_2, but some particulate matter or dusts may also affect vegetation.

The Mediterranean basin is characterized by high temperatures and solar radiation intensity during summer combined with stable air mass and high emission of air pollutants. These conditions favour massive photochemical production of O_3. Three classes of transport phenomena have been recognized as strongly influencing the background O_3 concentration in the southern Europe and the Mediterranean Basin: (i) transport of O_3 and its precursor over regional, continental or inter-continental scales; (ii) stratospheric intrusion events; (iii) transport of mineral dust from the Sahara desert. (Cristofanelli & Bonsoni, 2009). In Greece, hourly average O_3 concentrations higher than 100 nL L^{-1} were reported (EEA, 2009). These concentrations exceed the O_3 critical levels AOT40 (Accumulated exposure Over a Threshold of 40 nL L^{-1}) in terms of vegetation protection (Fuhrer et al., 1997).

Water scarcity in the Mediterranean basin appears as one of the main factors limiting agricultural development, particularly in the period 2000-2025. During the next 25 years, although irrigated areas will increase, sustainable quantities of fresh water supplies will be diverted from agriculture to meet the growing water demand in the municipal and industrial sectors in the Mediterranean region (Correia, 1999; Hamdi et al., 1995). In order to overcome water shortages and to satisfy the increasing water demand for agricultural development, the use of low quality (brackish, reclaimed, drainage) water is becoming important in many countries (Chartzoulakis, 2005). However, the underground aquifers are often contaminated from sea water in many coastal Mediterranean areas and highly saline water is often used for olive irrigation.

The effects of O_3 or salinity as single stress factors in plant growth and physiology have been well studied and this information can be used to assess their potential or actual impact on agricultural productivity. As knowledge of crop responses to ozone or salinity and the combination of these stresses becomes more advanced, it can support management programmes aiming to maintain productivity or to prevent or ameliorate environmental degradation. Effective action does not only depend on controlling the pollutant: the use of salt-tolerant crop strains, for example, enables agriculture to be adapted to obtain acceptable yields in areas with soils affected by natural or human-induced salinity.

Although management may be deployed to deal with particular environmental stresses, where expertise and resources are available, it is frequently focused on a single problem. The combined effects of different types of stress are not often well understood and integrated control and management is rare. Plants grow in both soil and air, and the simultaneous application of different stresses in the two environments affects plant growth in a more complex way than can be predicted by simply adding known responses to pollution in anyone environment. When a crop is exposed to salinity, there is very limited information available to assess whether simultaneous ozone exposure will have additive impacts on growth or whether more complex response patterns will be seen.

This study examined, for the two major Greek table olive cultivars 'Kalamata' and 'Konservolea' grafted on seedling rootstock, the combined effects of exposure to soil and air pollution as salinity and O_3. Understanding such interactions is important as a basis for crop development and agricultural and environmental management. The study focuses on cultivars from Greece where O_3 pollution and salinization both occur and where the issues associated with agriculture, environmental management, urban growth and industrial expansion are extensively present and important.

1.2. Chemistry of ozone formation

Ozone (O_3) is a strong oxidant naturally occurring gas present in both the stratosphere (the 'ozone layer', 10–40 km above the surface of the earth) and in the troposphere (0–10 km above the earth). Stratospheric O_3 protects the earth's surface from solar UV radiation. In the troposphere, the prevailing problem is the increasing O_3 concentrations that can be harmful to plants and animals. Emissions of nitrogen

oxides (NO_x) and volatile organic compounds (VOC) have created a potential for greatly increased tropospheric O_3 concentrations in much of the industrialized world. The major sources for NO_x and VOC are use of hydrocarbons for transportation, industry and energy production.

In sunlight, hv ($\lambda \leq 400$ nm), nitrogen dioxide (NO_2) dissociates into single atoms of oxygen (O) and nitrogen monoxide (NO):

$$NO_2 + hv \rightarrow NO + O \qquad (1)$$

The single atoms O combine with molecular oxygen (O_2) to form O_3:

$$O + O_2 \rightarrow O_3 \qquad (2)$$

However, NO reacts rapidly with O_3, which is then consumed in reaction (3) (Schlesinger, 1997):

$$NO + O_3 \rightarrow NO_2 + O_2 \qquad (3)$$

These three reactions, (1), (2) and (3), form a cycle which normally does not result in very high O_3 concentration. Another mechanism that involves VOC and competes with O_3 for NO is needed to obtain strongly elevated concentrations of tropospheric O_3. The majority of the VOCs are broken down in the atmosphere by a chain of reactions, some of which are dependent on light. If an organic compound of this kind is called RH, the reaction step we are most interested in may be described as follows. In the atmosphere, hydrocarbons are primarily broken down by reactions with free radicals. The most important is the hydroxyl radical $HO^{.}$ (the dot after chemical formula indicates that this particle has an unpaired electron, which is the main reason for its reactivity). Decomposition of hydrocarbons is initiated by:

$$HO^{.} + RH \rightarrow R^{.} + H_2O \qquad (4)$$

The free hydrocarbon radical $R^{.}$ reacts rapidly with O_2 to form a peroxy radical ($RO_2^{.}$):

$$R^{.} + O_2 \rightarrow RO_2^{.} \qquad (5)$$

It is this radical which subsequently plays a part in O_3 formation, since it can oxidize NO to NO_2 in competition with O_3:

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$$NO + RO_2^{\cdot} \rightarrow NO_2 + RO^{\cdot} \qquad\qquad\qquad (6)$$

Reaction (6) is important because NO is converted into NO_2 without O_3 being consumed as in reaction (3). Consequently, if suitable organic compounds are present in the air, NO_2 can be regenerated without O_3 consumption. However, a nitrogen oxide molecule cannot participate indefinitely in O_3 formation. This is due to the fact that there are competing processes which consume nitrogen oxides in the atmosphere. One of these is the deposition onto vegetation and other surfaces. Another is the chemical conversion of nitrogen oxides into other nitrogen compounds, which play no part in the generation of O_3, mainly nitric acid (Schlesinger, 1997).

1.2.1. Seasonality and daily variation of O_3 formation

Formation is not only dependent on the abundance of O_3 precursors but on the location of sources, meteorology and topography. High temperatures (>20 °C) and high solar radiation intensities are conducive to O_3 formation, providing the necessary energy to drive the reactions (Kovac-Andric et al., 2009).

The importance of climate in O_3 formation means that concentrations may show significant seasonal and daily variations. The timing of O_3 highs and lows is of significance in relation to the timing of crop development in different regions and means that high levels of O_3 may have a greater impact in particular regions.

The O_3 concentrations exhibited a diurnal and a seasonal fluctuation. High O_3 levels occurred from middle spring to middle autumn, when sunlight intensity was high enough to induce photochemical formations of O_3. The diurnal pattern was the typical one for urban areas; O_3 maximized during early afternoon hours, when sunlight also maximizes, and minimized during the night. The minimum O_3 levels occurred during the dawn and morning hours, when the O_3-destroying pollutants (mainly dust and NO_x) of anthropogenic origin (mainly traffic) began accumulating in the atmosphere, while the sunlight was not sufficient enough to induce photochemical formation of O_3 (Saitanis et al., 2004).

1.2.2. Transport of O_3

Tropospheric O_3 is a transboundary or even global air pollution problem. Emissions in one country have a decisive impact on O_3 concentrations in another country (Jackson, 2003). This is due to the fact that the chemical reactions between

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primary air pollutants, which are involved in O_3 formation, take some time and are regulated by sunlight, concentration changes, meteorological conditions and other factors.

O_3 and its precursors can be transported to rural areas up to several hundred kilometers from emission sources depending on weather conditions and transport mechanisms. O_3 concentrations will often be higher in rural areas that are downwind of urban areas than in the urban area itself. This is because as the polluted body of air moves away from the emission sources, O_3 continues to form and concentrations continue to rise as fewer scavenging mechanisms are operating than in the urban atmosphere (Comrie, 1994).

1.2.3. Future trends in O_3 concentration

Predicting future trends in O_3 concentration is difficult: the chemistry of formation is complex and there are numerous natural and human sources of the precursors. However, it is reasonable to assume that higher O_3 concentrations will become more widespread as the number of emission sources increases. Urban growth, population increases and industrial development will all lead to greater demands for energy generation, for transport and for agricultural land, which in turn result in forest clearance, with associated biomass burning and emissions of O_3 precursors. Furthermore projections based on the Intergovernmental Panel on Climate Change (IPCC) indicate that O_3 could increase by 20-25% between 2015 and 2050, and further increase by 40-60% by 2100, if current emission trends continue (Meehle et al., 2007).

1.3. Plant response to O_3 /Mode of action

The plant responses to O_3 may be viewed as the culmination of a sequence of physical, biochemical, and physiological events. Only the O_3 that diffuses into a plant through the stomata (which exert some control on O_3 uptake) to the active sites within a leaf impairs plant processes or performance. An effect will occur only if sufficient amounts of O_3 reach sensitive cellular sites that are subject to the various physiological and biochemical controls within the leaf cells. O_3 injury will not occur if (1) the rate and amount of O_3 uptake is small enough for the plant to detoxify or metabolize O_3 or its metabolites or (2) the plant is able to repair or compensate for the

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O_3 impacts (EPA, 1996; Tingey and Taylor, 1982). Therefore, a precondition for O_3 to affect plant function is that it must enter the stomata and be absorbed into the water lining the mesophyll cell walls. The response of each plant is determined by the amount of O_3 entering the leaves, which varies from leaf to leaf (EPA, 2006).

1.3.1. Stomatal O_3 uptake

O_3 uptake is a function of both ambient O_3 levels and stomatal conductance (Mauzerall et al., 2001). Plant injury is most closely related to the fraction of O_3 entering the plant through the stomata, i.e. the O_3 flux (Fredericksen et al., 1996; Sandermann et al., 1997). Plants exhibiting a higher rate of stomatal uptake undergo in many cases larger O_3 damage (Reich, 1987). O_3 itself is influencing stomatal aperture, because the stomata are injured so that they close prematurely and slow CO_2 movement into the leaf. Some toxic products might migrate into the chloroplast where they react and/or ionic balances are altered to induce metabolic shifts (Heath, 1994). Stomatal closure can also be the result of disturbances in the photosynthetic apparatus (Farage et al., 1991).

O_3 affects vegetation by direct cellular damage (especially to palisade mesophyll cells) once it enters the leaf through the stomata. Gaseous O_3 diffuses from the atmosphere, through the stomata, and dissolves in water surrounding the cells before entering the cells themselves (Mauzerall et al., 2001). The cellular damage is probably the result of changes in membrane permeability and may or may not result in visible injury or reduced growth or yield (Krupa and Manning, 1988). Stomata generally open in response to light and warmth and close in response to aridity, water stress, and high CO_2 (Mauzerall et al., 2001). A secondary response to O_3 is a reduction in stomatal conductance, as the stomata close in response to increased internal CO_2 that occurs because of the reduced photosynthetic activity caused by the O_3 (Reich, 1987, Runeckles and Palmer, 1987). Upon fumigation with O_3, a sharp decrease in stomatal conductivity is observed. However, the effect largely depends on a number of factors: first of all the O_3 concentration in the atmosphere, then, the exposure time; and finally, the peculiarities of plant species (Roshchina and Roshchina, 2003). More importantly, leaf age and environmental factors such as solar radiation, air temperature, relative humidity or leaf water status also influence the time that stomata are open and thus the potential damage caused by ozone (Drogoudi, 2000).

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1.3.2. Effects of O_3 on cellular level

O_3 is a strong oxidant molecule. Once inside the leaf, O_3 rapidly reacts with components of the cell walls, cellular membranes and apoplastic fluids. Upon these reactions, O_3 degrades into highly reactive oxygen species (ROS) such as superoxide ($O_2 \bullet^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\bullet OH$), and singlet oxygen ($^1O_2$). These ROS then go on to further reaction in the apoplast. Longer-lived ROS, in essence H_2O_2, which can diffuse through the plasma membrane via certain types of aquaporins (Bienert et al., 2007), can transmit the oxidant potential of O_3 into the cell. The apoplast is considered the primary site of O_3 action. However, O_3 and O_3-derived ROS have been shown to oxidize proteins, membranes, and other components in and outside plant cells (Brosché et al., 2009). Ozonolysis is suggested as the primary mechanism of O_3 action with lipids, and primarily yields aldehydes and peroxides (Mudd, 1996; Pryor, 1994). In addition, O_3 interactions at the plasma membrane could change the ionic balances within the cell and affect regulation of intracellular calcium levels, for example, which would initiate diverse responses in the cell, possibly leading to metabolic imbalances (Heath, 1999).

Plants have several antioxidant mechanisms that remove these toxic compounds, therefore protecting cell components and avoiding cellular damage. Apoplastic ascorbate (AsA) has been proposed to constitute the first line of defense against O_3 (Sharma and Davis, 1997). In addition to ascorbate, there are other antioxidant enzymes such as superoxide dismutase (SOD) that converts the superoxide anions into H_2O_2, catalase (CAT) that eliminates H_2O_2 and ascorbate peroxidase (APX) that reduces H_2O_2 into water. In addition, polyamines and phenolic compounds play a major role in the plant defense system against oxidative stress (Bors et al., 1989). Numerous studies have reported an increase of leaves' antioxidant enzymes activities in response to O_3 exposure (Chernikova et al., 2000; Larson, 1995; Rao et al., 1996; Sebastiani et al., 2002). In general, antioxidant enzyme activities in plant tissues show an age-related decline, and this may be one of the main factors that promote the onset of oxidative processes associated with senescence (Bender et al., 1994; Porchazkova and Wilhelmova, 2007).

1.3.3. Effects of O_3 on photosynthesis

Several studies have shown that the photosynthetic rate is significantly reduced by O_3 in sensitive crops (Flowers et al., 2007; Guidi et al. 2002; Long and Naidu 2002; Morgan et al. 2003). For instance, Morgan et al. (2003) reported that leaf photosynthetic rate in soybean was reduced 20% on average by exposure to 70 nL L^{-1} O_3 compared to carbon-filtered air. Similarly, photosynthetic rate of barley exposed to 180 nL L^{-1} O_3 decreased by 17% compared to 10 nL L^{-1} (Plazek et al., 2000). These effects are normally attributed to decreased stomatal conductance, direct oxidative damage to chloroplast, premature leaf senescence or a combination of these three mechanisms (Pell et al., 1997; Zheng et al., 2002). The relative contributions that stomatal and non-stomatal changes resulting from elevated O_3 make to photosynthetic reductions is not known and probably varies among crop species and with the timing, duration or level of O_3 exposure (Meyer et al., 1997).

However, the effects of O_3 on photosynthetic rate in fruit trees are variable and dependent on the plant species and O_3 exposure. Fumigation with ambient or near-ambient O_3 concentration reduced photosynthetic rate in two olive cultivars, Moraiolo and Frantoio, after 18 months of exposure (100 nL L^{-1} O_3) (Sebastani et al., 2002), in plum cv. Casselman (59 nL L^{-1} O_3) after four months of exposure (Retzlaff et al., 1992) and peach cv. Glohaven (34 nL L^{-1} O_3) only after 20 days of exposure (Paolacci et al., 1995). Contradictory results were reported by Retzlaff et al. (1991) for peach cv. O' Henry and nectarine cv. Fantasia; where the photosynthetic rate was not affected by 111 nL L^{-1} O_3 after 3.5 months of exposure. Also, in orange trees cv. Valencia, a seasonal mean O_3 concentration of 73 nL L^{-1} in ambient air for 4 years did not affect the photosynthetic rate (Olszyk et al., 1991).

A large body of literature published since 1996 shows that O_3 exposure reduces Rubisco concentrations, the main photosynthetic enzyme for dark reactions (Pell et al., 1997). Treatment of a variety of plants with O_3 at near-ambient levels results in a loss of Rubisco and of the mRNA coding for both subunits of Rubisco (rbcS, small and rbcL, large). In addition, oxidation of Rubisco by O_3-generated ROS may be an important factor in suppressing photosynthesis (Pell et al., 1997). Increased carbonyl concentrations of Rubisco are correlated with O_3 injury (Kanoun et al., 2002; Leitao et al., 2003). Because Rubisco plays such an important role in the production of carbohydrates, any loss may have severe consequences for the plant's productivity. O_3

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has also been shown to cause breakdown of photosynthetic pigments, including chlorophyll (Donnelly et al., 2001; Meyer et al., 2000; Morgan et al., 2003), which lead to a decrease in the efficiency of light harvesting and thylakoid electron transport, and, thus, availability of NADPH and ATP to drive the dark reactions of photosynthesis (Krupa et al.,2001).

Chlorophyll a fluorescence analysis is an effective non-destructive tool for the *in vivo* detection of stress to the photosynthetic apparatus. It is used extensively in the evaluation of O_3 impacts onto the photosynthetic apparatus (Chang and Yu, 2001; Farage and Long, 1999; Guidi et al., 1997). The principle of chlorophyll a fluorescence analysis is that the light energy absorbed by chlorophyll undergoes one of three fates: it can be used in photosynthesis, dissipated as heat, or be re-emitted as light. An increase in one of these processes will therefore cause a decrease in the other two. Changes in chlorophyll fluorescence, or re-emission of light, can provide information on changes in the efficiency of photosynthesis (photochemistry) and heat dissipation (non-photochemistry). As the reduction of photosynthetic rate would lead to other negative effects, such as reduced levels of carbohydrates and reduced growth, this analysis is useful in the early detection of plant stress induced by O_3 (Armond et al., 1980). In crop plants, O_3 stress has been shown to negatively affect the maximum (Fv/Fm) and effective (Φ_{PSII}) quantum yield of photosystem II (PSII) photochemistry, to decrease the relative fraction of open PSII reaction centers photochemical quenching coefficient (q_p), and to favour heat dissipation (non photochemical quenching, q_{NP}) (Calatayud et al., 2002; Castagna et al., 2001;). Similar observations have been shown in the case of seedlings of several tree species (Guidi et al., 2001; Ribas et al., 2005), although lack of response was reported as well (Maurer et al., 1997). A reduction in maximum quantum yield of PSII photochemistry (Fv/Fm) indicates photoinhibition (Calatayud and Barreno, 2004; Calatayud et al., 2003).

The most widely observed chemical change induced by O_3 in the chloroplast is the destruction of chlorophyll (Guderian et al., 1985).With an increase in O_3 stress, the ratio of chlorophyll a to chlorophyll b is usually reduced. This change probably results from the greater sensitivity of chlorophyll a to O_3 or from the inhibition of pigment synthesis (Reiling and Davison, 1994). O_3 action on leaves results in the increased formation of the superoxide anion radical in chloroplasts, which in turn causes the destruction of chlorophyll.

1.3.4. Effects of O_3 on permeability of membranes

The membrane damage caused by O_3 is readily discernible as disturbances to membrane permeability and regulatory functions. Such effects are especially noticeable in the plasmalemma (Roshchina and Roshchina, 2003). Most studies focus on the influences that O_3 has on the membrane permeability to water, ions and some organic substances (Evans and Ting, 1973). Exposure to O_3 (180 nL L^{-1}) for a few minutes results in rapid loss of cell turgor (Guderian, 1985). Increasing the concentration of O_3 or the duration of exposure causes transparent spots to appear on leaf plates as a result of water infiltration into the intercellular space (Tingey and Taylor, 1982). Both phenomena indicate that the plasmalemma has become more permeable to water as well as to some organic compounds. There are significant changes in the permeability of membranes to water, glucose and ions. O_3 increases the permeability of plasma membrane to the ions K^+, Ca^{2+}, Mg^{2+}, and their concentration in the extracellular medium is consequently increased (Roshchina and Roshchina, 2003).

Proteins and lipids are the membrane constituents that are the most sensitive to O_3 damage. Upon deacetylation of membranous phospholipids and galactolipids, fatty acids are liberated, which then can become the targets of O_3. The products of their oxidation include the hydroperoxides of fatty acids, as well as carbonic and other oxygenated compounds. The presence of these substances is an early marker of cell damage. The other membrane constituents subject to damage are the protein components included in the receptory, catalytic and other complexes. As a consequence of the adverse action of O_3, changes to the enzymatic system occur involving energetic and metabolic reactions, along with the receptors regulating ion permeability. Although these reactions have not yet been sufficiently studied, they may have future diagnostic applications (Roshchina and Roshchina, 2003).

1.3.5. Formation of stress ethylene

Ethylene has been shown to regulate many aspects of plant growth and development, including germination, cell elongation, and fruit ripening (Burg and Thimann, 1959). It is also believed to be involved in organ senescence induced by various stress factors.

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It has been shown that ethylene emission is an early and consistent phenomenon in O_3-sensitive plants. The involvement of ethylene in determining the degree of O_3 lesion formation has been demonstrated in several plant species, and the decrease of ethylene biosynthesis has been shown to reduce O_3-induced cell death (Tuomainen et al., 1997). It was once proposed that ethylene could react with gaseous O_3 and form cell damaging ROS and aldehydes, thus yielding toxic products that caused foliar injury (Elstner et al. 1985). However, recent studies indicate that instead of being directly toxic, ethylene may serve as a regulator of programmed cell death (PCD) and modulator of lesion formation in O_3-treated plants (Overmyer et al., 2000). Moeder et al. (2002) suggested that ethylene biosynthesis and perception are required for H_2O_2 accumulation and cell death that results in visible tissue damage in O_3-exposed tomato. In Arabidopsis, the initiation process of lesion formation in the plant is demonstrated to be independent of ethylene, followed by ethylene-dependent amplification of superoxide ion (O_2^-) accumulation, which is responsible for lesion-propagation processes (Overmyer et al., 2000).

1.3.6. Effects of O_3 on carbon partitioning

Understanding allocation strategies for photosynthates is essential for the prediction of long-term responses of whole plant to O_3. According to 'optimal partitioning models', adjustments in biomass allocation between above and below ground structures, in response to environmental stresses, may serve to balance resource acquisition and to maximize or sustain growth.

Since carbohydrates are the primary products of photosynthesis, a reduction in growth is to be expected if exposure to O_3 results in decreased photosynthesis. But the relative impacts on the growth of the different parts of the plant are the result of differential effects on the translocation of assimilates from the leaves, i.e., their distribution or partitioning. O_3 stress may induce adjustments via the reduction of leaf carbon assimilation and source or sink strength (Minchin et al., 1993). The reduction in source strength would reduce the availability of soluble sugars in source leaves for export (Andersen, 2003).

As the overall growth is a long-term process, various types of multiplier effects are to be expected. For example, changes in the partitioning between the leaves and the rest of the plant frequently reduce both the size and longevity of individual leaves

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and thereby influence the plant's long-term capacity for photosynthetic carbon gain (Mooney and Winner, 1988).

A frequently observed whole-plant response to gaseous air pollutants in general is that root growth is reduced more than shoot growth. O_3 is no exception, and there are numerous reports of O_3-induced increased shoot/root (S/R) dry matter ratios. The lists compiled by Miller (1987) and Cooley and Manning (1987) together cover 26 examples of such S/R reductions taken from studies with 21 different crops; only with peppers (*Capsicum annuum*; Bennett et al., 1979), millet (*Panicum miliaceum*; Agrawal et al., 1983), and peanut (*Arachis hypogaea*; Heagle et al., 1983) was no effect or a decreased ratio observed, while in bean (*Phaseolus vulgaris*; Ito et al., 1985) and annual ryegrass (*Lolium multiflorum*; Bennett and Runeckles, 1977), effects were only observed at the highest exposure levels used.

O_3 injury to leaves, which act as the main source of photosynthates for root growth, might explain decreases in root dry mass (Andersen, 2003; Cooley & Manning, 1987). However, the allometric shift in root biomass ratio in O_3-treated Pima cotton could not be reproduced by pruning canopy or lower-stem leaves to mimic the suppressive effect of O_3 on leaf area (Grantz & Yang, 2000). This suggests that inhibitory effects of O_3 on phloem loading, with consequent inhibition of translocation to roots, might be part of the reason why O_3 induces changes in biomass partitioning (Grantz & Farrar, 2000; Grantz & Yang, 2000).

The accumulation of carbon in the leaves of O_3 treated plants may also result from the greater demand of assimilates for repairing damage to the foliage, making the leaf a stronger sink. Also, the O_3-induced decrease in the root/shoot ratio may result from a more substantial decrease in the rate of photosynthesis in leaves of the lower canopy, that supply carbon mainly to roots, than of the higher canopy (Coleman et al., 1995; Ito et al., 1985; Reich, 1983). Moreover, in bean plants, O_3 (200 nL L^{-1} O_3 for 4 days, 24 h d^{-1}) affected the translocation pattern of assimilates more in the primary leaves that usually direct their assimilates to the roots than in the trifoliate leaves that usually supply assimilates to the upward translocating stream (Okano et al., 1984).

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1.4. O_3 critical levels

The potential for O_3 to damage vegetation has been known for over 40 years. One of the first confirmed reports of widespread foliar injury which could be attributed to O_3 was the so-called "weather fleck" of tobacco in the eastern United States (Heggestad and Middleton, 1959). In Europe it is now clearly established that O_3 found at ambient concentrations can cause a range of effects including visible leaf injury, growth and yield reductions, and altered sensitivity to biotic and abiotic stresses (Fuhrer and Achermann, 1994). Furthermore, because O_3 is a secondary pollutant with a regional distribution, these effects may occur over large areas of rural Europe (Fuhrer et al., 1997). The need to develop international control policies to reduce O_3 exposures, which are based on the effects of the pollutant, has led to attempts to define the so called critical levels of O_3 above which adverse effects on trees, crops and natural vegetation may occur.

Critical levels of air pollutants are defined as concentrations which, if exceeded, may cause adverse effects on such receptors as plants, ecosystems or materials, according to present knowledge (de Leeuw and Zantvoort, 1997). For O_3, they are calculated as the sum of the differences between hourly O_3 concentrations (in nL L^{-1}) and 40 nL L^{-1} per hour, when concentration exceeds 40 nL L^{-1} (AOT40 = Accumulated exposure Over a Threshold of 40 nL L^{-1}), this concept was agreed at a workshop in Kuopio, Finland in 1996 (Kärenlampi and Skärby, 1996). On the basis of statistical yield and biomass loss observed in controlled exposure experiments, mainly using open-top field chambers, critical limits have been set for agricultural crops, forest trees and semi-natural vegetation, with evolving definitions of periods during which the exposure is to be considered (Fuhrer and Achermann, 1994).

For agricultural crops, the long-term critical level was defined as an AOT40 value of 3000 nL L^{-1} h, calculated over a three months period (e.g. May- July) for daylight hours, because only small rates of O_3 deposition have been measured over agricultural crops and forests during night time. For forest trees, the proposed threshold was 6000 nL L^{-1} h, over a six months period (April-September), which would cover the time when trees are most sensitive (Kärenlampi and Skärby, 1996). Exceeding the critical levels can only be used as an indicator of the potential for damage to vegetation, rather than to predict damage from O_3. This is because high levels of O_3 correlate with high temperatures, solar irradiance and vapor pressure

deficit, and hence stomatal closure and lower O_3 uptake. Approaches are being developed to better quantify air pollution absorption through stomata in order to calculate dose vs plant response and characterize the plant strength as sink of ozone (Winner, 1994).

1.5. Effect of O_3 on evergreen woody species

Most investigations have shown that evergreen sclerophyllous species appear to be less sensitive to O_3 compared to deciduous species (Bussoti and Gerosa, 2002; EPA, 1996; Nali et al., 2004), and slower-growing species are less sensitive than faster-growing ones (EPA, 2006). As an example, experiments in the Great Smoky Mountains National Park found black cherry seedlings to demonstrate substantial decreases in biomass (Neufeld et al., 1995). However, exposure for up to three growing seasons did not decrease the biomass of eastern hemlock, table mountain pine, and Virginia pine seedlings exposed to O_3 under similar conditions in this location (Neufeld et al., 2000). In the Mediterranean basin, evergreen broadleaves are suggested as tolerant to O_3 pollution, because of their sclerophyllous leaves, low gas exchange rates (Bussotti and Gerosa, 2002; Grulke and Paoletti, 2005; Manes et al., 1998), and their constitutional and induced ability to tolerate oxidative stress by an active antioxidant pool (Nali et al., 2004). Sclerophyllous consist of coriaceous leaves, with 2-3 palisade layers, little intercellular air space, thick cuticle and cell walls, high stomatal density and development of veins per leaf surface unit and small stomatal size (De Lillis, 1991). This ecological strategy allows better stomatal control. When transpiration is limited, stomatal conductance to CO_2 and O_3 is also limited (Bussotti and Gerosa, 2002). An elevated stomatal density may lead to lower O_3 load per single stoma. As suggested for Betula clones, a more even distribution of O_3 inside the leaf tissue may account for lower injury (Pääkkönen et al., 1997).

Stomatal conductance is considered the metric of plant sensitivity to O_3 (Reich, 1987) and is relatively low in sclerophyllous evergreen broadleaves (Larcher, 1995). The prevailing weather conditions in the Mediterranean reduce stomatal conductance during summers, especially at midday (Tenhunen et al., 1987), so that the highest ambient O_3 levels coincide with the time that natural Mediterranean vegetation suffers the most water stress (Paoletti et al., 2005). Avoidance (Levitt, 1972) is therefore one

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reason of the discrepancy between high O_3 levels and slight sensitivity of sclerophyllous evergreen broadleaf species.

1.6. Effects of O_3 on olives

Olive cultivation is widespread throughout the Mediterranean basin and is important for the rural economy, local heritage, and environment. Mediterranean countries account for around 98% of the world's olive cultivation, estimated at about 9,000,000 hectares. There are more than 800 million olive trees currently grown throughout the world. Olive culture is growing rapidly, and expanding all over the world with an overall increase of 10% in area and 24% in total production during the last 10 years. (FAOSTAT, 2007).

Effects of O_3 on plants have been widely studied in annual and forest species. The effects of O_3 on olive trees have received limited attention compared to forest tree species. Inclán et al. (1999) observed that O_3 exposure (80 nL L^{-1}) for 5 months induced adverse effects on the biomass of wild olive trees grown freely in forests. Other studies with cultivated olive cultivars (Minnocci et al., 1999; Sebastiani et al., 2002) have shown that high O_3 concentrations (100 nL L^{-1}) during the olive growing season reduced photosynthetic activity and stomatal conductance, with a different sensitivity in the two cultivars (Frantoio and Moraiolo) tested. Moraiolo showed greater sensitivity than Frantoio. However, Ribas et al. (2005) have reported that exposure of wild olive trees to 100 nL L^{-1} O_3 concentration for two years had no effect on either biomass partitioning or photosynthetic activity. O_3 resistance of olive trees was linked to intrinsic characteristics such as greater foliar chlorophyll content, thicker spongy parenchyma or high photosynthetic rates.

1.7. Salinity Problem

Soil salinity has been increasing due to many factors, such as low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water, entry of sea water into freshwater, and poor agricultural practices (Foolad, 2004). The accumulation of salts because of irrigation with even slightly low quality water extensively affects agriculture. The soil can lose its pure water as a result of evaporation and transpiration and it becomes enriched with salts. The problem is intensified, if irrigation is done with water that has a high solute

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concentration. Moreover, many factors interact with salinity such as humidity, temperature, air pollution, light and soil fertility and influence the effect of salinity. It has been suggested that the detrimental effects of salinity stress on whole-plant and leaf physiology may be greater at the sunny than at the shaded sites, since light-induced increases in uptake and transport of salt (Tattini et al., 2006) may aggravate the problem of allocating potentially toxic ions in highly sensitive shoot organs and cellular compartments (Munns, 2005).

Expansion of agriculture to semi-arid and arid regions with the use of intensive irrigation will increase secondary salinization (Human-induced salinity) as a result of changes in the hydrologic balance of the soil between water applied (irrigation or rainfall) and water used by crops (transpiration). In order to overcome water shortages and to satisfy the increasing water demand for agricultural development, the use of low quality water due to limited supply of high-quality water, is becoming important in many countries (Chartzoulakis, 2005).

In the Mediterranean basin, plants are subjected to high temperature regimes and extreme water deficits during the dry season. Under these climatic conditions, salts tend to accumulate in the soil because of the high evaporative demand and insufficient leaching of ions, problems often exacerbated by the use of brackish irrigation water in areas of intensive agriculture (FAO, 1993).

Future global warming would likely exacerbate water demand for irrigation with the implications that crops would grow in hotter, drier, and more saline conditions. The ability of irrigated agriculture to meet future challenges would therefore depend on the progress of new research to enhance adaptation to these changes. Understanding the mechanisms of salinity tolerance at the molecular, cellular and whole plant level will improve crop performance and will probably allow the increased use of low quality water for irrigation with minimum adverse impacts on yield, soil productivity and environmental sustainability.

1.7.1. Definition of salinity

Soil salinity is defined as a measure of the total amount of soluble salt in soil. Soils with an electrical conductivity (EC) of saturation extracts above 4 dS m^{-1} are called saline soils (Marschner, 1995). Such an electrical conductivity is equivalent to approximately 40 mM NaCl and generates an osmotic pressure of approximately 0.2

MPa. This definition of salinity is derived from the EC that significantly reduces the yield of most crops.

1.7.2. Effects of salinity on agriculture

Our global water reserves consists primarily of saline waters, i.e., 96.5% is seawater and almost 1% is saline groundwater. This leaves only 2.5% of the global water reserves as fresh, non-saline water, of which two-thirds exist in the form of ice and only
about one-third is fluid fresh-water (Table 1). Thus, there is a limited amount of directly usable fresh water, contrary to the continuing increases in world's population and demand for fresh water. It is estimated that irrigated agriculture presently uses about 65% of the consumed water. However, the extent of water dedicated to irrigated agriculture is likely to be challenged, as pressure is mounting to meet increased demands for human consumption and industrial uses.

Soil salinity is an increasing threat for agriculture and is a major factor in reducing plant productivity throughout the world (Munns and Tester, 2008). The cost of salinity to agriculture is estimated to be about 12 billion $US a year, and is expected to increase as soils are further affected (Ghassemi et al., 1995). About 17% of the world's cropland is under irrigation, but irrigated agriculture contributes over 30% of the total agricultural production (Hillel, 2000). Thus, secondary salinization of irrigated lands is of major concern for global food production. Current estimates indicate that at least 20% of the irrigated lands are salt-affected (Ghassemi et al., 1995). Other estimates are considerably higher and indicate that up to 50% of all irrigated lands may be salt-affected (Flowers, 1999; Szabolcs, 1989). The coincidence of irrigation and salinization threatens the sustainability of high agricultural productivity (Flowers and Yeo, 1995). In addition, irrigation is not the only reason for land salinization, as the risk of seawater incursions can lead to tidal intrusion of saline water into rivers and aquifers in coastal areas (Flowers, 1999). Soil salinity affects an estimated 1 to 3 million hectares in the European Union, mainly in the Mediterranean countries, and is a major cause of desertification (FAO, 1996). It is estimated that about one half of the world's currently irrigated area of 270 million ha is located in the arid zones, like the Mediterranean basin. One of the most alarming processes causing desertification was found to be soil and water salinization due to improper irrigation management (Boonstara et al., 1997; Hamdy, 1996; Mengel, 1993). It is obvious that

in the Mediterranean basin where water use exceeds the natural recharge, reduction of groundwater level and circulation of salts are associated with irreversible salinization processes (Ben-Asher, 1993). The Mediterranean basin is predicted to become warmer and drier and increased demand for irrigation and water resources in general can only make matters worse.

Table 1. Global water supply (after Ghassemi et al., 1995).

Source	Volume		
	Millions of km³	Thousands of km³	% of total
Global water	1386		100
Sea water	1338		96.5
Saline groundwater	12.9		0.93
Ice	24.4		1.73
Fresh groundwater	10.6		0.77
Cycling (rainfall)	0.108		0.008
Annual rainfall		108	
Annual stream flow		45	
Human water use		5	

1.8. Plant responses to salinity

1.8.1. Halophytes vs Glycophytes

Based on general tolerance to salt stress, all plants can be divided into two major groups: a) halophytes, are native to saline soils (around 500 mM NaCl) and able to complete their life cycle in that environment (Colmer et al., 2006), and b) non-halophytes or glycophytes, are severely inhibited or even killed by 100–200 mM NaCl. However, there are great differences in the level of salt stress tolerance within both the halophytes (Flowers et al., 1977; Munns et al., 1983; Ungar, 1991) and the glycophytes (Greenway and Munns, 1980), which include sensitive, moderately tolerant and very tolerant species. Although halophytes represent only 2% of the terrestrial plant species, they are present in about half the higher plant families and exhibit a great diversity of plant forms (Glenn et al., 1999). Most of the agricultural crops are glycophytes, although some of them like sugar beet, barley, wheat etc. can tolerate salt to some extent.

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1.8.2. Salinity responses on a whole plant level

There is general agreement that whole plant growth responses to salinity are multigenic and that a better knowledge of the underlying physiology is required in order to understand why some species and varieties are more salt-resistant than others (Neumann, 1997). This is a complex task since plant growth responses to salinity can vary with: 1) the duration and degree of stress encountered (mild, moderate, severe), 2) the experimental system used, i.e. the plant organ, variety or species, and 3) the plant developmental stage. Some species are more tolerant at the seedling stage, while others exhibit greater tolerance during vegetative growth, flowering or fruiting (Subbarao and Johansen, 1994). Moreover, the growth inhibitory effects of salinity can also be affected by variation in the calcium or potassium ions in the saline root medium (Neumann, 1997). Salinity affects plants in different ways such as osmotic effects, specific-ion toxicity and/or nutritional disorders (Läuchli and Epstein, 1990). Thus, in order to understand the physiological mechanisms responsible for the salinity tolerance of plant species, it is necessary to know whether their growth is being adversely affected by osmotic effects or by the toxic effect of salt in the plant.

Munns (1993) has suggested that plant growth under salinity is inhibited through two phases. Initially (phase 1), growth is affected because of cellular responses to the osmotic effects. Subsequently (phase 2), growth is reduced due to the toxic effects of accumulated salts. Time-dependent changes of growth and development of plants exposed to salinity stress have been reviewed (Munns, 2002a). In the first few seconds or minutes, cells lose water and shrink, whereas, over hours, cells regain their volume but the expansion rates are limited. The second much slower effect, taking days, weeks or months, is the result of salt accumulation in leaves, leading to salt toxicity in the plant, primarily in the older leaves (i.e. salt-specific effect). This salt toxicity can result in leaf death and reduce total photosynthetic leaf area. As a result, there is a reduction in the supply of photosynthates to the plant, affecting the overall carbon balance necessary to sustain growth (Munns, 2002a). Salt toxicity primarily occurs in the older leaves where Na and Cl build up in the transpiring leaves over a long period of time, resulting in high salt concentration and leaf death. Leaf injury and death is probably due to the high salt load in the leaf that exceeds the capacity of salt compartmentation in the vacuoles, causing salt to build up in the cytoplasm to toxic levels (Munns, 2002a; 2005; Munns and Termaat, 1986; Munns et al., 2006). The rate at which leaves die and, thus, reduce their total

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photosynthetic leaf area determines the survival or not of the plant. If new leaves are produced at a rate greater than the rate at which old leaves die, there are enough photosynthesizing leaves for the plant to flower and produce seeds, although at reduced numbers. If, however, old leaves die faster than new leaves develop, the plant may not survive long enough to supply sufficient photosynthates to the reproductive organs and produce viable seeds. Based on this two-phase concept, the initial growth reduction for both salt sensitive and salt tolerant plants is caused by an osmotic effect of the salts in the medium outside the roots. In contrast, in the second phase, a salt-sensitive species or genotype differs from a more salt tolerant one by its inability to prevent salt from accumulating in transpiring leaves to toxic levels (Munns et al., 2006).

Because NaCl is the most soluble and widespread salt, it is not surprising that all plants have evolved mechanisms to regulate its accumulation and to select against it in favor of other nutrients commonly present in low concentrations, such as K^+ and NO_3^-. In most plants, Na^+ and Cl^- are effectively excluded by roots while water is taken up from the soil (Munns, 2005). Halophytes are able to maintain this exclusion at higher salinities than glycophytes. For example, sea barley grass, *Hordeum marinum*, excludes both Na^+ and Cl^- until at least 450 mM NaCl (Garthwaite et al., 2005). NaCl-salinity imposes at least three types of problems for higher plants: 1) the osmotic pressure in the external solution can exceed the osmotic pressure in the plant cells (low soil water potential), requiring an osmotic adjustment by the cells to avoid desiccation, 2) the uptake and transport of nutritional ions such as K^+ and Ca^{+2} can be disrupted by excess Na^+, 3) Na^+ and Cl^- can have direct toxic effects on membranes and enzyme systems at high levels (Ashraf, 1994a).

1.8.3. Salinity responses on an organ level

Roots

Root growth of glycophytes is generally affected less by salinity than vegetative shoot growth or fruit and seed production (Maas and Nieman, 1978). Depending on the species, the level of salinity stress, and the composition of the external solution, root growth may be stimulated [e.g. *Cynodon* sp.(Maas et al., 1986), *Chloris gayana* (Waisel, 1985)], inhibited [e.g. *Pisum sativum*, *Sorghum bicolor*, *S. halepenese* (Yang et al., 1999)], or unaffected [e.g. *Hordeum vulgare* (Delane et al., 1982)]. Likewise, the type of salt has a profound influence on root growth. At low

salinity, Weimberg et al. (1984) found that root growth of *Sorghum bicolor* was stimulated by KCl and K_2SO_4, was unaffected by Na_2SO_4, and was inhibited by NaCl. It is well-known that salinity with an adequate supply of calcium reduces shoot growth, particularly leaf area, more than root growth (Läuchli and Epstein, 1990). However, inadequate Ca^{+2} supplies under saline conditions can adversely affect membrane function and growth of the root within minutes (Cramer , 2002; Epstein, 1961; Läuchli and Epstein, 1970). When supplemental Ca^{+2} was added to a salinized medium, cell elongation of cotton roots was favored at the expense of radial cell growth, while cell production rates were maintained (Kurth et al., 1986). Additional studies with cotton roots revealed that supplemental Ca^{+2} partly alleviated the inhibition of the elongation rate due to high salinity but the shortening of the growth zone of the root caused by intense salt stress was not restored by supplemental calcium (Zhong and Läuchli, 1993). High salt stress increased the deposition rate of Na in the growing region of the root and hence decreased the selectivity for K^+ versus Na^+. The latter effect was partly mitigated by supplemental Ca^{+2}, but only in the apical 2 mm region (Zhong and Läuchli, 1994). The conclusion of these studies is that supplemental Ca^{+2} alleviates the inhibitory effect of salt on cotton root growth by maintaining plasma membrane selectivity of K^+ over Na (Läuchli, 1990; 1999; Zhong and Läuchli, 1994).

Shoots

The mechanisms by which salinity inhibits shoot growth may be grouped into the following categories (Lazof and Bernstein, 1999): 1) disturbed photosynthesis, 2) decline in turgor of expanding tissues and insufficient osmoregulation, 3) root sensing and down-regulation of shoot growth via a long distance signal, and 4) disturbance in mineral supply to the shoot. Reduction in shoot growth due to salinity is commonly expressed by stunted shoots (Läuchli and Epstein, 1990). As already described for roots, the effect of salt stress on shoot growth in several species can also be partly alleviated by supplemental Ca^{+2} (Cramer, 2002; Läuchli and Epstein, 1990). If, however, plants are exposed to high Na/Ca ratios, Ca-deficiency in the shoot can be induced, as for example demonstrated for developing corn leaves by Maas and Grieve (1987). The importance of supplemental Ca to alleviate salt stress effects in the shoot, has been clearly emphasized by Cramer (2002) and Munns (2002b), who

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recommended adding at least 5–10 mM Ca^{+2} to the medium for salinities of 100-150 mM NaCl, to counteract the inhibitory effect of high Na^+ concentrations on growth. Shoots of halophyte *Salicornia bigelovii* were larger and more succulent when grown in highly saline conditions (Parks et al., 2002).

1.8.4. Salinity responses on a cellular level

High salinity causes hyper osmotic stress and ion disequilibrium that produce secondary effects or pathologies (Hasegawa et al., 2000; Zhu, 2001). Fundamentally, plants cope by either avoiding or tolerating salt stress. That is plants are either dormant during the salt episode or they need cellular adjustments to tolerate the saline environment. Tolerance mechanisms can be categorized as those that function (a) to minimize osmotic stress or ion disequilibrium or (b) to alleviate the consequent secondary effects caused by these stresses. The chemical potential of the saline solution initially establishes a water potential imbalance between the apoplast and symplast that leads to turgor decrease, which, if severe enough, can cause growth reduction (Bohnert et al., 1995). Growth cessation occurs when turgor is reduced below the yield threshold of the cell wall. Cellular dehydration begins when the water potential difference is greater than what can be compensated for by turgor loss (Taiz and Zeiger, 2002). The cellular response to turgor reduction is osmotic adjustment. The cytosolic and organellar machinery of glycophytes and halophytes is equivalently Na^+ and Cl^- sensitive. Thus, osmotic adjustment is achieved in these compartments by accumulation of compatible osmolytes and osmoprotectants (Bohnert et al, 1995; Bohnert and Jensen, 1996). However, Na^+ and Cl^- are energetically efficient osmolytes for osmotic adjustment and are compartmentalized into the vacuole to minimize cytotoxicity (Blumwald et al., 2000; Niu et al., 1995). Since plant cell growth occurs primarily because of directional expansion mediated by an increase in vacuolar volume, compartmentalization of Na^+ and Cl^- facilitates osmotic adjustment that is essential for cellular development (Yokoi et al., 2002).

1.8.4.1. Osmolytes and osmoprotectants

During stress conditions plants need to maintain internal water potential below that of soil and maintain turgor and water uptake for growth (Tester and Davenport, 2003). This requires an increase in osmotica, either by uptake of soil solutes or by

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synthesis of metabolic (compatible) solutes. To accommodate the ionic balance in the vacuoles, cytoplasm accumulates compounds of low molecular mass (i.e. the compatible solutes) because they do not interfere with normal biochemical reactions (Zhifang and Loescher, 2003). With accumulation proportional to the change of external osmolarity within species-specific limits, protection of structures and osmotic balance supporting continued water influx (or reduced efflux) are accepted functions of osmolytes (Hasegawa et al., 2000). While some compatible osmolytes are essential elemental ions, such as K^+, the majority of them are organic solutes (Yokoi et al., 2002). However, the solutes that accumulate vary between plant species and a major category of organic osmotic solutes consists of simple sugars (mainly fructose and glucose), sugar alcohols (glycerol and methylated inositols) and complex sugars (trehalose, raffinose and fructans) (Bohnert and Jensen, 1996). Others include quaternary amino acid derivatives (proline, glycine betaine, β-alanine betaine, proline betaine, tertiary amines 1,4,5,6-tetrahydro-2-methyl-4-carboxyl pyrimidine), and sulfonium compounds (choline o-sulfate, dimethyl sulfonium propironate) (Yokoi et al., 2002).

1.8.4.2. Carbohydrates

Among the various organic osmotica, sugars contribute up to 50% of the total osmotic potential in glycophytes subjected to saline conditions (Cram, 1976). The accumulation of soluble carbohydrates in plants has been widely reported as a response to salinity or drought, despite the induced significant decrease in net CO_2 assimilation rate (Murakeozy et al., 2003). Carbohydrates, such as sugars (glucose, fructose, sucrose, and fructans) and starch, accumulate under salt stress (Parida et al., 2002) and play a leading role in osmoprotection, osmotic adjustment, carbon storage, and radical scavenging. A decrease in starch content and an increase in both reducing and non-reducing sugars and polyphenol levels have been reported in leaves of *Bruguiera parviflora* (Parida et al., 2002). In tomato leaves, the contents of soluble sugars and total saccharides are increased significantly, but the starch content is not affected (Khavarinejad and Mostofi, 1998). Ashraf and Tufail (1995) determined the total soluble sugar content in five sunflower accessions differing in salt tolerance; the salt tolerant lines had generally greater soluble sugars than the salt sensitive ones.

1.8.4.3. Proteins and salinity

Proteins that accumulate in plants under saline conditions may provide a storage form of nitrogen that is re-utilized later (Singh et al., 1987) and may play a role in osmotic adjustment. Such proteins may be synthesized *de novo* in response to salt stress or may be present constitutively at low concentration (Pareek-Singla and Grover, 1997). It has been concluded that a number of proteins induced by salinity are cytoplasmic; a fact that can cause alterations in cytoplasmic viscosity of the cells (Hasegawa et al., 2000). A higher content of soluble proteins has been observed in salt tolerant cultivars of barley, sunflower, finger millet, and rice (Ashraf and Harris, 2004). Agastian et al. (2000) have reported that soluble proteins increase at low salinity and decrease at high salinity in mulberry cultivars. On the contrary, Ashraf and Fatima (1995) found that salt tolerant and salt sensitive accessions of sunflower did not differ significantly in leaf soluble proteins.

1.8.4.4. Amino acids and amides and salinity

Amino acids (alanine, arginine, glycine, serine, leucine, and valine, proline, and the non-protein amino acids citrulline and ornithine) and amides (such as glutamine and asparagines) have also been reported to accumulate in plants subjected to salt stress (Mansour, 1998). Total free amino acids in the leaves have been reported to be higher in salt tolerant than in salt sensitive lines of sunflower (Ashraf and Tufail, 1995), safflower (Ashraf and Fatima, 1995), *Eruca sativa* (Ashraf, 1994b) and *Lens culinaris* (Hurkman et al., 1991). Proline accumulation is believed to play adaptive roles in plant stress tolerance. Proline has been proposed to act as a compatible osmolyte and serve in storing carbon and nitrogen (Hare and Cress, 1997). Proline is osmotically very active and contributes to membrane stability and mitigates the effect of NaCl on cell membrane disruption (Mansour, 1998). Salinity and drought are known to induce oxidative stress. Early *in vitro* studies showed that proline can be a ROS scavenger (Smirnoff and Cumbes 1989). Proline may act as a signaling/regulatory molecule able to activate multiple responses that are components of the adaptation process (Maggio et al., 2002). Petrusa and Winicov (1997) demonstrated that salt tolerant alfalfa plants rapidly doubled their proline content in roots, whereas in salt sensitive plants the increase was slow.

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Transgenic approaches in regard to proline accumulation in order to improve plant stress tolerance have appreciable results. Overproduction of proline by genetically manipulated tobacco plant showed tolerance to NaCl (Hong et al., 2000). Nanjo et al. (2003) demonstrated that introduction of antisense proline dehydrogenase cDNA in *Arabidopsis* overexpresses proline and showed tolerance to salinity (600 mmol NaCl).

1.8.4.5. Polyols and salinity

Polyols, the polyhydric alcohols, are among the compatible solutes involved in osmoregulation and are thought to play a role in plant salt tolerance (Bohnert and Shen, 1999). They exist in both acyclic and cyclic forms and are widely distributed in the plant kingdom. The most common polyols in plants include acyclic forms, mannitol, glycerol, sorbitol, and cyclic (cyclitols) forms ononitol and pinitol. In general, they accumulate in the cytoplasm of some halophytes to overcome the osmotic disturbances caused by high concentrations of inorganic ions compartmentalized in vacuoles. Polyols make up a considerable percentage of all assimilated CO_2 as scavengers of stress-induced oxygen radicals (Bohnert et al., 1995). Mannitol, a sugar alcohol that may serve as a compatible solute to cope with salt stress, is synthesized via the action of a mannose-6-phosphate reductase (M6PR) in celery (Zhifang and Loescher, 2003) and its accumulation increases when plants are exposed to low water potential. The accumulation is regulated by inhibition of competing pathways and decreased mannitol consumption and catabolism (Stoop et al., 1996). Studies using transgenic tobacco and *Arabidopsis* plants showed improved growth of plants accumulating mannitol under stress (Thomas et al., 1995).

1.8.4.6. Antioxidants and salinity

It is well documented that, when plants are subjected to many environmental stresses including salinity, induce an overproduction of reactive oxygen species (ROS) which include hydrogen peroxide, superoxide radical and hydroxyl radicals, and these compounds are thought to be responsible for the oxidative damage associated with plant stress. ROS are inevitable by-products of normal cell metabolism (Martinez et al., 2001). But under normal conditions production and destruction of ROS is well regulated in cell metabolism (Mittler, 2002). Oxidative

stress occurs when there is a serious imbalance between the production of ROS and antioxidative defence (Ahmad et al., 2008).

Reactive oxygen species (ROS) are regarded as the main source of damage to cells under biotic and abiotic stresses (Candan and Tarhan, 2003; Gara at al., 2003; Vaidyanathan et al., 2003). ROS are partially reduced forms of atmospheric oxygen, which are produced in vital processes such as photorespiration, photosynthesis and respiration (Mittler, 2002). These species of oxygen are highly cytotoxic and can seriously react with vital biomolecules such as lipids, proteins and nucleic acid, causing lipid peroxidation, protein denaturing and DNA mutation (Breusegem et al., 2001; Quiles and Lopez, 2004).

Salinity causes oxidative stress by inhibiting CO_2 assimilation, exposing chloroplasts to excessive excitation energy, which in turn increases the generation of ROS from triplet chlorophyll (Asada 1994; Gosset et al., 1994). As soon as the carbon fixation inside chloroplasts decreases, there is also a lower NADP availability to accept electrons from PSI, thus initiating O_2 reduction resulting in the ROS generation (Sudhakar et al., 2001). In addition, considering the fact that Cl^- is involved in electron flux during the H_2O oxidation, the Cl^- toxicity is likely to disrupt the normal electron flow to PSII, which in turn leads to excess electron leakage and increased production of ROS (Gosset et al., 1994).

Plants possess various protective mechanisms to control ROS, which are effective at different levels of stress-induced deterioration (Beak and Skinner, 2003). The enzymatic antioxidant system is one of the protective mechanisms including superoxide dismutase (SOD: EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and ascorbate peroxidase (APX; EC 1.11.1.11) (Mittler et al., 2004). SOD catalyses the dismutation of O_2- to H_2O_2, catalase (CAT) dismutates H_2O_2 to oxygen and water, and ascorbate peroxidase (APX) reduces H_2O_2 to water by utilizing ascorbate (ASC) as specific electron donor. These are considered the main enzymatic systems for protecting cells against oxidative damage (Gara et al., 2003). Plants with high levels of antioxidants have been reported to have greater resistance to oxidative damage (Spychalla and Desborough, 1990). Garratt et al. (2002) and Mittova et al. (2002) reported increased activities of the antioxidant enzymes in plants under salt stress. They found a correlation between these enzymes levels and salt tolerance. Many changes have been detected in the activities of antioxidant enzymes in plants exposed to salinity. The activity of antioxidant enzymes was reported to increase under saline conditions in

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shoot cultures of rice (Fadzilla et al., 1997), wheat (Meneguzzo and Navarilzzo, 1999) and pea (Hernandez et al., 1999), but decreased in wheat roots (Meneguzzo and Navarilzzo, 1999) or was unaffected as in the case of SOD in cucumber (Lechno et al., 1997). The differences in these results may be due to the fact that salinity effects depend on a number of factors, for example salt type, concentration, plant genotype, growth stage and environmental conditions (Shannon et al., 1994).

1.8.5. Effect of salinity on olives

Olive is a glycophytic species of intermediate tolerance to salinity (Rugini and Fedeli, 1990). Several studies have shown that olive is more tolerant than other widely grown fruit trees which are generally salt sensitive (Hartmann et al., 1966; Hoffman et al., 1989; Taha et al., 1972). FAO (1985) classifies olive trees as moderately tolerant to salinity suggesting a threshold of electrical conductivity (EC) of the soil saturation extract between 3 and 6 dS m^{-1}. This value can be high as 6-8 dS m^{-1} in soils with high calcium status (Chartzoulakis, 2005). Although the threshold chloride and sodium ions toxic concentration varied, as a result of different experimental conditions and tested genotypes, most studies reported that they are 2 mg g^{-1} of Cl^{-} and 4-5 mg g^{-1} Na^{+} on a leaf dry mass basis, and it was suggested that injury is better correlated with Na^{+} than with Cl^{-} (Al-Saket and Aesheh, 1987; Gucci and Tattini, 1997). Therios and Misopolinos (1988) reported that three-year old olive trees did not suffer salt stress at NaCl concentrations lower than 80 mM during a 90-day culture period. Irrigation with saline water at NaCl concentration of 100 mM has been considered to be a critical threshold level for reductions in olive tree growth (Chartzoulakis et al., 2002; Loreto and Bongi, 1987). However, olive trees can tolerate even higher EC values, when NaCl represents a small portion of the soluble salts. The type of salts contained in the irrigation water is also related to the degree of plant damage. Bartolini et al. (1991) reported that Na$_2$SO$_4$ was more deleterious to the general growth than NaCl.

Salinity tolerance in olive is cultivar-dependent. Several studies have reported large differences between different cultivars (Benlloch et al., 1991; Chartzoulakis et al., 2002; Perica et al., 2004; Tattini et al., 1992; Therios and Misopolinos, 1988). The growth of all tested cultivars is reduced by salinity stress to varying degrees, the cultivars 'Kalamata', 'Picual', 'Lechin', 'de Sevilla', and 'Megaritiki' proved to be

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tolerant, 'Cordal' 'Manzanillo', 'Frantoio', 'Koroneiki', 'Chemlali' and 'Hotjiblanca' as moderately tolerant, while 'Pajarero' 'Lechino', Chetoui' and 'Chalkidikis' are considered as moderate sensitive (Benlloch et al., 1991, 1994; Chartzoulakis, 2005; Chartzoulakis et al., 2002, 2004, Therios and Misopolinos, 1988).

1.8.5.1. Symptoms of salinity toxicity in olives

Typical symptoms of salt stress in olive trees are reduced growth, leaf tip burn, leaf chlorosis, leaf rolling, wilting of flowers, root necrosis, shoot dieback, and defoliation (Gucci and Tattini, 1997). Necrotic areas develop first at the distal end of mature leaves and then expand to the rest of the leaf. Tip burn tends to appear earlier in mature than in young leaves (Benlloch et al., 1991; Tattini et al., 1992). Tip burn occurs because the typically thick cuticle of olive leaves is much thinner at the apex, where necrosis of the fibrovascular tissue rapidly develops if exposed to salt stress (Cirulli and Laviola 1981). Leaf abscission occurs at high salt concentrations, but it is not necessarily related to the appearance of visual symptoms; that is abscising leaves may appear as green and healthy as those of untreated plants (Gucci and Tattini, 1997).

The concentration at which toxicity symptoms are likely to appear depends on various factors such as cultivar, plant age, growth medium, duration of exposure, rootstocks and environmental conditions. Young 'Kalamata' plants did not suffer any apparent injury when treated with 100 mM NaCl for 5 months (Chartzoulakis et al., 2002). Genotypic differences in the appearance of symptoms have been reported by several authors (Benlloch et al., 1991; Chartzoulakis et al., 2002; Therios and Misopolinos, 1988).

1.8.5.2. Effects of salinity on morphology and anatomy in olives

Salt-treated olive trees are usually characterized by smaller size, smaller leaves, shorter internodes, decreased number of shoots and leaves, and decreased leaf area than plants grown without saline stress (Chartzoulakis et al., 2002; Perica et al., 2008; Therios and Misopolinos, 1988). Root morphology is also affected since root branching is inhibited under saline conditions (Tattini et al., 1992).

Bongi and Loreto (1989) reported thicker cell walls and a 38% increase in spongy mesophyll thickness in potted plants treated with dilute seawater solution (250

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mM NaCl) for 90 days. Palisade mesophyll cell length was increased by 50% over control values, whereas no differences were observed in epidermal thickness (Bongi and Loreto, 1989). The increase in palisade cell length has been mainly attributed to Cl^- ion effect (Bernstein, 1975). The increase in mesophyll thickness and length of palisade cells in olive is similar to that reported for other glycophytic species (Longstreth and Nobel, 1979).

1.8.5.3. Effects of salinity on growth of olives

Most studies report that olive growth is adversely affected by moderate and high salinity (Bartoloni et al., 1991; Chatzoulakis et al., 2002; Perica et al., 2008; Tattini et al., 1992). Shoot growth is completely inhibited at NaCl concentration higher than 200 mM (Chartzoulakis et al., 2002; Tattini et al., 1995). Shoot growth is generally more inhibited than root growth (Klein et al., 1994; Perica et al., 2008; Tattini et al., 1992), so that the root-shoot ratio tends to increase in salt-stressed plants (Tattini et al., 1995). It is a common observation that the effect of salinity on growth depends on the cultivar, and the duration of exposure. Chartzoulakis et al. (2002) reported that the lowest dry mass reduction at 200 mM was measured in cultivar 'Kalamata' (48%), while for the other cultivars studied the reduction ranged from 65 to 72%.

1.8.5.4. Water relations and salinity in olives

Salinity affects the water relations of many higher plants so that salt stress often results in water deficit (Greenway and Munns, 1980; Shalhevet, 1993). The early response of woody crops to salinity is the reduction of leaf water potential (Ψ_w) and relative water content (RWC). However in olive trees changes in RWC, Ψ_w and water uptake occur at higher salinities than those causing comparable changes in other fruit trees species (Banus and Primo-Millo, 1992; Gucci et al., 1997). The decrease in RWC is probably a consequence of the high concentration of the external solution which causes osmotic stress and leaf dehydration. The high bulk modulus of elasticity of olive leaves (Chartzoulakis et al., 1999; LoGullo and Salleo, 1988) and leaf dehydration can explain the substantial drop in Ψ_w during salinity and its ability to recover upon relief of stress.

The salt-induced decrease of Ψ_w was accompanied by a decrease of osmotic potential (Ψ_π) resulting in turgor potential (Ψ_p) values of salinized plants similar or higher than the Ψ_p of the control plants. The decrease in Ψ_π mainly reflects the different ability of olive genotypes to exclude Na^+ and Cl^- ions from the shoot. Prolonged stress causes an increase in specific leaf mass and dry mass to fresh mass ratio, but no change in succulence (Bongi and Loreto 1989; Gucci et al. 1997).

1.8.5.5. Effects of salinity on photosynthesis in olives

Sclerophyllous olive leaves are characterized by a thick cuticle, compact mesophyll and stomata (only on the abaxial side) covered by a layer of peltate trichomes. Hence, cuticular, stomatal, and internal diffusive resistances are high and net CO_2 assimilation rate (A) is relatively low with respect to other C_3 species (Bongi et al., 1987; Bongi and Palliotti, 1994). Under salt stress, olive leaves become thicker and more succulent (Bongi and Loreto, 1989). Increasing leaf thickness may further reduce the mesophyll conductance by extending and making more tortuous the CO_2 pathway toward the chloroplasts (Evans et al., 1994; Syvertsen et al., 1995). Several studies have shown that the photosynthetic rate of olive is reduced by salinity stress (Bongi and Loreto, 1989; Chartzoulakis et al., 2002; Tattini et al., 1995). This reduction in photosynthetic rate could be ascribed to Na and Cl accumulation in the leaves under salt stress (toxic effect), and also to the decrease in mesophyll conductance due to increased leaf thickness (Syvertsen et al., 1995). The effect of salinity on CO_2 assimilation rate reduction varies with the salt concentration to which the plants are exposed and the cultivar. In general, the highest inhibition is observed in olive cultivars with inherently high photosynthesis and stomatal conductance (Loreto et al., 2003). It was found that the relative decrease in stomatal conductance of salt-treated plants is greater in the salt-tolerant cultivar 'Frantoio' than in the salt-sensitive 'Leccino' (Tattini et al, 1995). Loreto et al. (2003) showed that the low chloroplast CO_2 concentration set by both low stomatal and mesophyll conductances is the main limitation of photosynthesis in moderately salt-stressed olive.

Chlorophyll fluorescence has proved to be a useful, quantitative, rapid and non-invasive technique to study different aspects of photosynthesis. Measurements of chlorophyll fluorescence *in vivo* can provide a rapid means of detecting salt stress in

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leaves, including instances where photosynthesis is reduced in the absence of visible symptoms (Smillie and Nott, 1982). The ratio of variable fluorescence to maximal fluorescence (F_v/F_m) of dark adapted leaves is used commonly to assess the relative state of PSII. F_v/F_m is used frequently as an expression of photoinhibition (Critchley, 1998, Kitao et al., 2000; Krause et al., 1999; Schansker and van Rensen, 1999). Most of the relevant studies report a decrease in the F_v/F_m under salinity stress for several plant species (Dionisio-Sese & Tobita, 2000; Lee et al., 2004; Netondo et al., 2004). Similar results were obtained with olive (Melgar et al., 2008), where F_v/F_m significantly decreased after exposure to high salinity concentration (100 mM NaCl). These decreases in Fv/Fm and quantum yield of PSII, can be ascribed as down-regulation of PSII that reflect the protective or regulatory mechanism to avoid photodamage of photosynthetic apparatus (Demming-Adams and Adams, 1992). The loss in D_1 protein has been accounted for the inhibition in PSII activity and also the observed decline in Fv/Fm was caused by the degradation of D_1 protein, leading to impaired energy transfer to the PSII reaction center (Sudhir et al., 2005).

1.9. Ozone and interaction with other abiotic stresses

Under real field conditions, effects of O_3 on plants rarely occur in the absence of effects of other environmental influences and limiting factors. Thus, efforts to increase plant performance under future conditions by breeding or biotechnological progress will depend on the understanding of these interactions. Many of them are poorly understood and deserve more basic research attention.

Salinity is one of the stresses that can influence plant responses to O_3. However, the interaction of other environmental factors such as relative humidity, light intensity, air temperature and soil moisture can also modify the response to O_3. Soil moisture availability may influence the effects of O_3 on crop physiological processes. Reduced soil moisture may limit O_3 access by reducing stomatal conductance, and hence, protect cellular components from oxidant attack. However, recent findings suggest that, in some species, soil moisture stress may reduce rather than increase O_3 tolerance (Bungener et al., 1999). Other studies have shown no significant interaction between O_3 and water stress for growth and yield parameters-for example in spring wheat cv. Turbo (Fangmeier et al., 1994). This is in contrast to the increased sensitivity of growth and yield parameters of water-stressed soybeans to ambient O_3 levels (Heggestad et al., 1985).

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1.10. Combined effects of salinity and O_3 on plants

The combined effects of O_3 and salinity on physiology and growth of plants first received attention in the early 1970s in the U.S.A. Few experiments examined the response to combined salinity and O_3 exposure of commercially important crops; including beets (Ogata and Maas, 1973), pinto beans (Hoffman et al., 1973; Maas et al., 1973), and alfalfa (Hoffman et al., 1975). These and other studies are summarized in Table 2. However these studies focused on acute O_3 exposure at concentrations between 100-350 nL L^{-1}, in excess of those encountered in rural areas. Salt-treated plants that were exposed to O_3 exhibited less visible O_3 injury, and yield reductions were lower than non-saline plants exposed to ozone. These effects were suggested to be attributable to reduced O_3 dose as a result of saline-induced stomatal closure. In contrast, the only field study conducted showed no interaction between O_3 and salinity on growth and yield of alfalfa (Olszyk et al., 1988).

In a more recent investigation (Maggio et al., 2007) on the impact of salinity in combination with ambient O_3 concentration at 56 nL L^{-1} on tomato plant, it was reported that salinity reduced O_3 damage on total biomass and yield. In contrast, additive effects of salinity and O_3 were reported for a number of growth parameters and physiological processes such as photosynthesis, transpiration and stomatal conductance for five cultivars of rice (Welfare et al., 1996) (see also Table 2).

Experiments on the combined effects of salinity and other air pollutants, notably SO_2 on the growth and yield of wheat (Huang & Murray, 1993; Huang et al., 1994) and soybeans (Qifu & Murray, 1991) found variable responses depending on the stage of plant development and the concentration and duration of the stresses. While SO_2 or salinity individually reduced chlorophyll content, leaf area, plant dry mass and seed yield of soybeans, SO_2 and salinity interactions were not significant. However, SO_2-induced leaf injury was more severe in non-saline than salt-treated plants, providing evidence of a protective effect against SO_2 injury (Qifu & Murray, 1991). In wheat seedlings cv. Wilgoyne, exposure to a high salt concentration (100 mM NaCl) reduced SO_2 uptake and leaf sulphur concentrations due to increased stomatal resistance (Huang & Murray, 1993). At a lower salt concentration (50 mM NaCl), salinity did not provide an effective protection against SO_2 uptake by increasing stomatal resistance in the leaves during SO_2 fumigation, and as a result the effects of NaCl in combination with SO_2 (231 nL.L^{-1}) on growth were additive (Huang et al., 1994).

The combined effects of O_3 and salinity on olive cultivars or on any other woody species besides red maple have not been investigated and are the focus of this thesis.

Table 2. Studies on the combined effects of salinity and ozone in plants.

Plant species	Salinity treatment	Ozone treatment	Effects	Reference
Tomato	NaCl + CaCl$_2$ 7.0 mM	Ambient O$_3$ 12h/day for 75 days continuously	Salinity reduced ozone damage on total biomass and yield	Maggio et al (2007)
Wheat	NaCl 50 mM	O$_3$ 50 nL L^{-1} 8h/day	Antagonistic interaction between salinity and O$_3$	Hassan (2004)
Chickpea	NaCl 30 mM	O$_3$ 85 nmol mol^{-1} 6h/day for 25 days	Additive effects of Nacl & O$_3$ on growth	Welfare et al. (2002)
Rice	NaC125, 50mM	O$_3$ 88 nL L^{-1} 5h/day for 15 days intermittently over 34 days	Additive effects of NaCl & O$_3$ on photosynthesis, stomatal conductance, root dry wt., height, shoot [K]	Welfare et al. (1996)
Alfalfa	NaC1+CaC1$_2$ 30, 55mM	Ambient O$_3$ 13h/day for 130 days continuously	No interaction between O$_3$ and salinity	Olszyk et al. (1988)
Pinto bean	NaCl+CaC1$_2$ 50,100mM	O$_3$ 200 nL L^{-1}, S0$_2$ 200 nL L^{-1}, O$_3$+S0$_2$ 200+200, 7h/day, 4 days intermittently over 10 days	Salinity reduced injury by O$_3$ and O$_3$ + SO$_2$	Bytnerowicz and Taylor (1983)
Red Maple	NaCl 35, 70mM	O$_3$ 25 nL L^{-1} 8h/day for 42 days continuously	Genetic variability. Antagonistic effect on height	Dochinger and Townsend (1979)
Alfalfa	NaCl +CaC1$_2$ 50, 100, 150mM	O$_3$ 100, 150, 200 nL L^{-1} 2h/day for 21 days continuously	Increasing salinity reduced impact of O$_3$ on yield	Hoffman et al. (1975)
Pinto bean	NaC1+CaCl$_z$ 50, 100mM	O$_3$ 150, 250 & 350 nL L^{-1} 2h/day for 63 days continuously	Some antagonistic interactions at high O$_3$ & salt. No interaction on growth or gas exchange at low O$_3$ & salt	Hoffman et al. (1973)
Pinto bean	NaCl 50 & 100 mM	O$_3$ 150, 220, 300 nL L^{-1} for 0.5-6h/day for 14 days continuously	Salinity extended ozone tolerance threshold and reduced ozone injury	Maas et al. (1973)
Garden beet	NaCl +CaC1$_2$ 100 & 200 mM	O$_3$ 200 nL L^{-1} for 0.5-3.0 h/ day for 38 days continuously	Increasing salinity reduced impact of O$_3$ on yield	Ogata and Maas (1973)

Chapter 2. Materials and Methods

2.1. Open top chambers (OTC) and fumigation system

The study was carried out at the Agricultural Research Station, Velestino, University of Thessaly, in Thessaly valley, Central Greece. Six OTCs were set up for the experiment. Each circular OTC had a diameter of 2.5 m, an open top diameter of 2 m and a height of 2.8 m as described by Heagle et al. (1973). Each chamber was constructed of an iron frame covered with a HDPE sheet (180 μm thickness, >80% light penetration). Each OTC was continuously ventilated with an air ventilation unit bringing ambient outside air into the chamber at 1600 m^3 h^{-1}. Air was distributed via perforated tubes 15 cm in diameter positioned at a height of 70 cm along the chamber walls (the lower level of tree canopy). Mean air temperatures for two years (2006, 2008) were 21.8 °C for May, 28.2 °C for June, 28.5 °C for July, 28.1 °C for August, and 23.2 °C for September.

2.2. Plant material

Two-year-old uniform olive plants (*Olea europaea* L.) of two Greek table olive cultivars, 'Konservolea' and 'Kalamata', grafted on seedling rootstock were transplanted in 12 L pots containing a sand-perlite mixture 1:1 v/v for hydroponic culture late March. Eight plants of each cultivar were lightly pruned and placed inside each OTC. Seedling rootstock was produced using seeds from fruit of wild olives grown in the forests.

2.3. Experimental design and treatments

Three of the six OTCs used in this experiment received charcoal-filtered air and the other three received ambient ozone. Half of the pots (randomly chosen) inside each chamber received two liters of half strength Hoagland's solution containing 100 mM NaCl two to three times per week, while control pots received half strength Hoagland's solution. There were 4 replicate pots per treatment in each chamber and from each cultivar.

To avoid salt shock, the high salinity treated plants were given 25 mM NaCl at first, the dose increasing step-wise to 100 mM NaCl. The conductivity of the drainage water (leachate) was measured weekly to monitor salinity. In order to avoid salt

accumulation in the pots, all pots were flushed with half strength Hoagland's solution twice per month keeping the leachate conductivity close to 13 mS cm^{-1}. Ambient or charcoal-filtered air fumigation and salinity treatment were applied from April until October for two experimental years, 2006 and 2008. The plants were moved between replicate chambers every two weeks to offset any chamber differences.

2.4. Ozone monitoring

Ozone was monitored with two Eco Sensors ozone monitors (Model C-30ZX, Eco Sensors, Santa Fe, NM, USA), one placed in a chamber with charcoal-filtered air and another one in a chamber with non-filtered (ambient) air. The data from each ozone analyzer were logged in a data logger and collected weekly from May 1st to October 15th.

2.5. Measurement of leaf dry matter and specific leaf dry mass

On June 27, July 20, August 24 and September 27, two plants from each cultivar and treatment were chosen randomly in each chamber for sampling. Three leaves each of two different ages (last year's –old- leaves and newly developed –new-mature leaves) per plant were collected and placed in plastic bags (six replicates per leaf age and treatment). After collection, the bags were moved to the laboratory, where dry mass and chlorophyll content were estimated. If the analysis was not done on the same day, the samples were stored in plastic bags in the refrigerator overnight.

Leaf dry matter content was measured by using an electronic balance (Ohaus, Germany) accurate to four decimals points, 10 cm diameter glass Petri dishes, oven (Memmert, Germany) and 9 mm diameter borer. One circular disc from the base and one from the distal end of each leaf were removed. Each disc had an area of 0.636 cm^2. All six discs from each replicate were put in pre-weighted Petri dishes. The six discs were weighted immediately (fresh mass) using an electronic balance and dried in oven 80 °C for 24 h to constant weight (dry mass). Leaf dry mass was estimated as percent dry mass. Specific leaf mass (SLM) was calculated as the dry mass in mg per leaf area in cm².

2.6. Estimation of chlorophyll content

Chlorophyll content was measured using the method by Wintermans and Mots (1965). From the same leaf samples used for dry matter evaluation, 6 half discs were taken with 9 mm diameter borer and chopped up into small pieces. After measuring their fresh mass, the pieces were placed in screw top test tubes containing 15 mL of 95% ethanol. The tubes were closed and placed in a water bath at 80 °C for 1 h. They were then left to cool to room temperature in the dark. The absorption at 665 and 649 nm was measured for each sample using a quartz cuvette and a spectrophotometer (Milton Roy Spectronic 301, USA).

The concentration of chlorophyll a and b, expressed in $\mu g \ mL^{-1}$ ethanol, was calculated using the following formulas:

Chla: $13.7 \times A665 - 5.76 \times A649$ ($\mu g \ mL^{-1}$)

Chlb: $25.8 \times A649 - 7.6 \times A665$ ($\mu g \ mL^{-1}$)

The concentration of chlorophyll a and b is better expressed in $mg \ g^{-1}$ leaf dry mass, and was calculated using the following formulas:

$15 \times$ Chla/ leaf dry mass of 6 half discs $\times 1000$ ($mg \ g^{-1}$)

$15 \times$ Chlb/ leaf dry mass of 6 half discs $\times 1000$ ($mg \ g^{-1}$)

Total Chl = Chla + Chlb

2.7. Activity of leaf main antioxidant enzymes

On July 5, August 10, September 8 and October 11, 2006, and on July 11, September 9 and October 13, 2008, leaf samples were collected for enzyme analysis. Two plants from each cultivar and treatment were chosen randomly in each chamber for sampling. Three leaves each of two different ages (last year's –old- leaves and newly developed –new- mature leaves) per plant were collected and placed in plastic bags. Two h later the main midrib was removed from each leaf. Leaves were washed with distilled water, dried with tissue paper, and dipped in liquid nitrogen, ground using a mortar and pestle and stored <-20 °C until enzyme analysis.

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2.7.1. Estimation of Superoxide dismutase (SOD) activity

Enzyme extraction

Frozen powdered leaf tissue (0.2 g) was homogenized in pre-chilled mortar and pestle with 2 mL of ice-cold 100 mM potassium phosphate extraction buffer (pH 7), containing 2% (w/v) PVPP, 1 mM EDTA, and 1 mM PMSF. The homogenate was filtered through muslin cloth and centrifuged at 15000 g for 30 minutes at 4 °C. The supernatant was used as crude extract for enzyme activity assays. All steps for enzyme extraction procedure were carried out at 4 ℃.

SOD assay

SOD activity was determined by measuring the amount of enzyme required to cause 50% inhibition of nitroblue tetrazolium chloride (NBT), as described by Giannopolitis and Ries (1977). The assay mixture consisted of 50 mM phosphate buffer (pH 7.8), 3.3 mM EDTA, 13 mM methionine, 75 μM NBT, 50 μL enzyme extract and 2 μM riboflavin (to be added last) in a total volume of 3 mL in 15 mL screw top glass test tubes. Samples were vortexed for 20 sec and then the test tubes were kept at 25 °C for 10 min in a water bath. The reaction was started by exposing the samples to four 30 W white fluorescent lamps in a box (80 cm x 50 cm x 50 cm) with aluminum-foil-coated internal walls. The reaction was allowed to proceed for 15 minutes and was then stopped by switching off the light. The absorbance was measured at 560 nm using spectrophotometer (Milton Roy Spectronic 301, USA). Blanks and controls were run in the same manner but without illumination and enzyme, respectively. One unit of SOD was defined as the amount of enzyme that produced 50% inhibition of NBT reduction under assay conditions.

2.7.2. Estimation of catalase (CAT) activity

Enzyme extraction

Frozen powdered leaf tissue (0.2 g) was homogenized in pre-chilled mortar and pestle with 2 mL of ice-cold 100 mM potassium phosphate extraction buffer (pH 7), containing 2% (w/v) PVPP, 1 mM EDTA, 1 mM PMSF and 0.05% Triton-X. The homogenate was filtered through muslin cloth, centrifuged at 15000 g for 30 min at 4 °C, and the supernatant was used as crude extract for enzyme activity assays. All steps for enzyme extraction procedure were carried out at 4 ℃.

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CAT assay

CAT activity was determined by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm according to Aebi (1983). The assay mixture consisted of 50 μL of the enzyme extract, 50 mM phosphate buffer and 10.5 mM H_2O_2 in a total volume of 1.5 mL. The decrease of H_2O_2 was monitored by reading the absorbance at 240 nm at the moment of H_2O_2 addition and 3 min later with Shimadzu spectrophotometer (Shimadzu UV-1700, Shimadzu Co. Ltd, Japan) using quartz cuvette. The activity was calculated using the extinction coefficient (40 mM^{-1} cm^{-1}) for H_2O_2.

2.7.3. Estimation of ascorbate peroxidase (APX)

Enzyme extraction

Frozen powdered leaf tissue (0.2 g) was homogenized in pre-chilled mortar and pestle with 2 mL of ice-cold 100 mM potassium phosphate extraction buffer (pH 7), containing 2% (w/v) PVPP, 1 mM EDTA, 1 mM PMSF and 0.5 mM ascorbic acid. The homogenate was filtered through muslin cloth, centrifuged at 15000 g for 30 min at 4 °C, and the supernatant was used as crude extract for enzyme activity assays. All steps for enzyme extraction procedure were carried out at 4 ºC.

APX assay

APX activity was measured by the method of Nakano and Asada (1981). The assay mixture contained 50 μL of the enzyme extract, 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate, and 1.0 mM H_2O_2 in a total volume of 1.5 mL. The decrease in absorbance of the oxidized ascorbate at 290 nm was monitored with Shimadzu spectrophotometer (Shimadzu UV-1700, Shimadzu Co. Ltd, Japan) using quartz cuvette for two min. The activity was calculated using the extinction coefficient (2.8 mM^{-1} cm^{-1}) for ascorbate.

2.8. Measurement of various leaf gas and water exchange functions

On June 26, August 3, August 30 and October 8, 2008, a portable photosynthesis unit (LCi Photosynthesis System, ADC BioScientific Ltd, Hoddesdon, Herts, UK) was used to monitor leaf net photosynthetic and transpiration rates, stomatal conductance and some other calculated leaf physiological parameters. The measurements were taken in the morning between 9:00 and 11:00, when midday heat

stress was absent. Mature leaves fully exposed to light (two leaves from each tree, eight trees from each treatment) were selected.

2.9. Measurement of midday stem water potential

Midday stem water potential was measured periodically at the same day of measuring leaf photosynthesis. Two fully expanded leaves per tree (eight trees per treatment) located on branches near the main trunk were selected and covered with aluminum foil for about two h between 12:00 and 14:00 before excision. Then, the leaves were collected and water potential was measured using a pressure bomb chamber (SKPM 1400, Skye Instruments Ltd., Liandrindod Wells, UK). Care was taken to minimize water loss during the transfer of the leaves to the chamber by enclosing them in plastic bags immediately after excision.

2.10. Measurement of leaf chlorophyll fluorescence

On June 27, August 4 and August 31, 2008 chlorophyll fluorescence equipment (OS-30p fluorometer, BioScientific Ltd., ADC, UK) was used to measure leaf stress due to salinity and ozone treatments. In total, twelve leaves from four trees from each treatment (three leaves per tree) were selected. Before the measurement, leaves were kept in the dark for 30 min using special clips. Measurements were made by introducing the analysis probe to the leaf clip. The leaf clip shutter is then withdrawn exposing the dark adapted site to a saturating excitation light source provided by a 660 nm solid state source. The same leaves were labeled and used to measure chlorophyll fluorescence over the summer. Maximum quantum yield of PSII was estimated by the Fv/Fm ratio (Krause and Weis 1991).

2.11. Evaluation of stored sugar metabolism

On October 13, 2008 about 2-5 g from the new and old shoot, new leaves and roots were sampled from four plants per treatment and stored at <-20 °C before freeze-drying in a lyophilizer (model 20 SRC-X; Virtis Co. Inc., New York, USA). Samples were ground using mortar and pestle to pass a 40 mesh screen. The method of Nzima et al. (1997) for estimation of stored sugar metabolism was followed.

Carbohydrate extraction

Soluble sugars were extracted from each of twelve samples per treatment for each organ sample (20 mg of leaf sample, 100 mg of old shoot sample, 100 mg of new shoot sample and 20 mg of root sample) The respective qualtity of each organ was added to 10 mL of 80% (v/v) methanol and homogenized for 20 s using a polytron (IKA-Labortechnik, Staufen, Germany). The extraction was repeated three times, each time using 10 mL of the methanol. The homogenates were centrifuged for 5 min at maximum speed in a centrifuge (Hettich Universal, Tuttlinger, Germany), the supernatant was decanted and all three 10-mL supernatants were combined. The methanol was evaporated to 3 to 5 mL using a water bath at 80 ºC, and then the volume was increased to 25 mL by adding deionized water. The methanol-water-soluble fractions were deproteinated using 2 mL each of 2% zinc sulfate and 2% barium hydroxide solutions and then filtered through G6 fiberglass after the precipitate had settled. Extracts were diluted with 10 mL deionized water and stored in a freezer until immediately before determining the concentrations of glucose equivalents.

Hydrolysis of starch to glucose units

The residue after methanol extraction was resuspended in 2 mL of 0.5 M NaOH and incubated for 45 min at 60 ºC in a water bath to hydrolyze starch. Once the suspensions were cool, their pH was adjusted to ~ 4.6 using 1 M HC1 and 0.5 M NaOH. The volumes were made up to 5 mL with 0.2 M of sodium acetate-acetic acid buffer of pH 4.6. Five hundred μL of amyloglucosidase (EC 3.2.1.3) was added to each suspension, mixed and incubated for 15 h at 45 ºC in a water bath to break starch chains into glucose units. The suspensions were centrifuged, the supernatant was collected, and their volumes were made up to 5 mL with 0.2 M of sodium acetate-acetic acid buffer. Starch extracts were deproteinated with 0.5 mL each of 2% zinc sulfate and 2% barium hydroxide solutions, filtered, and stored in a freezer.

Sugar determination

Glucose equivalents of the methanol-water-soluble carbohydrates and starch glucose were used to determine concentrations using the anthrone method. Ten mL of 2 g of anthrone reagent in 1 L of concentrated sulfuric acid were mixed with 5 mL of plant extract and heated for 10 min in boiling water. The solutions were cooled in ice for 10 min and assayed for glucose equivalents by measuring absorbance at 630 nm using a spectrophotometer (Milton Roy Spectronic 301, NY, USA). Anthrone and

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glucose standard solutions were freshly prepared every assay day. Glucose equivalents were calculated from glucose standard curves, and the concentration of starch was obtained by multiplying the concentrations of the starch glucose equivalents by 0.9. All concentrations were expressed on a dry mass basis.

2.12. Measurement of leaf sodium and potassium

Leaf sodium and potassium were measured using the method described by Cottenie et al. (1982). On October 13, 2008 new mature leaves (four leaves from each tree, twelve trees per treatment) were dried in the oven at 100 °C for 48 h and ground to a fine powder. Then 0.5 g samples were ashed for 5 h at 500 °C , digested in a mixture of HCl and HNO_3 (1:3), and then Na^+ and K^+ contents in the digest were determined using a flame photometer (Jenway, PFP7, Dunmow, UK).

2.13. Fresh and dry matter partitioning in tree parts

On October 15, 2006 and 2008 all plants (twelve trees per treatment) were uprooted from the pots and separated into plant parts. The bulk of the sand-perlite mixture was removed from the roots by high pressure water flushing. Plants were divided into roots, trunk, old shoots, new shoots, old leaves, and new leaves, weighed immediately to obtain fresh mass, and then placed in open paper bags in a greenhouse for drying. Dry mass was measured after complete dryness. Shoot length and total leaf area were measured for all samples.

2.14. Estimation of total new leaf area

We measured the leaf area of 30 leaves per tree using a scanner, dried the leaves at 100 °C until constant mass and calculated the ratio leaf area per unit dry mass. For plant leaf area estimation, we collected all leaves of each plant grown in the same year, dried at 100 °C and total leaf area was estimated multiplying the total leaf dry mass by the leaf area/dry mass ratio (Chartzoulakis et al., 2002).

2.15. Statistical analysis

All data were analysed using analysis of variance over two, three or four parameters (cultivar, treatment, time, leaf age) with the SPSS statistical package (SPSS 16.0, Chicago, IL). There were 6, 12 or 16 replications or otherwise shown for each mean presented. Least significant difference is presented for 5% error.

Chapter 3. Results

3.1. Ozone concentrations

Ozone levels showed a typical diurnal profile, with the highest concentrations recorded at midday and during the early afternoon, which are the day hours with maximum radiation and air temperature (Fig. 1). The mean daylight concentrations of O_3, expressed for 12 h daylight (9:00-21:00 h), in the non-filtered chambers over the experimental periods (from May 1st to October 15th in 2006 and 2008) were 70 nL L^{-1} and 72 nL L^{-1}, respectively (Fig. 2). The maximum 12 h concentrations reached 90 nL L^{-1} in 2006 and 93 nL L^{-1} in 2008. The cumulative O_3 exposure during daylight hours, expressed as AOT40 (accumulated exposure over a threshold of 40 nL L^{-1}), calculated over the periods from May 1st to October 15th in 2006 and 2008 had values of 53092 nL L^{-1} h in 2006 and 54176 nL L^{-1} h in 2008. In the chambers with filtered air, mean daylight concentration of O_3 was 26 nL L^{-1} in 2006 and 17 nL L^{-1} in 2008 and maximum values reached 27 nL L^{-1} in both years, thus AOT40 values were zero.
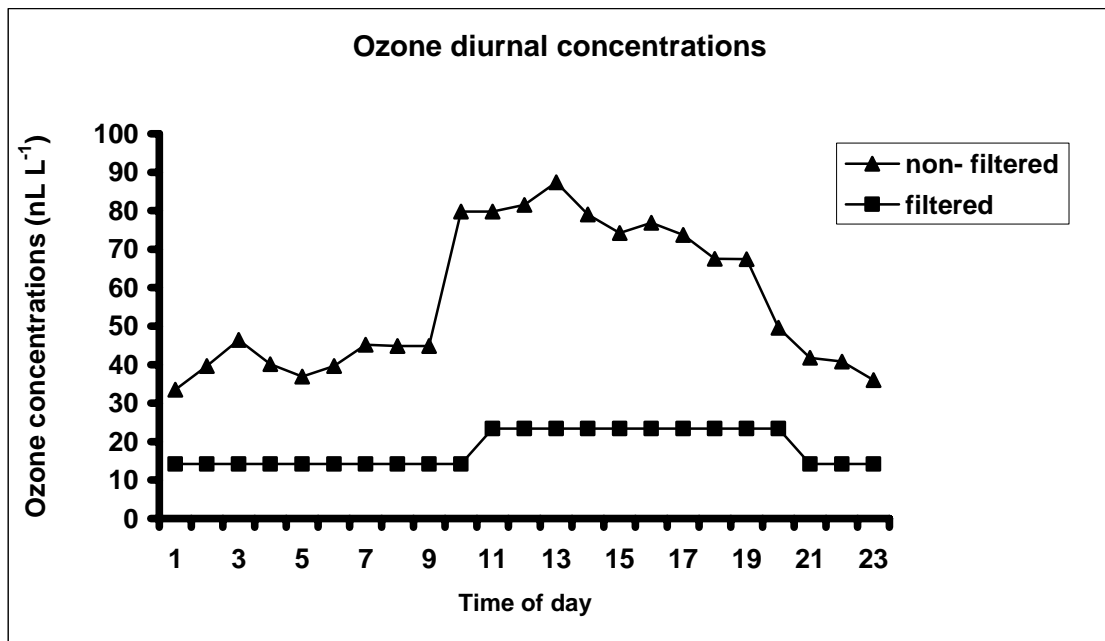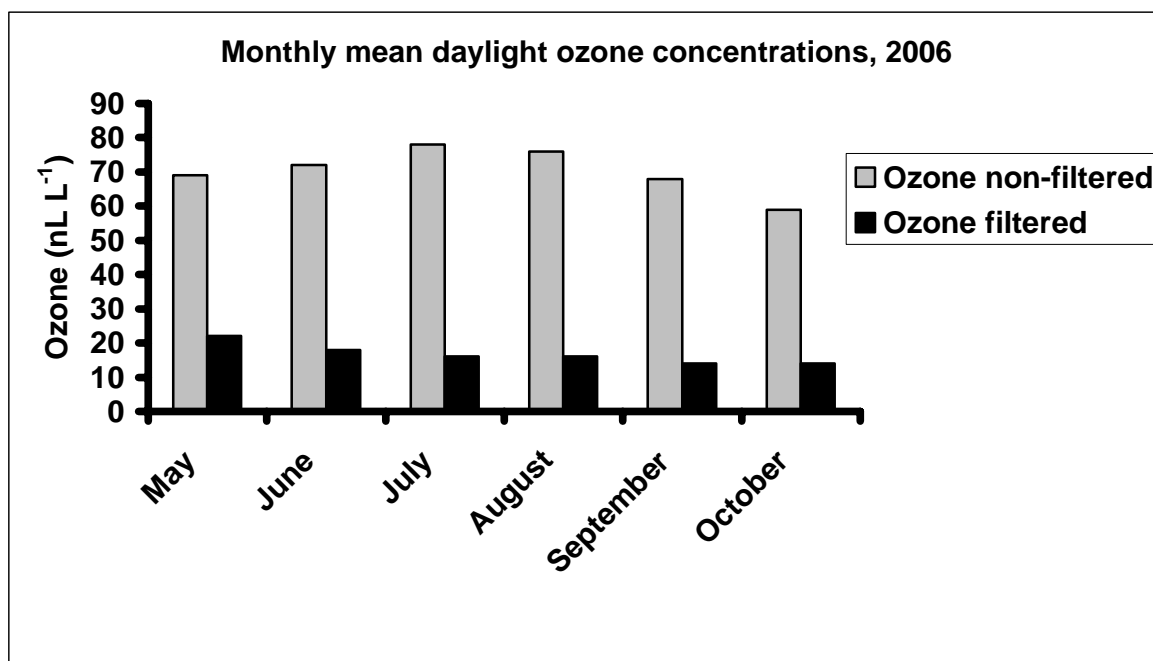

Fig. 1. Ozone concentrations during the day on July 15, 2008 in non-filtered and filtered chambers.

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A

Monthly mean daylight ozone concentrations, 2006

B

Monthly mean daylight ozone concentrations, 2008
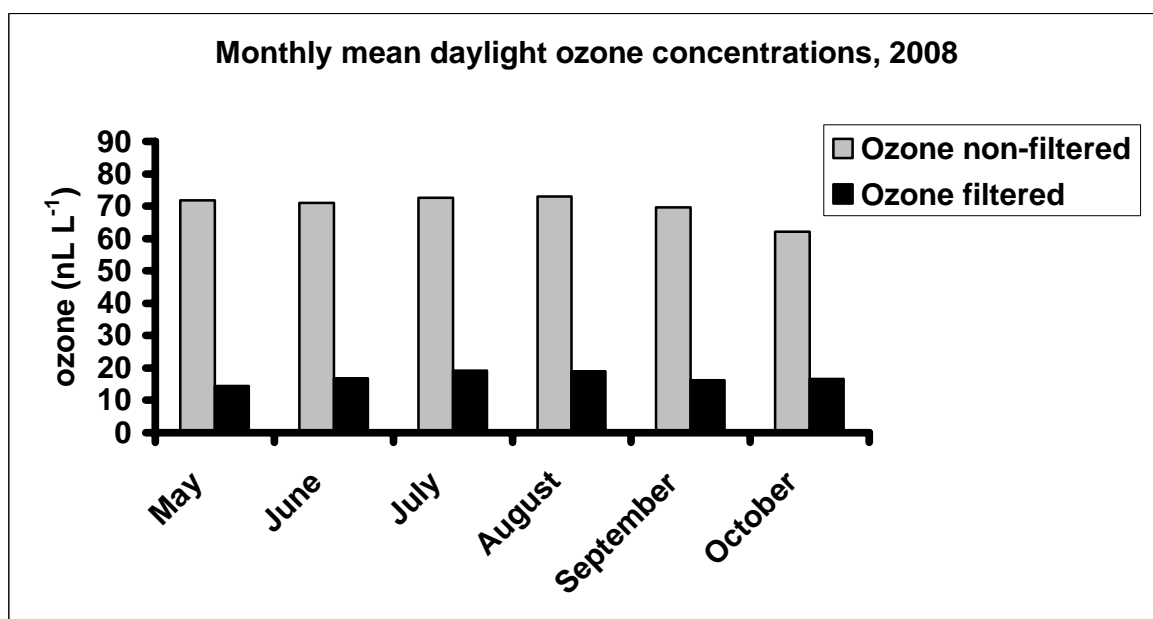
Fig. 2. Monthly average O_3 concentrations in non-filtered and filtered chambers in 2006 (A) and 2008 (B).

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3.2. Leaf characteristics, 2006 data

3.2.1. Leaf percent dry matter

Leaves from 'Kalamata' olive trees had higher percent dry matter (DM) than leaves from 'Konservolea' olive trees in June and September, while in August and July the leaves from both cultivars had similar percent DM (Table 3). This trend was found in most treatments and in both leaf ages.

Leaf percent DM in 'Konservolea' olive trees increased in July, but decreased in August and remained unchanged in September keeping levels higher than in June (Table 3). This trend was found in all treatments except of the combination treatment of high salinity plus ambient ozone (WW), where leaf percent DM increased in July and remained unchanged until September.

Leaf percent DM in 'Kalamata' olive trees increased progressively in July and August and remained unchanged in September (Table 3). This trend was found in all treatments except of WW treatment, where leaf percent DM increased in July, remained unchanged in August and further increased in September.

In 'Konservolea' olive trees, the high salinity plus charcoal-filtered air (WN) and WW treatments decreased leaf percent DM compared to control (low salinity plus charcoal-filtered air) and the low salinity plus ambient ozone (NW) treatments in July and August (Table 3). In June and September (when the weather was cooler), salinity-treated plants had similar leaf percent DM to control plants.

In 'Kalamata' trees, the two high salinity treatments (WN and WW) decreased leaf percent DM compared to control and NW treatments in August and somewhat in September (Table 3). In June and July, the leaves of all treatments had similar percent DM. NW did not affect leaf percent DM in both cultivars tested.

In both cultivars, new leaves had lower percent DM than last year's leaves in June and differences diminished during summer and in September (Tables 3). NW treatment may have affected leaf percent DM of old leaves, as leaves of both ages in NW-treated trees had similar percent DM.

3.2.2. Specific leaf mass

Leaves from 'Kalamata' olive trees had higher specific leaf mass (SLM) than leaves from 'Konservolea' olive trees in June and September, while in July and August the leaves from both cultivars had similar SLM (Tables 3). This trend was found in all treatments and in both leaf ages.

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SLM in 'Konservolea' olive trees increased in July and remained unchanged until September. This trend was found in all treatments except of the NW treatment, where SLM increased in July and August and decreased in September (Table 3).

SLM in 'Kalamata' olive trees remained unchanged in June and July and increased in August and September. This trend was found for all treatments except of WW treatment, where SLM increased in July and August and reached the highest value in September (Table 3).

In 'Konservolea' olive trees, NW treatment decreased SLM only slightly compared to the other treatments in June, while in July WW treatment decreased SLM compared to the other treatments. In August and September the leaves of all treatments had similar SLM (Table 3).

In 'Kalamata' trees, the leaves of all treatments had similar SLM in June and July, while, in August, WN treatment decreased SLM compared to control and NW treatments and, in September, WN treatment decreased SLM compared to control (Table 3).

In both cultivars, new leaves had lower SLM than last year's leaves in all treatments and dates except of July, where leaves from both ages had similar SLM (Table 3).

3.2.3. Leaf chlorophyll a content

Leaves from 'Konservolea' olive trees had lower chlorophyll a content (Chl a) than leaves from 'Kalamata' olive trees only in June and September. In July and August, the leaves from both cultivars had similar Chla (Table 4). This trend was found in all treatments and in both ages.

In 'Konservolea' olive trees, the leaves of the control treatment had similar Chl a content in all dates. In WN and WW treatments, leaf Chl a content decreased from June to July and remained unchanged until September. In NW treatment, leaf Chl a content remained constant from June to July, decreased in August and increased again in September reaching similar values to the ones in June (Table 4).

In 'Kalamata' olive trees, in the control treatment, leaf Chl a content remained unchanged from June to July, decreased in August and increased again in September reaching similar values to the ones in June. In NW and WN treatments, leaf Chl a content decreased from June to July, remained constant in August and increased again in September reaching similar values to the ones in June. In WW treatment, leaf Chl a

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content decreased progressively from June to August and slightly increased in September reaching lower values than the ones in June (Table 4).

In 'Konservolea' olive trees, both WN and WW treatments decreased leaf Chl a content compared to control over the time period studied except in June, where, WW treatment did not have significant effect on leaf Chl a content compared to control. NW treatment slightly decreased leaf Chl a content compared to control in June and August, while in July and September NW treatment did not affect leaf Chl a compared to control (Table 4).

In 'Kalamata' olive trees, WN treatment decreased leaf Chl a content compared to control in June and July, while, in August and September, WN treatment did not affect leaf Chl a content compared to control. WW treatment slightly increased leaf Chl a content compared to control in June, while in July, WW treatment slightly decreased leaf Chl a content compared to control. In August and September, WW treatment did not affect leaf Chl a compared to control. NW treatment did not affect leaf Chl a content over the time period studied except in July, where NW treatment decreased leaf Chl a content compared to control (Table 4).

In both cultivars, new leaves had higher Chl a content than last year's leaves in all dates except of September where leaves from both ages had similar leaf Chl a content (Table 4).

3.2.4. Leaf chlorophyll b content

Leaves from 'Konservolea' olive trees had higher chlorophyll b content (Chl b) than leaves from 'Kalamata' only in June, while, in July and August, the leaves from both cultivars had similar Chl b content. In September, leaves from 'Konservolea' olive trees had lower Chl b content than leaves from 'Kalamata' trees. This trend was found in leaves from both ages (Table 4).

Leaves from 'Konservolea' olive trees, had similar Chl b content over the time period studied in the control treatment. In NW treatment, leaf Chl b content decreased slightly from June to July, increased in August and decreased again in September. In WN treatment, leaf Chl b content decreased from June to July, remained unchanged in August and decreased again in September. In WW treatment, leaf Chl b content decreased from June to July, increased in August and decreased again in September (Table 4).

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In 'Kalamata' olive trees, leaf Chl b content decreased from June to July, remained unchanged in August and decreased again in September reaching levels lower than the ones in June in the control treatment. In NW treatment, leaf Chl b content remained unchanged from June to July, increased in August and decreased again in September in. In WN treatment, leaf Chl b content remained unchanged from June to July, increased in August and decreased in September reaching similar values to the ones in June. In WW treatment, leaf Chl b content increased somewhat in July and remained unchanged until September (Table 4).

In 'Konservolea' olive trees, WN and WW treatments decreased leaf Chl b content compared to control and NW treatments in June, while in July all treatments decreased leaf Chl b content compared to control, with the smallest reduction in leaf Chl b content occurring in plants exposed to NW treatment. In August, mainly WN and, to a lesser extend, WW treatments decreased leaf Chl b content compared to control and NW treatments. In September, WN and WW treatments decreased leaf Chl b content compared to control and NW treatments (Table 4).

In 'Kalamata' olive trees, all treatments decreased leaf Chl b content compared to control in June with the largest reduction occurring in plants exposed to WW treatment. In July, August and September, WN and WW treatments decreased leaf Chl b content compared to control and NW treatments, but in August the largest reduction occurred in plants exposed to WW treatment (Table 4).

In both cultivars, new leaves had lower leaf Chl b content than last year's leaves in all dates and treatments except in July, where leaves from both ages had similar leaf Chl b content (Table 4).

3.2.5. Leaf Total Chlorophyll content

Leaves from 'Konservolea' olive trees had lower leaf total chlorophyll content (Tchl) than leaves from 'Kalamata' olive trees in June and September, which was due to higher Chl a content. But in June and August, leaves from both cultivars had similar Tchl content (Fig. 3). In control and NW treatments leaves from both cultivars had similar Tchl content, while in WN and WW treatments, leaves from 'Konservolea' olive trees had lower Tchl content than leaves from 'Kalamata' olive trees (Table 5). New leaves from both cultivars had similar leaf Tchl content, while last year's leaves in 'Konservolea' olive trees had slightly lower TChl content than last year's leaves in 'Kalamata' olive trees (Table 5).

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Leaves from 'Konservolea' olive trees had similar Tchl content over the time period studied in the control and NW treatments, while in WN and WW treatments leaf Tchl content decreased from June to July and remained unchanged until September (Fig. 4, Table 5).

In 'Kalamata' olive trees, leaves in all dates had similar Tchl content in NW and WW treatments. In the control treatment, leaf Tchl content progressively decreased from June to August and then slightly increased in September reaching values lower than the ones in June. In WN treatment, leaf Tchl content decreased from June to July, remained unchanged in August and increased again in September reaching similar values to the ones in June (Fig. 4, Table 5).

In 'Konservolea' olive trees, WN and WW treatments decreased leaf Tchl content compared to control over the time period studied. The effect of WW treatment was smaller than WN treatment only in June. NW treatment slightly decreased leaf Tchl content compared to control in June; the reduction was more pronounced in July, and differences disappeared gradually in August and September (Fig. 4, Table 5).

In 'Kalamata' olive trees, WN and WW treatments decreased leaf Tchl content compared to control in all dates. The effect of WW treatment was smaller than WN treatment only in July. NW treatment decreased leaf Tchl content compared to control in June and July, while in August NW treatment did not affect leaf Tchl content compared to control. In September, NW treatment slightly decreased leaf Tchl content compared to control (Fig. 4, Table 5).

In both cultivars, leaves from both ages had similar leaf Tchl content in all dates and treatments (Table 5).

3.2.6. Chlorophyll a/b ratio

Leaves from both cultivars had similar leaf chlorophyll a over chlorophyll b ratio (Chl a/b) in July, August and September. In June, leaves from 'Konservolea' olive trees had lower Chl a/b ratio than leaves from 'Kalamata' olive trees. In the control and NW treatments, leaves from both cultivars had similar Chl a/b ratio. In WN treatment, leaves from 'Konservolea' had higher leaf Chl a/b ratio than leaves from 'Kalamata' olive trees, while in WW treatment, leaves from 'Konservolea' had lower leaf Chl a/b ratio than leaves from 'Kalamata' olive trees. This trend was found in leaves from both ages (Table 5).

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In 'Konservolea' olive trees, the leaves had similar leaf Chl a/b ratio in all dates in the control and WW treatments. In NW treatment, leaf Chl a/b ratio decreased only in August. In WN treatment, leaf Chl a/b ratio remained unchanged from June to August and increased in September (Table 5).

In 'Kalamata' olive trees, leaf Chl a/b ratio in June and August was higher than in July and September in the control treatment. In NW treatment, leaf Chl a/b ratio in July and August was higher than in June and September. In WW treatment, leaf Chl a/b ratio decreased in July and further decreased in August and September (Table 5).

In 'Konservolea' olive trees, leaves of all treatments had similar leaf Chl a/b ratio in June. In July, WN and NW increased leaf Chl a/b ratio compared to control; WW treatment did not affect leaf Chl a/b ratio compared to control. In August, WN and WW treatments did not affect leaf Chl a/b ratio compared to control, while NW treatment slightly decreased leaf Chl a/b ratio compared to control. In September, WN treatment increased leaf Chl a/b ratio compared to control, while NW and WW treatments did not affect leaf Chl a/b ratio compared to control (Table 5).

In 'Kalamata' olive trees, WN and NW treatments slightly increased leaf Chl a/b ratio compared to control, while in WW treatment the increase was more pronounced compared to control and other treatments in June. In July, WN treatment did not affect leaf Chl a/b ratio compared to control; NW treatment slightly decreased leaf Chl a/b ratio compared to control; and WW treatment did not affect leaf Chl a/b ratio compared to control. In August and September, leaves of all treatments had similar leaf Chl a/b ratio to control (Table 5).

In both cultivars, new leaves had higher leaf Chl a/b ratio than last year's leaves in June and differences diminished during summer. In September, leaves from both ages had similar leaf Chl a/b ratio (Table 5).

Table 3. Leaf dry matter (DM) and specific leaf mass (SLM) of this year's (N) and one year old (O) leaves from 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2006.

Month	Treatment	DM (%)				SLM (mg cm^{-2})			
		Konservolea		Kalamata		Konservolea		Kalamata	
		O	N	O	N	O	N	O	N
June	C	47.5	42.4	52.1	43.6	23	17.6	26.2	18.2
	WN	47.6	42.8	50.3	44.8	24.4	18	25.4	19.4
	WW	45.2	44.1	50.3	43.6	21	18.8	26.6	18
	NW	43.7	42.2	50	45.3	20,.2	17.4	27.2	19
July	C	52.5	54.9	48.3	52.6	25.4	24.6	23.8	23.2
	WN	49.9	53.5	50.1	52.6	24.4	25	24.4	23.4
	WW	47.8	53.2	49.1	52.4	22.6	23.4	25	23.6
	NW	51.7	55.9	48.6	52.9	25.8	25.8	25.2	23.6
August	C	51.7	50.6	55.4	51.3	25.6	22.6	30	22.4
	WN	50.5	48.4	50.9	49.4	26.6	22.4	25.8	23
	WW	50.1	49.4	52.5	50.4	25.2	22.4	26.4	22.4
	NW	52.7	48.8	53.9	53.9	27.2	21.4	27.6	25.6
September	C	50.8	47.4	53.7	52	25.4	21.8	30.2	24
	WN	50.2	47.6	51.3	48.1	26.4	23.0	28	24
	WW	50.7	51.3	51.6	58.9	27	23.2	27.4	27.2
	NW	50	48.8	55.5	53.7	24	22.2	30.6	27
Significance									
	Time	***				***			
	Treatment	*				NS			
	Leaf age	**				***			
	Cultivar	***				***			
	LSD$_{0.05}$	3.5				1.7			

Significance levels: NS not significant, * significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.001$.

Table 4. Leaf chlorophyll a and b content of this year's (N) and one year old (O) leaves from 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2006.

| Month | Treatment | Chl a (mg g^{-1}DM) | | | | Chl b (mg g^{-1}DM) | | | |
| | | Konservolea | | Kalamata | | Konservolea | | Kalamata | |
		O	N	O	N	O	N	O	N
June	C	2.32	2.41	2.22	2.4	1.44	1.42	1.8	1.59
	WN	1.87	2.18	2.01	2.16	1.36	1.07	1.14	1.15
	WW	2.16	2.27	2.3	2.71	1.31	1.36	1.04	0.67
	NW	2.02	2.29	2.4	2.27	1.47	2.29	1.24	1.39
July	C	2.09	2.48	2.64	2.09	1.58	1.44	1.13	1.82
	WN	1.63	1.92	1.7	2.02	1.22	0.84	1.03	1.08
	WW	1.55	1.82	1.96	2.45	1.13	1.04	1.17	0.84
	NW	2.38	2.1	1.88	2.14	1.2	1.3	1.49	1.37
August	C	1.9	2.43	1.96	1.91	1.84	1.22	1.6	1.43
	WN	1.62	1.71	1.65	1.75	0.93	1.18	1.3	1.32
	WW	1.5	1.71	1.87	1.67	1.42	1.09	1.19	1.17
	NW	1.76	2.03	2.03	1.98	1.89	1.6	1.7	1.38
September	C	2.12	2.29	2.26	2.36	1.59	1.27	1.43	1.31
	WN	2.02	1.81	2.21	1.99	0.65	0.99	1.28	1.04
	WW	1.74	1.79	2.03	2.02	0.94	0.92	1.31	1.04
	NW	2.34	2.23	2.28	2.15	1.41	1.3	1.34	1.15
Significance									
	Time	***				***			
	Treatment	***				***			
	Leaf age	**				***			
	Cultivar	*				NS			
	LSD$_{0.05}$	0.4				0.3			

Significance levels: NS not significant, * significant at $P< 0.05$, ** significant at $P< 0.01$, *** significant at $P< 0.001$.

Table 5. Leaf total chlorophyll content and the ratio chl a/b of this year's (N) and one year old (O) leaves from 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2006.

| Date | Treatment | TChl (mg g^{-1} DM) | | | | Chl a/b | | | |
| | | Konservolea | | Kalamata | | Konservolea | | Kalamata | |
		O	N	O	N	O	N	O	N
June	C	3.76	3.38	4.02	3.99	1.62	1.7	1.23	1.53
	WN	3.23	3.25	3.15	3.31	1.39	2.04	1.76	1.88
	WW	3.47	3.63	3.34	3.38	1.69	1.67	2.21	4.01
	NW	3.49	3.67	3.64	3.66	1.41	1.67	1.94	1.62
July	C	3.67	3.92	3.74	3.81	1.32	1.72	2.33	1.21
	WN	2.84	2.77	2.74	3.1	1.35	2.28	1.65	1.86
	WW	2.68	2.87	3.12	3.29	1.37	1.75	1.68	3.49
	NW	3.59	3.39	3.37	3.52	2.15	1.63	1.27	1.57
August	C	3.74	3.65	3.56	3.34	1.07	2.07	1.22	1.34
	WN	2.55	2.89	2.95	3.07	1.77	1.45	1.29	1.32
	WW	2.92	2.8	3.06	2.84	1.06	1.57	1.57	1.42
	NW	3.65	3.53	3.74	3.35	0.99	1.35	1.19	1.44
September	C	3.71	3.56	3.69	3.67	1.37	1.8	1.59	1.8
	WN	2.67	2.8	3.49	3.04	3.17	1.85	1.73	1.91
	WW	2.68	2.71	3.34	3.07	1.85	1.93	1.6	1.94
	NW	3.75	3.53	3.62	3.3	1.66	1.72	1.69	1.87
Significance									
	Time	***				***			
	Treatment	***				***			
	Leaf age	NS				**			
	Cultivar	**				NS			
	LSD$_{0.05}$	0.35				0.83			

Significance levels: NS not significant, ** significant at $P < 0.01$, *** significant at $P < 0.001$.

Fig. 3. Changes over the 2006 summer periods of leaf total chlorophyll content (Tchl) of 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock. The values are means of 48 replications and overall $LSD_{0.05}$ was 0.13.

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A
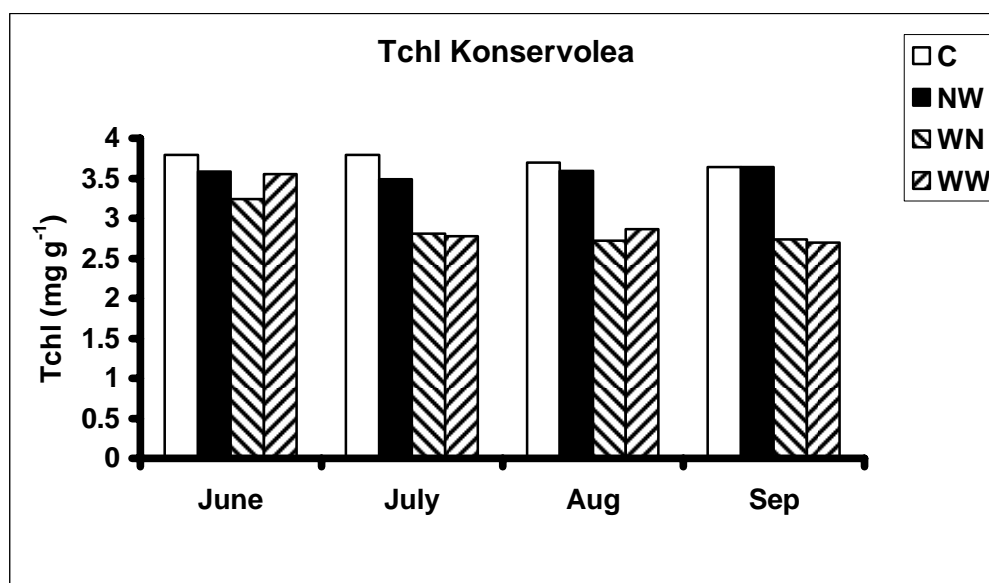
B
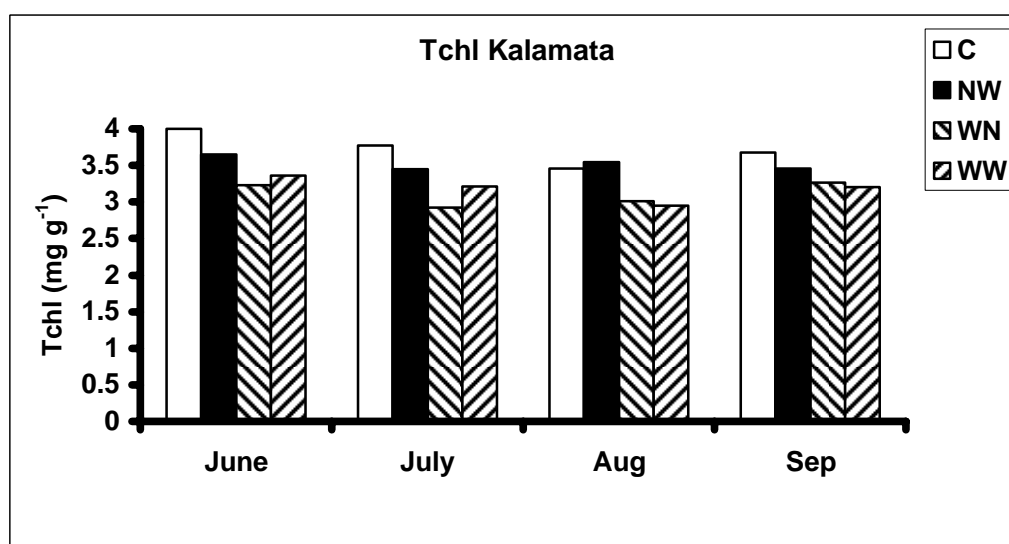
Fig. 4. Changes over the 2006 summer period of leaf total chlorophyll content (Tchl) of 'Konservolea' (A) and 'Kalamata' (B) olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution and filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN). The values are means of 12 replications and overall $LSD_{0.05}$ was 0.25.

3.3. Leaf Characteristics, 2008 data

3.3.1. Leaf percent dry matter

Leaves from 'Konservolea' olive trees had lower percent DM than leaves from 'Kalamata' olive trees in June and September, while in July and August, leaves from both cultivars had similar percent DM. In all treatments, leaves from 'Konservolea' olive trees had slightly lower percent DM than leaves from 'Kalamata' olive trees, except of WW treatment, where leaves from 'Konservolea' olive trees had slightly higher percent DM than leaves from 'Kalamata' trees. In both leaf ages, leaves from 'Konservolea' olive trees had slightly lower percent DM than leaves from 'Kalamata' olive trees (Table 6).

Leaf percent DM in 'Konservolea' olive trees increased from June to July, remained constant in August and significantly dropped again in September reaching similar values to the ones in June in the control treatment. In NW treatment, leaf percent DM gradually increased from June to August and significantly decreased in September. In WN treatment, leaf percent DM decreased from June to July, and remained unchanged until September. In WW treatment, leaf percent DM gradually decreased from June to August, and remained unchanged in September (Table 6).

In 'Kalamata' olive trees, the leaves had similar percent DM in all dates in the control treatment. In NW treatment, leaf percent DM remained constant from June until August, and increased in September. In WN treatment, leaf percent DM decreased from June to July, slightly decreased further in August, and then slightly increased in September in levels lower than in July. In WW treatment, leaf percent DM decreased from June to July and remained constant thereafter until September (Table 6).

In 'Konservolea' olive trees, WN and WW treatments decreased leaf percent DM compared to control in July, August and September, while in June WN treatment slightly increased leaf percent DM compared to control. The effect of WW treatment was smaller than WN treatment only in July. NW treatment slightly decreased leaf percent DM compared to control in June, while in July the reduction was more pronounced. In August and September, NW treatment did not affect leaf percent DM (Table 6).

In 'Kalamata' olive trees, the leaves of all treatments has similar percent DM in June. WN and WW treatments decreased leaf percent DM compared to control in July, August and September. The effect of WW treatment was smaller than WN

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treatment in July, while in August, WW treatment effect was higher than WN treatment; and in September, both treatments had similar effect. NW treatment increased leaf percent DM compared to control only in September (Table 6).

In both cultivars, new leaves had slightly lower percent DM than last year's leaves in all dates except in June, where leaves from both ages had similar percent DM. The above was true for the control and NW treatments, but in WN and WW treatments, leaves from both ages had similar percent DM (Table 6).

3.3.2 Specific leaf mass

Leaves from both cultivars had similar SLM. This trend was found in all dates, all treatments and in both leaf ages (Table 6).

SLM in 'Konservolea' olive trees slightly increased from June to July, remained constant in August and slightly decreased again in September reaching similar values to the ones in June in the control treatment. In NW treatment, SLM slightly increased gradually from June to August, and slightly decreased again in September. In WN and WW treatments, SLM did not change over the measurement period (Table 6).

In 'Kalamata' olive trees, leaves had similar SLM in all dates in the control and WN treatments. In NW treatment, SLM slightly increased from June to July, remained constant in August and slightly increased again in September. In WW treatment, SLM remained constant from June to July, increased slightly in August and slightly decreased again in September (Table 6).

In 'Konservolea' olive trees, leaves of all treatments had similar SLM in June. WN and WW treatments decreased SLM compared to control in July, August and September. The effect of WW was similar to the effect of WN in all dates. NW treatment slightly increased SLM compared to control in August and September, while in July NW treatment did not affect SLM compared to control (Table 6).

In 'Kalamata' olive trees, WN and WW treatments slightly decreased SLM compared to control in June. In July, only WN treatment slightly decreased SLM compared to control, while WW treatment did not affect SLM. In August, WN treatment decreased SLM compared to control, while WW treatment slightly increased SLM compared to control. In September, both WN and WW treatments decreased SLM compared to control in a similar manner. NW treatment did not affect SLM in June, while, in July, August and September, SLM was slightly increased by NW treatment compared to control (Table 6).

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In both cultivars, new leaves had lower SLM than last year's leaves in all dates and treatments (Table 6).

3.3.3. Chlorophyll a content

Leaves from both cultivars had similar Chl a content in June, while in July, August and September, leaves from 'Konservolea' olive trees had lower Chl a content than leaves from 'Kalamata' olive trees. Leaves from 'Konservolea' olive trees had slightly lower Chl a content than leaves from 'Kalamata' olive trees in all treatments except of the control treatment, where leaves from both cultivars had similar Chl a content (Table 7). This trend was found in both leaf ages.

In 'Konservolea' olive trees, leaf Chl a content remained constant from June to July, decreased in August and increased again in September reaching similar values to the ones in June in the control treatment. In NW treatment, leaf Chl a content remained constant from June to July, decreased in August and increased again in September in values smaller than the ones in June. In WN treatment, leaf Chl a content increased from June to July, decreased in August and remained constant in September. In WW treatment, leaf Chl a content slightly increased from June to July, slightly decreased in August, and slightly increased again in September reaching levels higher than the ones in June (Table 7).

In 'Kalamata' olive trees, leaf Chl a content increased from June to July, decreased in August and increased again in September reaching levels higher than the ones in June in the control treatment. In NW treatment, leaf Chl a content increased from June to July, slightly decreased in August and slightly increased again in September reaching levels higher than the ones in June. In WN and WW treatments, leaf Chl a content increased from June to July, decreased in August and remained constant in September (Table 7).

In 'Konservolea' olive trees, WN and WW treatments decreased leaf Chl a content compared to control in all dates except in September, where WW treatment slightly decreased leaf Chl a content compared to control. The effect of WW treatment was slightly higher than the effect of WN treatment in July, while in August and September both treatments had similar effect. NW treatment did not affect leaf Chl a content in June compared to control. In July, NW treatment increased leaf Chl a content compared to control, while in August and September, NW treatment slightly decreased leaf Chl a content compared to control (Table 7).

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In 'Kalamata' olive trees, WN and WW treatments decreased leaf Chl a content compared to control in all dates. The effect of WW treatment was slightly lower than the effect of WN treatment in July and August, while in June and September both treatments had similar effect. NW treatment did not affect leaf Chl a content in all dates except in August, where NW treatment increased leaf Chl a content compared to control (Table 7).

In both cultivars, leaves from both ages had similar leaf Chl a content in all dates except in June, where new leaves had lower leaf Chl a content than last year's leaves (Table 7).

3.3.4. Chlorophyll b content

Leaves from both cultivars had similar leaf Chl b content in all dates and treatments except in July, where leaves from 'Konservolea' olive trees had higher Chl b content than leaves from 'Kalamata' olive trees (Table 7). This trend was found in both leaf ages.

In 'Konservolea' olive trees, leaf Chl b content remained constant from June to July, increased in August and decreased again in September in the control treatment. In NW and WN treatments, leaves in all dates had similar Chl b content. In WW treatment, leaf Chl b content slightly decreased from June to July, increased in August and decreased again in September reaching similar values to the ones in June (Table 7).

In 'Kalamata' olive trees, leaf Chl b content in the control treatment slightly decreased from June to July, increased in August and decreased again in September reaching higher values than the ones in June. In NW treatment, leaf Chl b content decreased from June to July, increased in August and decreased again in September reaching higher values than the ones in June. In WN treatment, leaves had similar Chl b content in all dates. In WW treatment, leaf Chl b content remained constant from June to July, slightly increased in August and slightly decreased again in September (Table 7).

In 'Konservolea' olive trees, WN and WW treatments decreased leaf Chl b content compared to control in all dates. The effect of both treatments was similar except in August where the effect of WW treatment was slightly lower than the effect of WN treatment. NW treatment did not affect leaf Chl b content compared to control

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in all dates except in August, where NW treatment slightly decreased leaf Chl b content compared to control (Table 7).

In 'Kalamata' olive trees, WN and WW treatments decreased leaf Chl b content compared to control in all dates. Both treatments had similar effect in all dates except in August, where the effect of WW treatment was higher than the effect of WN. NW treatment decreased leaf Chl b content compared to control in June and July. In August, NW treatment increased leaf Chl b content compared to control and in September NW treatment did not affect leaf Chl b content compared to control (Table 7).

In both cultivars, leaves from both ages had similar Chl b content in July and August. In June, new leaves had higher Chl b content than last year's leaves, while the opposite was true in September, when new leaves had lower Chl b content than last year's leaves (Table 7).

3.3.5. Total Chlorophyll content

Leaves from 'Konservolea' olive trees had significantly lower Tchl content than leaves from 'Kalamata' olive trees. This difference between the two cultivars was mainly found in August and September, as in June and July, the difference was minimal in all treatments (Fig. 5). These differences between the two cultivars were mainly found in the last year's leaves, as in the new leaves the differences disappeared (Table 8).

In 'Konservolea' olive trees, leaf Tchl content in the control treatment remained constant over the time period studied. In NW treatment, leaf Tchl content slightly increased from June to July, decreased in August and remained constant in September. In WN treatment, leaf Tchl content increased from June to July and remained constant thereafter. In WW treatment, leaf Tchl content remained constant from June to July, increased in August and remained constant until September (Fig. 6, Table 8).

In 'Kalamata' olive trees, leaf Tchl content in the control treatment gradually increased from June to September. In NW treatment, leaf Tchl content slightly increased from June to July, further increased in August reaching the highest values, and slightly decreased in September reaching levels higher than the ones in June. In WN treatment, leaf Tchl content gradually increased from June to August and

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remained constant in September. In WW treatment, leaf Tchl content increased from June to July and remained constant until September (Fig. 6, Table 8).

In 'Konservolea' olive trees, WN and WW treatments decreased leaf Tchl content compared to control over the time period studied. The effect of WW treatment was slightly smaller than the effect of WN treatment in August and September, while in June and July both treatments had similar effect. NW treatment increased leaf Tchl content compared to control only in July, while in August, NW treatment decreased leaf Tchl content compared to control (Fig. 6, Table 8).

In 'Kalamata' olive trees, WN and WW treatments decreased leaf Tchl content compared to control over the time period studied. The effect of WW treatment was slightly smaller than the effect of WN only in July, while in the other dates, both treatments had similar effect. NW treatment decreased leaf Tchl content compared to control in June and July, while in August, NW treatment increased leaf Tchl content compared to control. In September, NW treatment did not affect leaf Tchl content compared to control (Fig. 6, Table 8).

In both cultivars, leaves from both ages had similar leaf Tchl content over the summer, but in September, new leaves had slightly lower leaf Tchl content than last year's leaves (Table 8).

3.3.6. Chlorophyll a/b ratio

Leaves from 'Konservolea' olive trees had lower Chl a/b ratio than leaves from 'Kalamata' olive trees in June and July, while in August and September, leaves from both cultivars had similar Chl a/b ratio. Leaves from 'Konservolea' olive trees had lower Chl a/b ratio than leaves from 'Kalamata' olive trees in both leaf ages and all treatments except of the control treatment, where leaves from both cultivars had similar Chl a/b ratio (Table 8).

In 'Konservolea' olive trees, leaf Chl a/b ratio remained constant from June to July, decreased in August and slightly increased in September in the control and NW treatments. In WN treatment, leaf Chl a/b ratio increased from June to July, decreased in August and again slightly increased in September reaching similar values to the ones in June. The same trend was found in WW treatment with a difference in September, where the increase was more pronounced (Table 8).

In 'Kalamata' olive trees, leaf Chl a/b ratio in the control treatment increased from June to July, decreased in August and increased again in September reaching

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similar values to the ones in June. In NW and WN treatments, leaf Chl a/b ratio increased from June to July, decreased in August and increased again in September in levels lower than the ones in June. In WW treatment, leaf Chl a/b ratio increased from June to July, decreased in August and remained constant in September (Table 8).

In 'Konservolea' olive trees, WN and WW treatments decreased leaf Chl a/b ratio compared to control in June. The opposite was true in July where both treatments increased leaf Chl a/b ratio compared to control. The effect of both treatments was similar. In August and September, leaves from all treatments had similar Chl a/b ratio. NW treatment did not affect leaf Chl a/b ratio compared to control (Table 8).

In 'Kalamata' olive trees, WN and WW treatments increased leaf Chl a/b ratio compared to control in June and July. The effect of WN treatment was higher than the effect of WW treatment only in July. In August, leaf Chl a/b ratio increased only by WW treatment compared to control. In September, leaves from all treatments had similar leaf Chl a/b ratio. NW treatment increased leaf Chl a/b ratio compared to control in June and July, while in August, NW treatment did not affect leaf Chl a/b ratio compared to control (Table 8).

In both cultivars, leaves from both ages had similar leaf Chl a/b ratio in all dates except in June, where new leaves had lower leaf Chl a/b ratio than last year's leaves (Table 8).

Table 6. Leaf percent dry matter (DM) and specific leaf mass (SLM) of this year's (N) and one year old (O) leaves from 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2008.

Month	Treatment	DM (%)				SLM (mg cm^{-2})			
		Konservolea		Kalamata		Konservolea		Kalamata	
		O	N	O	N	O	N	O	N
June	C	47.5	51.2	51.7	49.5	23	23.6	25.6	23.4
	WN	50.1	51	51.4	50.4	24.6	21.4	26.6	21.8
	WW	52.2	50.2	49.9	51.4	24.6	22	24.2	21.6
	NW	48.3	48.5	50.9	52.2	24	23.4	26	20.8
July	C	51.7	50.2	51.7	51.3	24.2	25.8	25.6	21.6
	WN	47	46.4	51.4	49.6	23.6	22	26.6	24.2
	WW	49.6	47.9	48.4	47.9	23.4	21.4	23.6	21
	NW	49	50.2	45.4	47.1	24.4	26.8	23.2	24
August	C	51.4	49.5	50.9	49.9	24.4	25.8	25.6	21.4
	WN	47.2	42.3	50.3	49.9	23.8	22	26.4	24.8
	WW	47.2	46.4	45.1	45.5	22.6	21	22.2	20.2
	NW	53.2	51	47	47.2	26.8	26	24.2	24.2
September	C	50.6	49.9	52.1	49.5	26.4	21.2	24	25.6
	WN	45.2	46	53.7	51	22	21.6	26.8	25.8
	WW	46.7	46.8	47.3	46.4	21.6	22.6	23.8	22
	NW	50.3	50.1	47.2	46.6	26	23.2	22.2	20.8
Significance									
	Time			***				***	
	Treatment			*				NS	
	Leaf age			**				***	
	Cultivar			NS				NS	
	LSD$_{0.05}$			3.5				1.7	

Significance levels: NS not significant, * significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.001$.

Table 7. Leaf chlorophyll a and b content of this year's (N) and one year old (O) leaves from 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2008.

Month	Treatment	Chl a (mg g^{-1} DM)				Chl b (mg g^{-1} DM)			
		Konservolea		Kalamata		Konservolea		Kalamata	
		O	N	O	N	O	N	O	N
June	C	2.35	2.23	2.21	1.83	1.05	1.39	1.16	1.39
	WN	1.71	1.41	2.33	1.72	0.83	1.11	0.73	1.27
	WW	1.67	1.49	1.97	1.44	0.88	1.14	0.64	1.11
	NW	2.6	2.02	2.14	1.4	1.09	1.28	0.76	1.01
July	C	2.29	2.15	2.31	2.56	1.18	1.3	1.23	1.06
	WN	1.93	1.99	2.37	2.51	0.93	0.76	0.75	0.76
	WW	1.98	1.75	2.17	2.16	0.75	0.9	0.7	0.65
	NW	2.47	2.5	2.04	2.47	1.13	1.36	0.92	0.71
August	C	1.75	2.15	2.03	1.94	1.54	1.62	1.73	1.6
	WN	1.61	1.69	2.1	2.32	1.19	1.3	1.93	1.78
	WW	1.69	1.67	1.76	1.67	1.51	1.2	1.36	1.32
	NW	1.71	1.83	2.05	1.91	1.44	1.49	0.96	1.27
September	C	2.11	2.19	2.31	2.3	1.34	1.33	1.57	1.33
	WN	1.77	1.73	2.44	2.32	1.15	1.07	1.56	1.34
	WW	2	1.91	1.81	1.93	1.27	0.98	1.19	1.1
	NW	2.11	2.01	2.1	1.75	1.37	1.22	1.05	1.13
Significance									
	Time		***				***		
	Treatment		***				***		
	Leaf age		*				***		
	Cultivar		**				NS		
	LSD$_{0.05}$		0.4				0.3		

Significance levels: NS not significant, * significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.001$.

Table 8. Leaf total chlorophyll content and chlorophyll a to b ratio (Chl a/b) of this year's (N) and one year old (O) leaves from 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2008.

Month	Treatment	TChl (mg g^{-1} DM)				Chl a/b			
		Konservolea		Kalamata		Konservolea		Kalamata	
		O	N	O	N	O	N	O	N
June	C	3.4	3.62	3.37	3.23	2.24	1.64	2.16	1.32
	WN	2.53	2.53	3.07	3.00	2.07	1.27	3.18	1.35
	WW	2.55	2.64	2.61	2.55	1.9	1.3	3.09	1.31
	NW	3.69	3.29	2.90	2.42	2.39	1.58	2.83	1.39
July	C	3.48	3.45	3.54	3.62	1.95	1.71	1.89	2.49
	WN	2.87	2.75	3.12	3.27	2.07	2.62	3.18	3.29
	WW	2.73	2.64	2.88	2.81	2.62	2.04	3.09	3.34
	NW	3.60	3.86	2.96	3.18	2.18	1.85	2.21	3.48
August	C	3.29	3.77	3.76	3.53	1.14	1.34	1.88	1.22
	WN	2.81	2.99	4.04	4.1	1.38	1.29	1.09	1.3
	WW	3.20	2.87	3.11	2.98	1.12	1.38	1.41	1.27
	NW	3.15	3.31	3.01	3.19	1.19	1.23	2.2	1.52
September	C	3.45	3.52	3.88	3.62	1.57	1.66	1.48	1.74
	WN	2.91	2.80	4.00	3.65	1.53	1.62	1.57	1.73
	WW	3.27	2.88	3.00	3.04	1.58	1.96	1.5	1.75
	NW	3.49	3.23	3.14	2.88	1.54	1.64	2.03	1.57
Significance									
	Time	***				***			
	Treatment	***				***			
	Leaf age	NS				**			
	Cultivar	NS				***			
	LSD$_{0.05}$	0.35				0.83			

Significance levels: NS not significant, ** significant at P< 0.01, *** significant at P< 0.01.

Fig. 5. Changes over 2008 summer period of leaf total chlorophyll content (Tchl) of 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock. The values are means of 48 replications and overall $LSD_{0.05}$ was 0.19.

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A
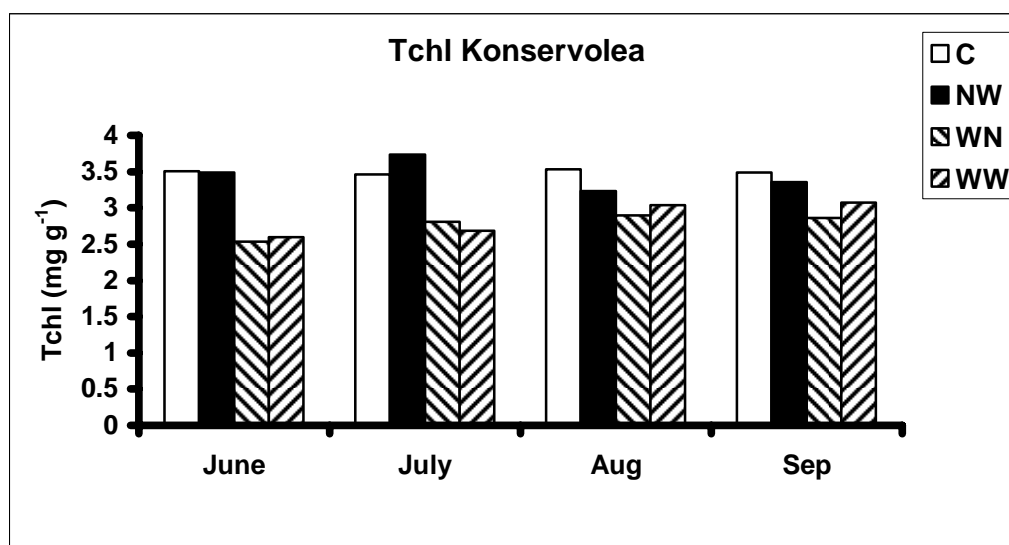
B
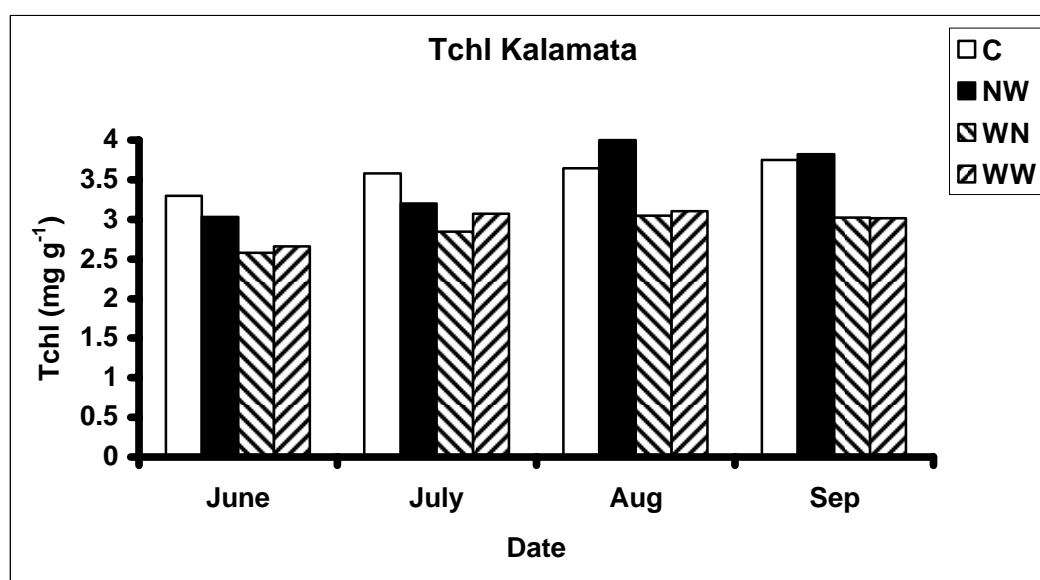
Fig. 6. Changes over the 2008 summer period of leaf total chlorophyll content (Tchl) of 'Konservolea' (A) and 'Kalamata' (B) olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution and filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN). The values are means from 12 replications and overall $LSD_{0.05}$ was 0.009 for (A) and 0.36 for (B).

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3.4. Leaf antioxidant enzymes activities, 2006 data

3.4.1. Superoxide dismutase (SOD) activity

Leaves from 'Konservolea' olive trees had significantly higher SOD activity than leaves from 'Kalamata' olive trees, but their actual values were relatively close (Table 9). This difference between the two cultivars was mainly found in young leaves and late in season (during the September and October measurements).

SOD activity in 'Konservolea' leaves decreased in August, increased again and reached the highest values in September and dropped substantially again in October (Table 9). This trend was found in the control and high salinity treatments with slight activity reduction in October in the control leaves and substantial reduction in the salinity treatments. In the NW treatment SOD activity did not decrease in October. SOD changes over time were exactly the same for 'Kalamata' leaves except that the above trend was present in the NW treatment as well.

In 'Konservolea' leaves, WN and WW treatments increased SOD activity compared to control and NW treatments in July, August and September (Table 9). The opposite was true in October, when leaves from WN and WW treatments had lower SOD activity than leaves from the control and NW treatments. The same differences in treatments were found with 'Kalamata' leaves except in October, when leaves from WN and WW treatments had lower SOD activity than leaves mainly from the control trees.

This year's (new) leaves had higher SOD activity than last year's (old) leaves in both cultivars and at all dates and treatments (Table 9).

3.4.2. Catalase activity

Overall, leaves from 'Konservolea' olive trees had significantly higher catalase (CAT) activity than leaves from 'Kalamata' olive trees, but their actual values were relatively close (Table 9). This difference between the two cultivars was mainly found in control leaves and in August.

CAT activity in 'Konservolea' leaves from the control trees increased in August and remained unchanged until October. In leaves from WN and WW treatments, CAT activity did not change over the summer but decreased in October and in leaves from NW treatment did not change over the time period studied (Table 9).

CAT activity in 'Kalamata' leaves from the control trees increased only in October compared to summer levels; in leaves from WN and WW treatments did not

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change over the summer, but decreased in October; and in leaves from NW treatment did not change over the time period studied (Table 9).

WN and WW treatments in 'Konservolea' leaves increased CAT activity compared to leaves from the control and NW treatments in July, August and September (Table 9). The opposite was true in October when leaves from WN and WW treatments had lower CAT activity than leaves from the control and NW treatments. The same differences in treatments were found with 'Kalamata' leaves (Table 9).

New leaves had higher CAT activity than old leaves in both cultivars and at all dates and treatments (Table 9).

4.4.3. Ascorbate peroxidase

Overall, leaves from 'Konservolea' olive trees had similar ascorbate peroxidase (APX) activity to leaves from 'Kalamata' olive trees (Table 10). But leaves from 'Konservolea' olive trees had higher APX activity than leaves from 'Kalamata olive trees only in young leaves and in October.

There was not a clear trend in APX activity changes over time in the various treatments in both cultivars tested, but, overall, in the leaves from WN and WW treatments, APX activity decreased in October and, in the leaves from control and NW treatments, increased in October or remained unchanged over time (Table 10).

WN and WW treatments in 'Konservolea' leaves increased APX activity compared to leaves from control and NW treatments in July, August and September and only from control in October (Table 10). The same differences in treatments were found with 'Kalamata' leaves in July, August and September (Table 10), but in October, WN and WW treatments had higher APX activity than leaves mainly from NW treatment.

New leaves had higher APX activity than old leaves in both cultivars and at all dates and treatments (Table 10).

Table 9. Antioxidant enzyme (superoxide dismutase SOD and catalase CAT) activities of this year's (N) and one year old (O) leaf extracts from 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2006.

Month	Treatment	SOD (units/g FW)				CAT (units/g FW)			
		Konservolea		Kalamata		Konservolea		Kalamata	
		N	O	N	O	N	O	N	O
July	C	196	190	194	192	120	106	117	103
	WN	224	207	228	200	245	102	239	99
	WW	225	193	227	202	233	114	227	111
	NW	192	191	192	193	123	111	120	109
August	C	188	182	189	182	144	121	120	105
	WN	227	179	230	179	258	106	244	101
	WW	227	180	230	180	247	124	248	113
	NW	200	184	200	184	112	108	123	110
September	C	202	200	202	197	142	118	117	105
	WN	235	210	229	213	252	104	240	99
	WW	234	210	230	200	241	122	244	111
	NW	200	201	199	197	109	105	121	107
October	C	199	188	198	194	141	118	141	119
	WN	176	169	175	175	94	78	99	77
	WW	181	172	164	168	97	81	99	83
	NW	220	194	170	176	131	103	135	105
Significance									
Time		***				***			
Treatment		***				***			
Leaf age		***				***			
Cultivar		***				NS			
$LSD_{0.05}$		6.8				21.2			

Significance levels: NS not significant, *** significant at $P < 0.001$.

Table 10. Ascorbate peroxidase activity (APX) of this year's (N) and one year old (O) leaf extracts from 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2006.

Month	Treatment	APX (units/ g FW)			
		Konservolea		Kalamata	
		N	O	N	O
July	C	1.27	0.86	1.27	0.83
	WN	2.57	0.56	2.53	0.56
	WW	2.65	0.60	2.62	0.56
	NW	1.19	0.84	1.19	0.84
August	C	1.31	0.88	1.31	0.82
	WN	2.65	0.58	2.61	0.56
	WW	2.69	0.60	2.69	0.58
	NW	1.21	0.86	1.23	0.86
September	C	1.39	0.88	1.29	0.84
	WN	2.71	0.60	2.67	0.64
	WW	2.77	0.62	2.73	0.66
	NW	1.31	0.86	1.31	0.82
October	C	1.29	0.76	1.51	0.92
	WN	2.45	0.54	2.23	0.70
	WW	2.12	0.56	1.81	0.68
	NW	2.27	0.70	1.05	0.88
Significance					
Time			***		
Treatment			***		
Leaf age			***		
Cultivar			NS		
$LSD_{0.05}$			0.26		

Significance levels: NS not significant, *** significant at P< 0.001.

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3.5. Leaf antioxidant enzymes activities, 2008 data

3.5.1. Superoxide dismutase (SOD) activity

Overall, leaves from 'Konservolea' olive trees had similar SOD activity to leaves from 'Kalamata' olive trees (Table 11). In particular, young 'Konservolea leaves had higher SOD activity than young 'Kalamata' leaves and 'Konservolea' leaves late in season had higher SOD activity than 'Kalamata' leaves.

SOD activity in 'Konservolea' leaves increased and reached the highest values in September and dropped substantially again in October (Table 11). This trend was found in all treatments with slight activity reduction in October in the control leaves and substantial reduction in WN and WW treatments. SOD changes over time were exactly the same for 'Kalamata' leaves (Table 11).

WN and WW treatments in 'Konservolea' leaves increased SOD activity compared to leaves from control and NW treatments in July and September (Table 11). The opposite was true in October. The same differences in treatments were found with 'Kalamata' leaves (Table 11).

New leaves had higher SOD activity than old leaves only in WN and WW treatments and in July and September in both cultivars. New and old leaves had similar SOD activity in the low salinity treatments and in October (Table 11).

3.5.2. Catalase activity

Leaves from 'Konservolea' olive trees had similar CAT activity to leaves from 'Kalamata' olive trees despite the treatment, leaf age and sampling period (Table 11).

CAT activity in 'Konservolea' leaves from the control trees remained unchanged over the sampling period; in leaves from WN and WW treatments did not change over the summer but decreased in October; and in leaves from NW treatment transiently decreased in September and increased again in October reaching the July levels (Table 11).

CAT activity in 'Kalamata' leaves from the control and NW treated trees transiently decreased in September and increased again in October and in leaves from WN and WW treatments decreased in October (Table 11).

WN and WW treatments in 'Konservolea' leaves increased CAT activity compared to leaves from control and NW treatments in July and September (Table 11). The opposite was true in October when leaves from the high salinity treatments

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had lower CAT activity than leaves from the control and NW treatments. The same differences in treatments were found with 'Kalamata' leaves (Table 11).

New leaves had higher CAT activity than old leaves in both cultivars and at all dates and treatments (Table 11).

3.5.3. Ascorbate peroxidase

Overall, leaves from 'Konservolea' olive trees had similar ascorbate peroxidase (APX) activity to leaves from 'Kalamata' olive trees (Table 12). But leaves from 'Konservolea' olive trees had higher APX activity than leaves from 'Kalamata olive trees in young leaves and in October.

There was not a clear trend in APX activity changes over time in the various treatments in both cultivars tested, but, overall, in the leaves from WW treatment, APX activity decreased in October; in the leaves from WN, did not change over time; and, in the leaves from control and NW treatments, increased in October or remained unchanged over time (Table 12).

WN and WW treatments in 'Konservolea' leaves increased APX activity compared to leaves from control and NW treatments in July and September and only from control in October (Table 12). The same differences in treatments were found with 'Kalamata' leaves in July and September (Table 12), but in October, WN and WW treatments had higher APX activity than leaves mainly from the NW treatment.

New leaves had higher APX activity than old leaves in both cultivars and at all dates and treatments (Table 12).

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Table 11. Antioxidant enzyme (superoxide dismutase SOD and catalase CAT) activities of this year's (N) and one year old (O) leaf extracts from 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2008.

Month	Treatment	SOD (units/g FW)				CAT (units/g FW)			
		Konservolea		Kalamata		Konservolea		Kalamata	
		N	O	N	O	N	O	N	O
July	C	185	178	182	180	126	114	124	109
	WN	214	196	218	191	251	111	243	106
	WW	215	182	217	190	239	121	231	118
	NW	181	179	180	181	130	119	126	117
September	C	210	209	218	216	130	107	107	94
	WN	243	228	249	226	239	92	226	88
	WW	244	216	248	225	227	111	229	99
	NW	215	214	216	217	99	95	111	96
October	C	204	184	203	193	132	108	130	109
	WN	171	165	173	173	84	69	88	67
	WW	177	168	159	165	87	71	89	73
	NW	228	190	168	172	121	91	126	93
Significance									
Time		***				***			
Treatment		***				***			
Leaf age		***				***			
Cultivar		NS				NS			
$LSD_{0.05}$		6.5				22.3			

Significance levels: NS not significant, *** significant at $P < 0.001$.

Table 12. Ascorbate peroxidase activity (APX) of this year's (N) and one year old (O) leaf extracts from 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock and growing with half-strength h Hoagland's solution with charcoal filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2008.

Month	Treatment	APX (units/ g FW)			
		Konservolea		Kalamata	
		N	O	N	O
July	C	1.15	0.86	1.19	0.78
	WN	2.53	0.56	2.47	0.56
	WW	2.61	0.58	2.57	0.54
	NW	1.17	0.82	1.17	0.82
September	C	1.15	0.80	1.25	0.80
	WN	2.59	0.58	2.53	0.52
	WW	2.61	0.54	2.65	0.54
	NW	1.19	0.80	1.17	0.82
October	C	1.27	0.76	1.49	0.92
	WN	2.43	0.54	2.21	0.70
	WW	2.10	0.58	1.81	0.68
	NW	2.27	0.70	1.05	0.88
Significance					
Time			***		
Treatment			***		
Leaf age			***		
Cultivar			NS		
$LSD_{0.05}$			0.25		

Significance levels: NS not significant, *** significant at P< 0.001.

3.6. Leaf gas and water exchange functions, 2008 data

Incident photosynthetically active radiation (PAR) measured using the photosynthesis measurement instrument ranged between 1200 to 1500 $\mu mol\ m^{-2}\ s^{-1}$ during the time period studied reaching the highest value in June when the day length is the largest of the year. There were no differences in PAR between the two cultivars. Olive leaves photosynthesize maximally at PAR higher than 1000 - 1200 $\mu mol\ m^{-2}\ s^{-1}$, thus the photosystems during all measurements had more than required available light for photosynthesis.

Leaves from 'Konservolea' olive trees had significantly lower net photosynthetic rate (Pn) than leaves from 'Kalamata' olive trees but the actual difference was only 3%. The same pattern was found with stomatal conductance (Gs) in which leaves from 'Konservolea' olive trees had significantly lower Gs and transpiration rate than leaves from 'Kalamata' olive trees (Fig. 7, Fig.10, Table 13). Water use efficiency (WUE) was 10% higher in 'Konservolea' olive trees than in 'Kalamata' olive trees as leaves from 'Kalamata' olive trees had higher transpiration rate (E) than leaves from 'Konservolea' olive trees (Tables 13 and 14).

In both cultivars, leaf Pn progressively decreased from June to August and remained unchanged until October (Fig. 7). This reduction in leaf Pn was associated with leaf Gs and E gradual reduction from June to October, while WUE remained constant from June until August and increased in October as a result of the more pronounced reduction in leaf E than in leaf Pn in October (Table 13 and 14).

In 'Konservolea' olive trees, WN and WW treatments decreased leaf Pn, E and Gs by 44%, 50% and 42%, respectively, compared to the control treatment over the time period studied (Fig. 8, Fig. 10). Leaf WUE was not affected by WN and WW treatments, result possibly showing that Pn decreased due to E decrease without other damage to the photosynthetic apparatus (Table 14). NW treatment decreased leaf Pn and Gs only by 6% and 15% compared to the control treatment in June. This difference remained in July only for leaf Gs. In July, August and October for Pn and in August and October for Gs, NW-treated trees had similar leaf Pn and Gs to control trees. Leaf E was not clearly affected by NW treatment compared to the control treatment over the time period studied (Table 13). Therefore, NW treatment did not affect leaf WUE compared to the control treatment over the time period studied (Table 14). No significant difference was found between the effect of WN and WW

treatments in all the above parameters in both cultivars and over the time period studied (Tables 13 and 14).

In 'Kalamata' olive trees, WN and WW treatments decreased leaf Pn, E and Gs by 41%, 30% 46%, respectively, compared to the control treatment over the time period studied (i.e. from June to October) (Fig. 9, Fig. 10, Table 13). This reduction in leaf Pn and E resulted in significant reduction in leaf WUE late in the season (August and October) (Tables 13 and 14). In June, NW treatment decreased leaf Pn and Gs only by 4% and 10%, respectively, but the leaf E was not affected by NW treatment compared to the control treatment. From July to October, NW treatment did not affect leaf Pn, Gs and E compared to the control treatment. Therefore overall, leaf WUE was not affected by NW treatment compared to the control treatment (Table 14).

Leaves from 'Konservolea' olive trees had similar quantum yield (QY) to the leaves from 'Kalamata' olive trees (Table 14). Overall, there was not a significant change in QY over time in leaves from the various treatments in both cultivars tested (Table 14). Overall, WN and WW treatments decreased leaf QY compared to the control treatment due to reduced Pn rate. Leaves from NW treatment had similar QY to the leaves from control treatment (Table 14).

3.7. Midday stem water potential, 2008 data

Midday stem water potential (SWP) in both cultivars was similar (Table 15).

In 'Konservolea' olive trees, SWP increased from June to July and remained constant in August in the control treatment. In WN and WW treatments, SWP increased from June to July and decreased in August reaching values similar to the ones in June. In NW treatment, SWP increased from June to July and remained constant in August. The same trend was found in 'Kalamata' olive trees (Table 15).

In 'Konservolea' olive trees, SWP in WN and WW treatments was lower than the control treatment over the sampling period. NW treatment did not affect SWP compared to the control treatment over the sampling period. The same trend was found in 'Kalamata' except in July where, NW treated-trees had slightly lower SWP than the control trees. No significant difference was found between the effect of WN and WW treatments in both cultivars and over the sampling period (Table 15).

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Table 13. Leaf transpiration rate, stomatal conductance and photosynthetic rate of 'Konservolea' (Kon) and 'Kalamata' (Kal) olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2008.

Month	Treatment	E (mmol m^{-2} s^{-1})		Gs (mol m^{-2} s^{-1})		Pn (μmol m^{-2} s^{-1})	
		Kon	Kal	Kon	Kal	Kon	Kal
June	C	3.7	4.2	0.18	0.21	13.0	14.8
	WN	2.5	2.8	0.09	0.11	7.7	8.2
	WW	2.5	3.0	0.09	0.12	7.8	8.3
	NW	3.5	3.9	0.15	0.2	12.3	13.
July	C	3.8	3.8	0.17	0.18	12..6	12.4
	WN	2.3	2.4	0.08	0.09	7.5	7.8
	WW	2.3	2.3	0.09	0.08	7.3	7.2
	NW	3.6	3.5	0.16	0.16	12.2	11.9
August	C	3.8	3.1	0.16	0.14	11.4	11.8
	WN	1.6	2.3	0.08	0.09	6.5	7.0
	WW	1.7	2.3	0.08	0.12	6.3	6.9
	NW	3.4	3.7	0.14	0.15	11.2	11.5
September	C	2.6	3.2	0.13	0.15	11.6	11.4
	WN	1.6	2.4	0.06	0.08	6.8	6.8
	WW	1.7	2.3	0.07	0.08	6.8	6.7
	NW	2.8	2.8	0.12	0.13	11.7	11.7
Significance							
Time		***		***		***	
Treatment		***		***		***	
Cultivar		***		***		***	
LSD$_{0.05}$		0.4		0.019		0.7	

Significance levels: NS not significant, *** significant at $P < 0.001$.

Table 14. Leaf water use efficiency (WUE), quantum yield (QY) and leaf temperature (T_{leaf}) of 'Konservolea' (Kon) and 'Kalamata' (Kal) olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2008.

Month	Treatment	WUE (mmol mol^{-1})		QY (mol/100mol)		T_{leaf} (°C)	
		Kon	Kal	Kon	Kal	Kon	Kal
June	C	3.7	3.6	0.97	1.0	32.9	32.7
	WN	3.2	3.0	0.55	0.54	33.0	33.1
	WW	3.2	2.8	0.53	0.58	33.2	33.6
	NW	3.5	3.3	0.84	0.89	32.9	32.9
July	C	3.3	3.3	0.88	0.90	34.0	33.2
	WN	3.3	3.3	0.55	0.59	33.4	32.7
	WW	3.2	3.2	1.03	0.65	32.7	33.4
	NW	3.4	3.5	1.12	1.05	33.6	32.7
August	C	3.0	4.1	0.78	0.95	32.9	33.2
	WN	4.0	3.2	0.53	1.47	33.2	32.7
	WW	3.8	3.2	0.55	0.49	32.6	33.5
	NW	3.5	3.1	0.80	0.75	33.7	34.3
September	C	4.5	3.7	0.78	0.86	31.1	32.9
	WN	5.0	2.9	0.55	0.59	31.9	33.9
	WW	4.1	2.9	0.52	0.52	31.2	34.0
	NW	4.1	4.2	0.82	0.82	31.3	31.6
Significance							
Time		***		NS		***	
Treatment		**		***		NS	
Cultivar		***		NS		*	
LSD$_{0.05}$		0.6		0.4		1.1	

Significance levels: NS not significant, * significant at $P<0.05$, ** significant at < 0.01, *** significant at $P< 0.001$.

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Table 15. Midday stem water potential for 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2008.

Month	Treatment	Stem water potential (MPa)	
		Konservolea	Kalamata
June	C	-2.50	-2.55
	WN	-3.12	-3.07
	WW	-3.12	-3.09
	NW	-2.54	-2.45
July	C	-1.95	-1.92
	WN	-2.54	-2.48
	WW	-2.56	-2.55
	NW	-2.05	-2.16
August	C	-2.18	-2.13
	WN	-3.06	-3.08
	WW	-3.06	-3.09
	NW	-2.19	-2.26
Significance			
Time		***	
Treatment		***	
Cultivar		NS	
LSD $_{0.05}$		0.16	

Significance levels: NS not significant, *** significant at P< 0.001.
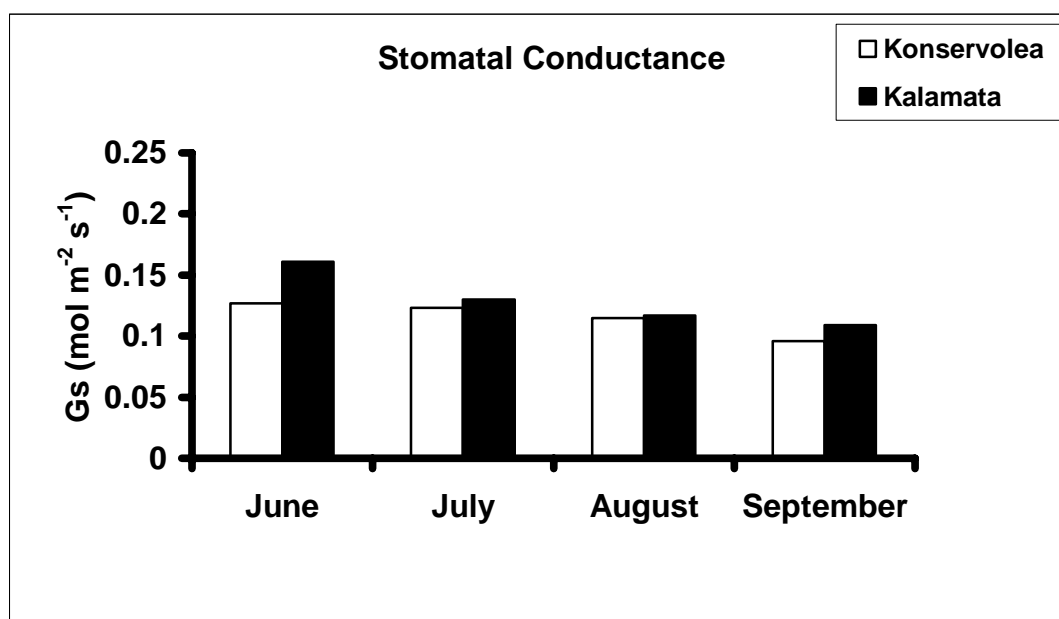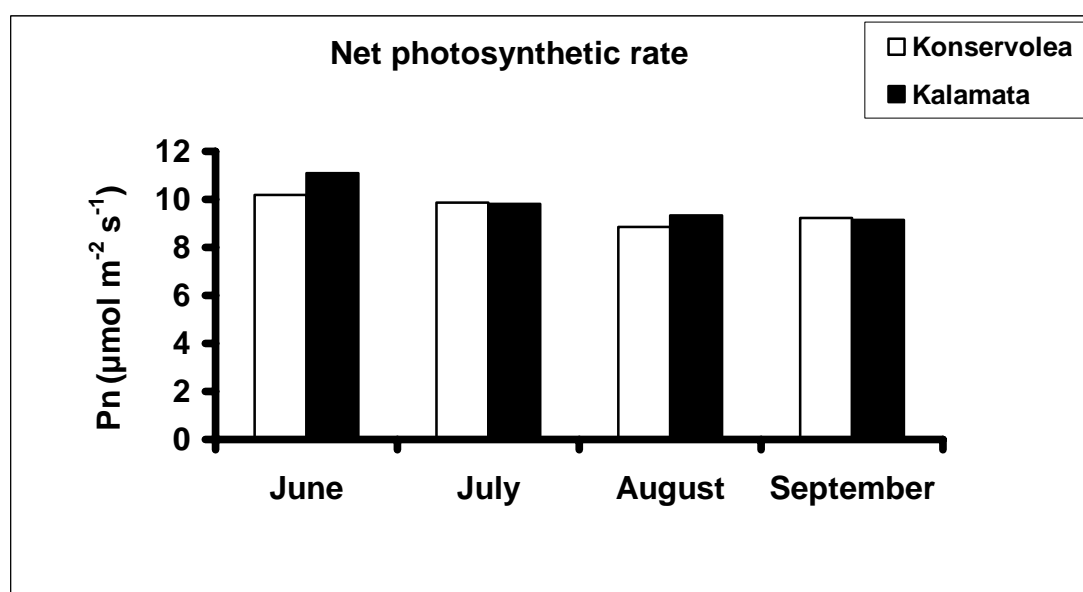
A

Stomatal Conductance

□ Konservolea
■ Kalamata

Gs (mol m^{-2} s^{-1})

0.25
0.2
0.15
0.1
0.05
0

June   July   August   September

B

Net photosynthetic rate

□ Konservolea
■ Kalamata

Pn (μmol m^{-2} s^{-1})

12
10
8
6
4
2
0

June   July   August   September

Fig. 7. Canges over the 2008 summer period of leaf stomatal conductance (Gs) (A) and leaf net photosynthetic rate (Pn) of 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock. The values are mean from 64 replications and overall $LSD_{0.05}$ 0.009 for Gs and 0.36 for Pn.

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A
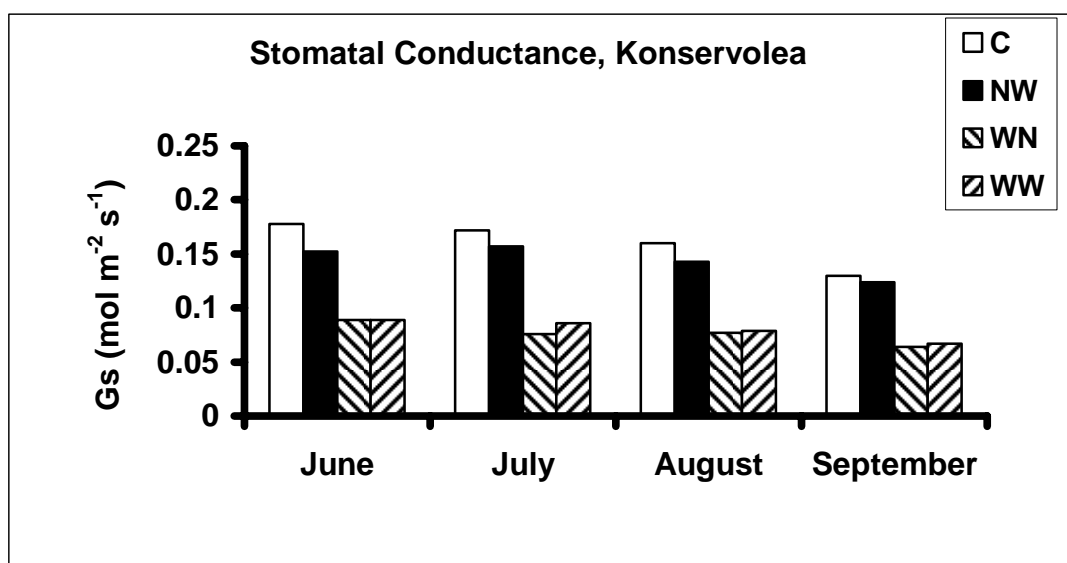
B
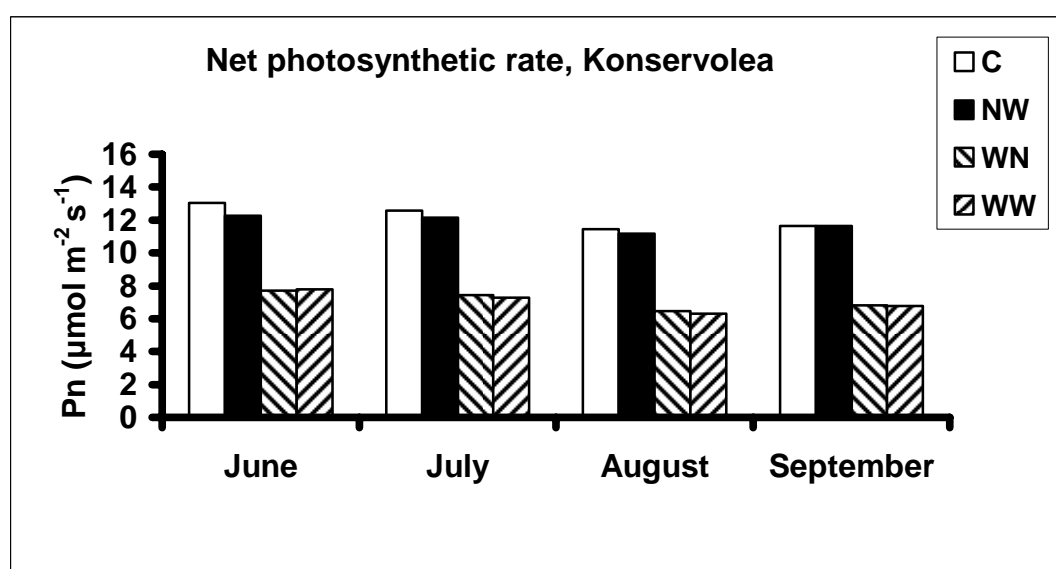
Fig. 8. Changes over the 2008 summer period of leaf stomatal conductance (Gs) (A)' and leaf net photosynthesis rate (Pn) (B) of (Kon) 'Konservolea' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution and filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN). The values are means from 16 replications and overall $LSD_{0.05}$ was 0.019 for Gs and 0.7 for Pn.

A
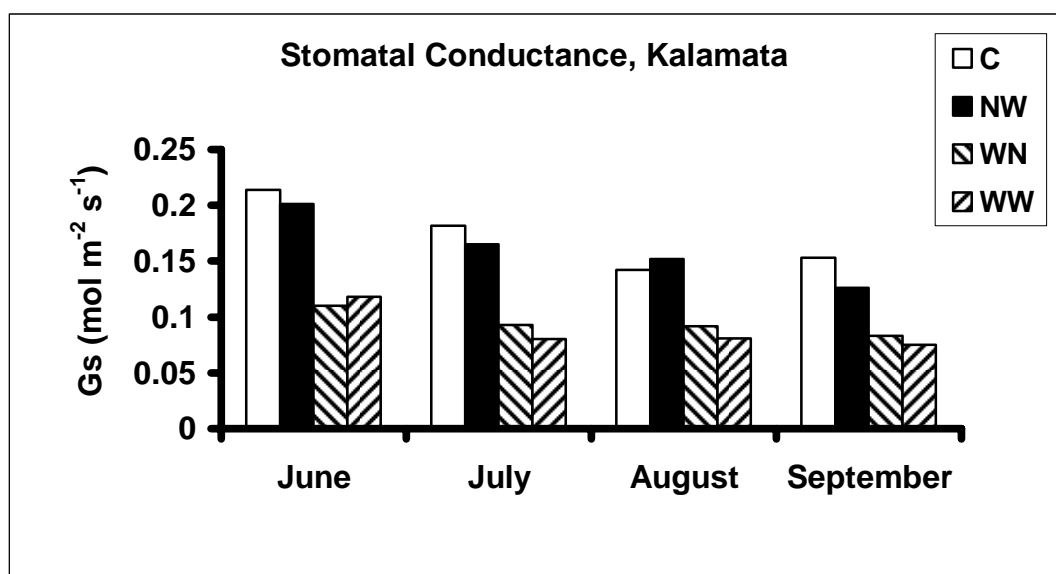
B
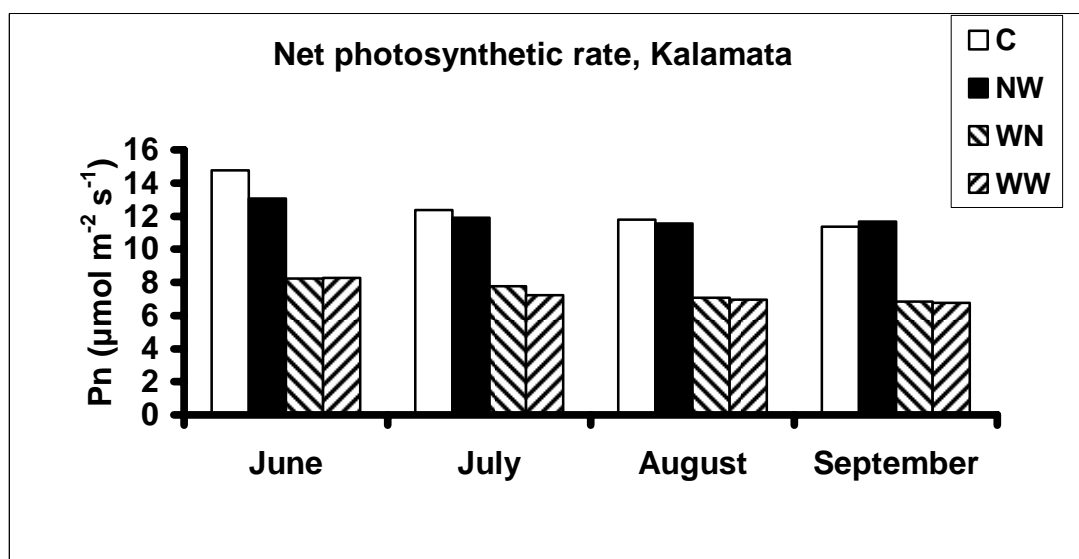
Fig. 9. Changes over the 2008 summer period of leaf stomatal conductance (Gs) (A)' and leaf net photosynthetic rate (Pn) (B) of 'Kalamata' (Kal) olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution and filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN). The values are means from 16 replications and overall $LSD_{0.05}$ was 0.019 for Gs and 0.7 for Pn.

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A
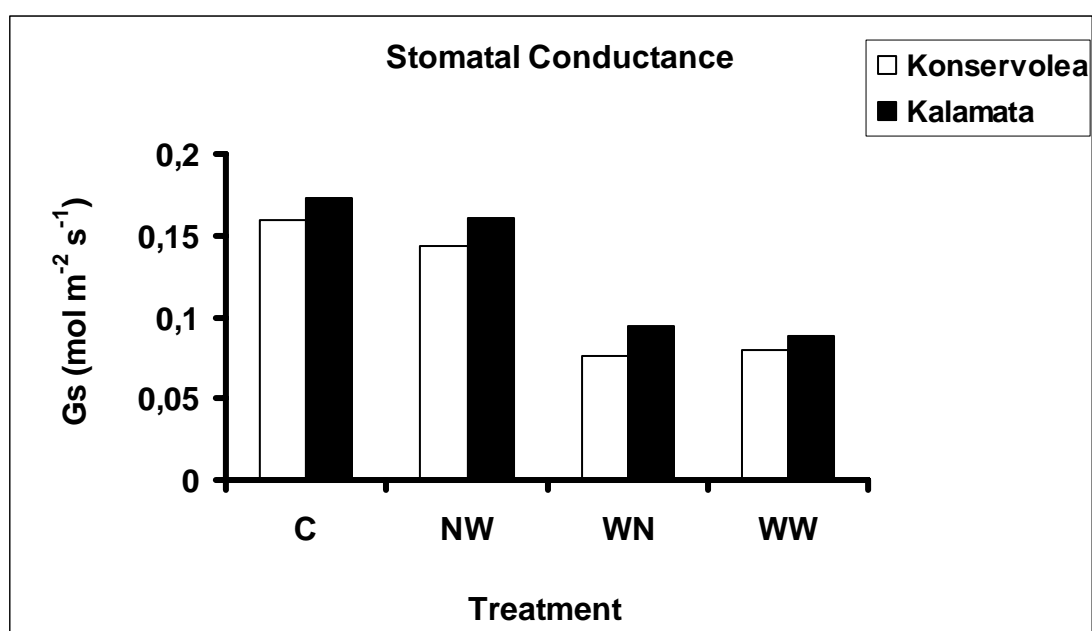
B
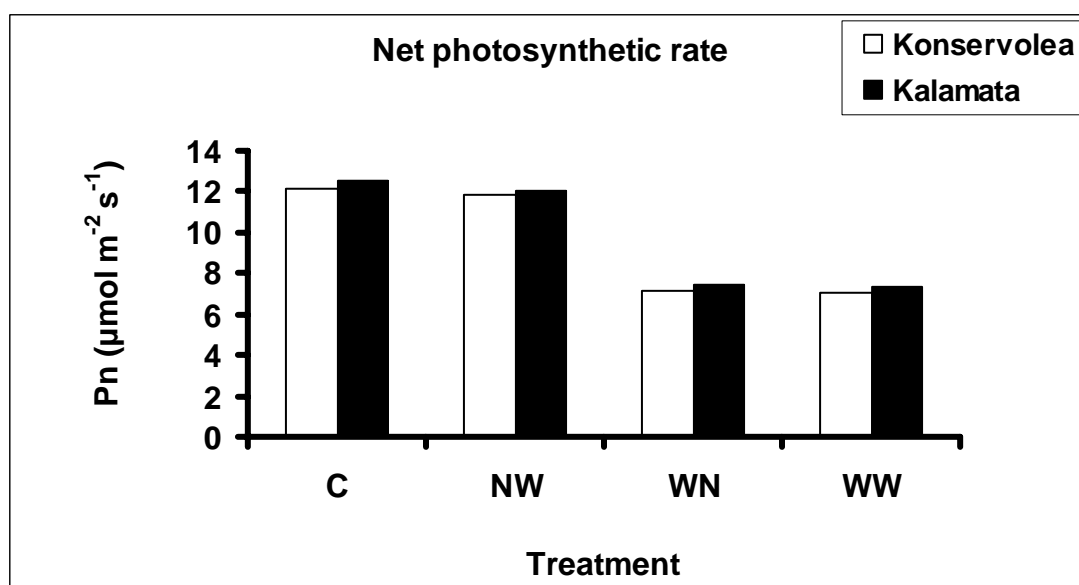
Fig.10. Changes of leaf stomatal conductance (Gs) (A) and leaf net photosynthetic rate (Pn) (B) of 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution and filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN). The values are means from 64 replications and overall $LSD_{0.05}$ 0.009 for Gs and 0.36 for Pn.

3.8. Chlorophyll fluorescence, 2008 data

3.8.1. F_0 (minimal fluorescence)

Overall, F_0 of leaves from 'Konservolea' olive trees was 6% higher than F_0 of leaves from 'Kalamata' olive trees in June and July, while in August leaves from both cultivars had similar F_0 (Table 16).

Leaf F_0 in 'Konservolea' olive trees progressively decreased from June to August in the control treatment. In WN treatment, leaf F_0 increased from June to July and decreased again in August in values similar to the ones in June. In WW treatment, leaf F_0 increased from June to July and slightly decreased in August. In NW treatment, leaf F_0 remained constant from June to July and sharply decreased in August (Table 16).

Leaf F_0 in 'Kalamata' olive trees, decreased from June to July and remained constant in August in the control treatment. In WN treatment, leaf F_0 increased from June to July and decreased in August reaching values higher than the ones in June. In WW treatment, leaf F_0 increased from June to July and decreased in August in values higher than the ones in June. In NW treatment, leaf F_0 remained constant from June to July and decreased in August (Table 16).

In 'Konservolea' olive trees, WW treatment decreased leaf F_0 compared to the control treatment only in June, while, in July, WW treatment did not affect leaf F_0 compared to the control treatment. In August, WW treatment increased leaf F_0 compared to the control treatment. WN treatment decreased leaf F_0 compared to the control treatment in June, while in July, WN treated trees had higher leaf F_0 than the control trees. In August, WN treatment slightly increased leaf F_0 compared to the control treatment. NW treatment slightly increased leaf F_0 compared to the control treatment in June, while in July, the increase was more pronounced. In August, NW treatment did not affect leaf F_0 compared to the control treatment (Table 16).

In 'Kalamata' olive trees, WW treatment decreased leaf F_0 compared to the control treatment in June, while in July and August, WW treatment increased leaf F_0 compared to the control treatment. WN treatment decreased leaf F_0 compared to the control treatment in June, while in July, WN treatment increased leaf F_0 compared to the control treatment. In August, WN treatment did not affect leaf F_0 compared to the control treatment. NW treatment did not affect leaf F_0 compared to the control treatment in June and August, while in July, NW treatment increased leaf F_0 compared to the control treatment (Table 16).

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3.8.2. F_m (maximal fluorescence)

Leaves from 'Konservolea' olive trees had higher leaf F_m than leaves from 'Kalamata' olive trees in June and July, while in August, leaves from both cultivars had similar leaf F_m (Table 16).

Leaf F_m in 'Konservolea' olive trees, progressively decreased from June to August in the control treatment. In WN treatment, leaf F_m decreased from June to July and again slightly decreased in August. In WW treatment, leaf F_m decreased from June to July and remained constant in August. In NW treatment, leaf F_m slightly decreased from June to July and decreased further in August (Table 16).

Leaf F_m in 'Kalamata' olive trees, slightly decreased from June to July and further decreased in August in the control treatment. In WN treatment, leaf F_m slightly increased from June to July and decreased in August. In WW treatment, leaf F_m remained constant over the time period studied. In NW treatment, leaf F_m progressively decreased from June to August (Table 16).

In 'Konservolea' olive trees, WW and WN treatments decreased leaf F_m compared to the control treatment over the time period studied. NW treatment increased leaf F_m compared to the control treatment in June and July; while in August, NW treatment did not affect leaf F_m compared to the control treatment (Table 16).

In 'Kalamata' olive trees, WW and WN treatments decreased leaf F_m compared to the control treatment over the time period studied. NW treatment increased leaf F_m compared to the control treatment in June. In July and August, NW treatment did not affect leaf F_m compared to the control treatment (Table 16).

3.8.3. F_v/F_m (ratio of variable to maximal fluorescence)

Leaves from 'Konservolea' olive trees had similar F_v/F_m to the leaves from 'Kalamata' olive trees over the time period studied (Table 16).

Leaf F_v/F_m in 'Konservolea' olive trees remained constant from June to August in the control and NW treatments. In WN and WW treatments, leaf F_v/F_m increased from June to July and remained constant in August (Table 16). The same trend was found in 'Kalamata' olive trees.

In 'Konservolea' olive trees, WN and WW treatments decreased leaf F_v/F_m compared to the control treatment over the time period studied. This decrease to values away from 0.8 shows a significant stress on the photosystems, which could use less sunlight for photosynthesis compared to non-saline plants. NW treatment did not

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affect leaf F_v/F_m compared to the control treatment over the time period studied (Table 16). The same trend was found in 'Kalamata' olive trees.

Table 16. Leaf chlorophyll fluorescence parameters of 'Konservolea' (Kon) and 'Kalamata' (Kal) olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2008. F_0: minimum fluorescence yield, Fm: maximum fluorescence yield, Fv/Fm: ratio of variable to maximum fluorescence.

Month	Treatment	F_0		Fm		Fv/Fm	
		Kon	Kal	Kons	Kal	Kon	Kal
June	C	100.8	87.4	574	503.7	0.82	0.83
	WN	80.4	66.8	402.3	343.5	0.80	0.80
	WW	77.7	70.9	395.7	365	0.80	0.80
	NW	106.4	93.9	618.3	549.4	0.83	0.83
July	C	87.6	79.6	508.9	478.7	0.83	0.83
	WN	94.3	92.3	347.2	357.5	0.73	0.74
	WW	88.2	90.7	328.7	353.7	0.73	0.74
	NW	101.5	88.7	585.6	480.9	0.83	0.81
August	C	75.8	78.2	425.3	448.2	0.82	0.82
	WN	80.8	79.8	321	315.3	0.75	0.75
	WW	83.5	85.2	329.7	334	0.75	0.74
	NW	74.3	76.7	409.4	426.9	0.82	0.82
Significance							
Time		***		***		***	
Treatment		***		***		***	
Cultivar		***		***		NS	
LSD$_{0.05}$		6.6		38		0.02	

Significance levels: NS not significant, *** significant at P< 0.001.

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3.9. Stored Carbohydrate Content, 2008 data

3.9.1. Old shoot neutral sugars

Old shoots from 'Konservolea' olive trees had similar neutral sugars content to the old shoots from 'Kalamata' olive trees in the control and WN treatments. In WW treatment, old shoots from 'Konservolea' olive trees had higher neutral sugars content than old shoots from 'Kalamata' olive trees. The opposite was true in NW treatment where old shoots from 'Konservolea' olive trees had lower neutral sugars content than the old shoots from 'Kalamata' olive trees (Table 17).

In 'Konservolea' olive trees, WW treatment increased old shoot neutral sugars content compared to the control treatment. No differences were found in old shoot neutral sugars content between control and WN and NW treatments (Table 17). In' Kalamata' olive trees, NW treatment increased old shoot neutral sugars content compared to the control treatment. No differences were found in old shoot neutral sugars content between control and WN and NW treatments (Table 17).

3.9.2. Old shoot starch

Starch content in old shoots from 'Konservolea' olive trees was 19% higher than in shoots from 'Kalamata' olive trees. This trend was found in all treatments except of the NW treatment, where old shoots from both cultivars had similar starch content (Table 17).

In 'Konservolea' olive trees, WN treatment had slightly lower old shoot starch content than the control treatment, while WW treatment decreased old shoot starch by 14% compared to the control treatment. Furthermore, NW treatment decreased old shoot starch content by 24% compared to the control treatment (Table 17). In 'Kalamata' olive trees, WW treatment decreased old shoot starch content by 20% compared to the control treatment, but WN treated trees had slightly lower old shoot starch content than the control trees. NW treatment did not affect old shoot starch content compared to the control treatment (Table 17).

3.9.4. New shoot neutral sugars

Neutral sugars content of new shoots from 'Konservolea' olive trees was 10% lower than in new shoots from 'Kalamata' olive trees in all treatments except of WW treatment where new shoots from both cultivars had similar neutral sugar content (Table 17).

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In 'Konservolea' olive trees, WN treatment slightly increased new shoots neutral sugars content compared to the control treatment, while WW treatment increased new shoots neutral sugars content by 19% compared to the control treatment. NW treatment slightly decreased new shoots neutral sugars content compared to the control treatment (Table 17). In 'Kalamata' olive trees, WN and WW treatments slightly increased new shoots neutral sugars content compared to the control treatment. NW treatment did not affect new shoots neutral sugars content compared to the control treatment (Table 17).

3.9.5. New shoot starch

New shoots from both cultivars had similar starch content in all treatments except of the control treatment where new shoots from 'Konservolea' olive trees had higher starch content than the new shoots from 'Kalamata' olive trees (Table 17).

In 'Konservolea' olive trees, new shoots from all treatments had similar starch content (Table 17). In 'Kalamata' olive trees, WN and NW treatments decreased new shoot starch content by 14% compared to the control treatment. WW treatments decreased new shoot starch content by 22% compared to the control treatment (Table 17).

3.9.6. Leaf neutral sugars

Leaves from both cultivars had similar neutral sugars content in all treatments (Table 17).

In 'Konservolea' olive trees, WN and WW treatments increased leaf neutral sugars by 35% compared to the control treatment. NW treatment did not affect leaf neutral sugars content compared to the control treatment (Table 17). In 'Kalamata' olive trees, WN and WW treatments increased leaf neutral sugars content by 27% compared to the control treatment. NW treatment did not affect leaf neutral sugars content compared to the control treatment (Table 17).

3.9.7. Leaf starch

Leaf starch content from 'Konservolea' olive trees was lower by 21% than in 'Kalamata' olive trees (Table 17).

In 'Konservolea' olive trees, WN and WW treatments decreased leaf starch content compared to the control treatment. No significant differences were found

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between the two high salinity treatments. NW treatment slightly decreased leaf starch content compared to the control treatment (Table 17). In 'Kalamata' olive trees, WN, WW and NW treatments decreased leaf starch content by 25% 43% and 15%, respectively, compared to the control treatment (Table 17).

3.9.9. Root neutral sugars

Root neutral sugars content in 'Konservolea' olive trees were 19% lower than in 'Kalamata' olive trees in the control and NW treatments. In WN and WW treatments, roots from both cultivars had similar neutral sugars content (Table 17).

In 'Konservolea' olive trees, WW and WN treatments decreased root neutral sugars content by 43% compared to the control treatment. NW treatment did not affect root neutral sugars content compared to the control treatment (Table 17). In 'Kalamata' olive trees, WN and WW treatments decreased root neutral sugars content by 57% compared to the control treatment. NW treated trees had slightly lower root neutral sugars content than the control trees (Table 17).

3.9.10. Root starch

Roots from both cultivars had similar starch content in all treatments except of the control treatment, where root starch content from 'Konservolea' olive trees was higher by 22% than in 'Kalamata' olive trees (Table 17).

In 'Konservolea' olive trees, WN and WW treatment increased root starch content by 55% compared to the control treatment. NW treatment did not affect root starch content (Table 17). In 'Kalamata' olive trees, WN and WW treatments increased root starch content by 39% compared to the control treatment. Again NW treatment did not affect root starch content (Table 17).

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Table 17. Stored carbohydrate content of plant parts from 'Konservolea' (Kon) and 'Kalamata' (Kal) olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) during the summer 2008.

Treatment	Old shoots Glucose (mg g^{-1} DM)		Old shoots Starch (mg g^{-1} DM)		New shoots Glucose (mg g^{-1} DM)		New shoots Starch (mg g^{-1} DM)	
	Kon	Kal	Kon	Kal	Kon	Kal	Kon	Kal
C	53.6	57.4	44.5	34.7	66.9	76.0	25.6	33.5
WN	52.3	52.6	41.2	32.2	75.0	82.7	27.8	29.0
WW	69.5	53.5	38.3	27.6	83.0	81.9	26.8	25.9
NW	59.4	70.8	33.6	33.0	63.0	79.8	26.2	28.7
Significance								
Treatment	**		***		*		NS	
Cultivar	NS		***		**		**	
LSD $_{0.05}$	10		4		11		3.8	

Treatment	Leaf Glucose (mg g^{-1} DM)		Leaf Starch (mg g^{-1} DM)		Root Glucose (mg g^{-1} DM)		Root Starch (mg g^{-1} DM)	
	Kon	Kal	Kon	Kal	Kon	Kal	Kon	Kal
C	168.4	188.4	85.3	108.2	64.9	80.2	21.2	27.4
WN	259.0	258.6	55.4	80.8	36.9	34.4	47.4	45.0
WW	256.0	261.3	53.7	60.9	38.8	37.0	42.6	45.6
NW	186.8	179.6	75.0	92.0	58.5	72.6	26.2	25.8
Significance								
Treatment	***		***		***		***	
Cultivar	NS		***		*		NS	
LSD $_{0.05}$	22.8		14.3		12.5		6.2	

Significance levels: NS not significant, * significant at $P<0.05$, ** significant at $P<0.01$, *** significant at $P<0.001$.

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3.10. Dry matter percent in different tree parts, 2006 data

3.10.1. New leaves percent dry matter (NLPDM)

NLPDM from 'Konservolea' olive trees were 5% lower than NLPDM from 'Kalamata' olive trees in NW and WN treatments. In WW and control treatments, both cultivars had similar NLPDM (Table 18).

In 'Konservolea' olive trees, WN, WW and NW treatments decreased NLPDM by around 8% compared to the control treatment (Table 18). In 'Kalamata' olive trees, WN treatment slightly decreased NLPDM compared to the control treatment. WW treatment decreased NLPDM by 8% compared to the control treatment. NW treatment did not affect NLPDM compared to the control treatment (Table 18).

3.10.2. Old leaves percent dry matter (OLPDM)

Both cultivars had similar OLPDM in WW and NW treatments. In the control treatment, OLPDM from 'Konservolea' olive trees were 11% lower than OLPDM from 'Kalamata' olive trees. The opposite was true in the WN treatment, where OLPDM from 'Konservolea' olive trees was 9% higher than OLPDM from 'Kalamata' olive trees (Table 18).

In 'Konservolea' olive trees, all treatments did not affect OLPDM compared to the control treatment (Table 18). In 'Kalamata' olive trees, WN and WW treatments decreased OLPDM by 15% and 10%, respectively, compared to the control treatment. NW-treated trees had slightly lower OLPDM than the control trees (Table 18).

3.10.3. New shoot percent dry matter (NSPDM)

NSPDM from 'Konservolea' olive trees was 7% lower than NSPDM from 'Kalamata' olive trees in the control and NW treatments. In WN and WW treatments, both cultivars had similar NSPDM (Table 18).

In 'Konservolea' olive trees, all treatments did not affect NSPDM compared to the control treatment (Table 18). In 'Kalamata' olive trees, WN and WW treatments decreased NSPDM by 7% compared to the control treatment. NW treatment did not affect NSPDM compared to the control treatment (Table 18).

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3.10.4. Old shoot percent dry matter (OSPDM)

OSPDM from 'Konservolea' olive trees was 10% lower than OSPDM from 'Kalamata' olive trees in all treatments except of the control treatment, where both cultivars had similar OSPDM (Table 18).

In both cultivars, all treatments had similar OSPDM (Table 18).

3.10.5. Trunk percent dry matter (TPDM)

TPDM from 'Konservolea' olive trees was 10% lower than TPDM from 'Kalamata' olive trees in the control and WN treatments. In WW and NW treatments, the difference between the cultivars diminished (Table 18).

In 'Konservolea' olive trees, all treatments had similar TPDM (Table 18). In 'Kalamata' olive trees, NW treatment decreased TPDM by 6% compared to the control treatment. WW-treated trees had slightly lower TPDM than the control trees. WN treatment slightly increased TPDM compared to the control treatment (Table 18).

3.10.6. Root percent dry matter (RPDM)

RPDM from 'Konservolea' olive trees was 8% lower than RPDM from 'Kalamata' olive trees in all treatments (Table 18).

In 'Konservolea olive trees, NW treatment slightly decreased RPDM compared to the control treatment. WN treatment did not affect RPDM compared to the control. WW-treated trees had slightly higher RPDM than the control trees (Table 18). In 'Kalamata' olive trees, NW treatment slightly decreased RPDM compared to the control treatment. WN and WW treatments slightly increased RPDM compared to the control treatment (Table 18).

Table 18. Dry matter percent in different tree parts from 'Konservolea' (Kon) and 'Kalamata' (Kal) olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) in 2006.

Treatment	New shoots (% DM)		New leaves (% DM)		Old shoots (% DM)	
	Kon	Kal	Kon	Kal	Kon	Kal
C	47.0	50.6	48.1	47.0	52.2	54.9
WN	46.0	47.0	44.0	46.0	51.5	56.7
WW	47.2	47.7	43.4	44.2	49.5	56.3
NW	46.8	51.0	44.4	47.6	49.9	58.8
Significance						
Treatment	**		***		NS	
Cultivar	***		NS		***	
LSD$_{0.05}$	2		2.4		4.0	

Treatment	Old leaves (% DM)		Trunk (% DM)		Root (% DM)	
	Kon	Kal	Kon	Kal	Kon	Kal
C	48.4	54.2	57.2	62.2	33.2	36.5
WN	50.3	45.9	56.5	63.5	33.8	37.2
WW	47.4	49.0	58.4	60.6	34.4	37.4
NW	50.3	51.2	57.2	58.6	32.3	35.0
Significance						
Treatment	NS		NS		*	
Cultivar	NS		***		***	
LSD$_{0.05}$	4.1		2.8		1.9	

Significance levels: NS not significant, * significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.001$.

3.11. Dry matter content in different tree parts, 2006 data

3.11.1. Total tree dry matter (TTDM)

TTDM of 'Konservolea' olive trees was 30% lower than TTDM of 'Kalamata' olive trees in all treatments (Table 19).

In 'Konservolea' olive trees, WN and WW treatments decreased TTDM by around 29% compared to the control treatment. NW treatment did not affect TTDM compared to the control treatment (Table 19). In 'Kalamata' olive trees, WW and WN treatments decreased TTDM by 26% and 14%, respectively, compared to the control treatment. NW-treated trees had slightly higher TTDM than the control trees (Table 19).

3.11.2. Shoot to root ratio dry matter (SRRDM)

SRRDM from 'Konservolea' olive trees was 18% higher than SRRDM from 'Kalamata' olive trees in all treatments (Table 19).

In 'Konservolea' olive trees, WN treatment increased SRRDM by 21% compared to the control treatment. WW and NW treatments did not affect SRRDM compared to the control treatment (Table 19). In 'Kalamata' olive trees, all treatments had similar SRRDM (Table 19).

3.11.3. New leaves dry matter (NLDM)

NLDM from 'Konservolea' olive trees were 24% lower than NLDM from 'Kalamata' olive trees in all treatments (Table 19, Fig. 11A).

In both cultivars, WN and WW treatments decreased NLDM by around 31% compared to the control treatment. NW treatment did not affect NLDM compared to the control treatment (Table 19, Figs. 12A, 13A).

3.11.4. Old leaves dry matter (OLDM)

Overall, OLDM from 'Konservolea' olive trees were 36% lower than 'Kalamata' olive trees in all treatments (Table 19, Fig. 11A).

In both cultivars, all treatments had similar OLDM (Table 19, Figs. 12A, 13A).

3.11.5. New shoot dry matter (NSDM)

NSDM from 'Konservolea' olive trees was 25% higher than NSDM from 'Kalamata' olive trees in all treatments (Table 19, Fig. 11A).

In both cultivars, WN and WW treatments decreased NSDM by around 50% compared to the control treatment. NW treatment did not affect NSDM compared to the control treatment (Table 19, Figs. 12A, 13A).

3.11.6. Old shoot dry matter (OSDM)

OSDM from 'Konservolea' olive trees was 48% lower than OSDM from 'Kalamata' olive trees in all treatments (Table 19, Fig. 11A).

In 'Konservolea' olive trees, all treatments had similar OSDM (Table 19, Fig. 12A). In 'Kalamata' olive trees, WN and NW treatments increased OSDM by 17% and 32%, respectively, compared to the control treatment. WW treatment did not affect OSDM compared to the control treatment (Table 19, Fig. 13A).

3.11.7. Trunk dry matter (TRDM)

TRDM from 'Konservolea' olive trees was 23% lower than TRDM from 'Kalamata' olive trees in all treatments (Table 19, Fig. 11A).

In 'Konservolea' olive trees, WW treatment decreased TRDM by 19% compared to the control treatment. WN treatment slightly decreased TRDM compared to the control treatment. NW treatment did not affect TRDM compared to the control treatment (Table 19, Fig. 12A). In 'Kalamata' olive trees, WW treatment decreased TRDM by 20% compared to the control treatment. WN and NW treatments did not affect TRDM compared to the control treatment (Table 19, Fig. 13A).

3.11.8. Root dry matter content (RDM)

RDM from 'Konservolea olive trees was 38% lower than RDM from 'Kalamata' olive trees in all treatments (Table 19, Fig. 11A).

In 'Konservolea' olive trees, WN and WW treatments decreased RDM by 43% and 28% compared to the control treatment. NW treatment did not affect root dry matter content compared to the control treatment (Table 19, Fig. 12A). In 'Kalamata' olive trees, WN and WW treatments decreased RDM by 13% and 21%, respectively, compared to the control treatment. NW treatment did not affect RDM compared to the control treatment (Table 19, Fig. 13A).

Table 19. Dry matter content of different tree parts from 'Konservolea' (Kon) and 'Kalamata' (Kal) olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) in 2006.

Treatment	New shoots DM (g)		New leaves DM (g)		Old shoots DM (g)		Old leaves DM (g)	
	Kon	Kal	Kon	Kal	Kon	Kal	Kon	Kal
C	34.0	26.4	48.6	59.2	26.0	39.4	10.5	17.0
WN	16.3	13.3	33.5	47.0	19.4	47.6	9.7	16.5
WW	17.5	12.9	33.2	40.0	25.5	34.8	11.3	16.6
NW	36.0	24.0	43.8	62.8	22.0	58.8	10.4	15.6
Significance								
Treatment	***		***		**		NS	
Cultivar	***		***		***		***	
LSD $_{0.05}$	3.7		7.2		8.1		3.4	

Treatment	Trunk DM (g)		Root DM (g)		Total tree DM (g)		Shoot /Root DM	
	Kon	Kal	Kon	Kal	Kon	Kal	Kon	Kal
C	61	78	73	104	253	337	2.5	2.3
WN	52	74	42	91	173	290	3.2	2.2
WW	50	62	52	83	188	249	2.6	2.0
NW	61	78	72	107	245	349	2.4	2.3
Significance								
Treatment	***		***		***		*	
Cultivar	***		***		***		***	
LSD $_{0.05}$	9.5		9.8		45.9		0.4	

Significance levels: * significant at P<0.05, * significant at P<0.05, ** significant at P<0.01, *** significant at P< 0.001.

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3.12. Dry matter partitioning, 2006 data

3.12.1. New leaves percent dry matter of the total tree dry matter (NLPC)

NLPC from 'Konservolea olive trees was 8% higher than NLPC from 'Kalamata' olive trees in WN and WW treatments. In NW and control treatments, both cultivars had similar NLPC (Table 20, Fig. 11B).

In 'Konservolea olive trees, WW treatment decreased NLPC by 9% compared to the control treatment. WN treatment slightly increased NLPC compared to the control treatment and NW-treated trees had slightly lower NLPC than the control trees (Table 20, Fig. 12B). In 'Kalamata' olive trees, WW and WN treatment decreased NLPC by 11% compared to the control treatment. NW treatment did not affect NLPC compared to the control treatment (Table 20, Fig. 13B).

3.12.2. Old leaves percent dry matter of the total tree dry matter (OLPC)

OLPC from 'Konservolea olive trees was 9% lower than OLPC from 'Kalamata' olive trees. This difference was only found in the control treatment. In the other treatments both cultivars had similar OLPC (Table 20, Fig. 11B).

In 'Konservolea' olive trees, WW and WN treatments increased OLPC by 29% compared to the control treatment. NW-treated trees had similar OLPC to the control trees (Table 20, Fig. 12B). In 'Kalamata' olive trees, WW treatment increased OLPC by 24% compared to the control treatment. WN treatment slightly increased OLPC compared to the control treatment. NW-treated trees had slightly lower OLPC than the control trees (Table 20, Fig. 13B).

3.12.3. New shoot percent of total tree dry matter (NSPC)

Overall, NSPC from 'Konservolea' olive trees was 47% higher than NSPC from 'Kalamata' olive trees and this difference was found in all treatments (Table 20, Fig. 11B).

In both cultivars, WW and WN treatments decreased NSPC by 30% compared to the control treatment; while NW-treated trees had similar NSPC to the control trees (Table 20, Figs. 12B, 13B).

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3.12.4. Old shoot percent of total tree dry matter (OSPC)

Overall, OSPC from 'Konservolea olive trees was 26% lower than OSPC from 'Kalamata' olive trees and this difference was found in all treatments (Table 20, Fig. 11B).

In 'Konservolea' olive trees, WW treatment increased OSPC by 24% compared to the control treatment. WN treatment slightly increased OSPC compared to the control treatment. NW-treated trees had slightly lower OSPC than the control trees (Table 20, Fig. 12B). In 'Kalamata' olive trees, WN and NW treatments increased OSPC by 25% compared to the control treatment. WW-treated trees had slightly higher OSPC than the control trees (Table 20, Fig. 13B).

3.12.6. Trunk percent dry matter of total tree dry matter (TRPC)

Overall, TRPC from 'Konservolea' olive trees was 8% higher than TRPC from 'Kalamata' olive trees and this difference was found in all treatments except of the control treatment, where both cultivars had similar TRPC (Table 20, Fig. 11B).

In 'Konservolea' olive trees, WN treatment increased TRPC by 21% compared to the control treatment. WW and NW treatments did not affect TRPC compared to the control treatment (Table 20, Fig. 12B). In 'Kalamata' olive trees, WW and WN treatments slightly increased TRPC compared to the control treatment. NW-treated trees had slightly lower TRPC than the control trees (Table 20, Fig. 13B).

3.12.7. Root percent dry matter of total tree dry matter (RTPC)

Overall, RTPC from 'Konservolea' olive trees was 14% lower than RTPC from 'Kalamata' olive trees and this difference was found in all treatments (Table 20, Fig. 11B).

In 'Konservolea' olive trees, WN treatment decreased RTPC by 16% compared to the control treatment. WW and NW treatment did not affect RTPC compared to the control treatment (Table 20, Fig. 12B). In 'Kalamata' olive trees, all treatments did not affect RTPC compared to the control treatment (Table 20, Fig. 13B).

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Table 20. Dry matter partitioning (percent dry matter of total tree dry matter) from different tree parts from 'Konservolea' (Kon) and 'Kalamata' (Kal) olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) in 2006.

Treatment	New leaves (%)		Old leaves (%)		New shoots (%)	
	Kon	Kal	Kon	Kal	Kon	Kal
C	19.3	18.3	4.1	5.1	13.5	8.1
WN	19.4	16.2	5.6	5.6	9.5	4.8
WW	17.6	16.0	6.0	6.7	9.4	5.2
NW	18.0	18.1	4.2	4.4	14.8	7.1
Significance						
Treatment	***		***		***	
Cultivar	**		NS		***	
LSD$_{0.05}$	1.5		0.9		1.5	

Treatment	Old shoots %		Trunk %		Root %	
	Kon	Kal	Kon	Kal	Kon	Kal
C	10.2	12.2	24.0	24.0	28.8	32.2
WN	11.1	16.3	30.3	25.5	24.0	31.4
WW	13.4	14.0	26.2	25.1	27.4	33.0
NW	9.0	16.4	25.0	22.5	29.2	31.5
Significance						
Treatment	***		***		**	
Cultivar	**		**		***	
LSD$_{0.05}$	2.2		2.4		2.5	

Significance levels: NS not significant, ** significant at < 0.01, *** significant at P< 0.001.

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A

Dry matter content, 2006

Legend: □ Konservolea ■ Kalamata

Y-axis: DM (g), scale 0 to 100

Categories: New leaves, Old leaves, New shoots, Old shoots, Trunk, Root

B

Dry matter partitioning, 2006

Legend: □ Konservolea ■ Kalamata

Y-axis: % of total tree DM, scale 0 to 30

Categories: New leaves, Old leaves, New shoots, Old shoots, Trunk, Root

Fig. 11. Dry matter content (A) and dry matter partitioning (B) of different tree parts of 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock in 2006.

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A

Dry matter content, Konservolea, 2006

Legend: □ C, ■ NW, ◩ WN, ▨ WW

Y-axis: DM g (0–80)

X-axis categories: New leaves, Old leaves, New shoots, Old shoots, Trunk, Root

B

Dry matter partitioning, Konservolea, 2006

Legend: □ C, ■ NW, ◩ WN, ▨ WW

Y-axis: % of total tree dry matter (0–35)

X-axis categories: New leaves, Old leaves, New shoots, Old shoots, Trunk, Root

Fig. 12. Dry matter content (A) and dry matter partitioning (B) of different tree parts of "Konservolea" olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution and filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) in 2006.

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A

B

Fig. 13. Dry matter content (A) and dry matter partitioning (B) of different tree parts of "Kalamata" olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution and filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) in 2006.

3.13. Dry matter percent in different tree parts, 2008 data

3.13.1. New leaves dry matter percent (NLPDM)

New leaves from both cultivars had similar dry matter percent and this was found in all treatments (Table 21).

In 'Konservolea' olive trees, WN and WW treatments decreased NLPDM by 8% compared to the control treatment. NW-treated trees had slightly lower NLPDM than the control trees (Table 21). In 'Kalamata' olive trees, all treatments had similar NLPDM (Table 21).

3.13.2. Old leaves dry matter percent (OLPDM)

Old leaves from both cultivars had similar dry matter percent and this was found in all treatments (Table 21).

In 'Konservolea' olive trees, all treatments had similar OLPDM (Table 21). In 'Kalamata' olive trees, WN treatment decreased OLPDM by 7% compared to the control treatment. WW-treated trees had slightly lower OLPDM than the control trees and NW treatment did not affect OLPDM compared to the control treatment (Table 21).

3.13.3. New shoot dry matter percent (NSDMP)

NSDMP from 'Konservolea' olive trees was 5% lower than NSDMP from 'Kalamata' olive trees in the control and NW treatments. In WN and WW treatments, new shoots from both cultivars had similar NSDMP (Table 21).

In 'Konservolea' olive trees, all treatments had similar NSDMP (Table 21). In 'Kalamata' olive trees, WN and WW treatments decreased NSDMP by 8% and 5%, respectively, compared to the control treatment. NW treatment did not affect NSDMP compared to the control treatment (Table 21).

3.13.4. Old shoot dry matter percent (OSDMP)

Overall, OSDMP from 'Konservolea' olive trees was 11% lower than OSDMP from 'Kalamata' olive trees in all treatments except of the control treatment, where old shoots from both cultivars had similar OSDMP (Table 21).

In 'Konservolea' olive trees, all treatments had similar OSDMP (Table 21). In 'Kalamata' olive trees, WN and WW treatments slightly increased OSDMP compared

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to the control treatment. NW-treated trees had 8% higher OSDMP than the control trees (Table 21).

3.13.5. Trunk dry matter percent (TRDMP)

The trunk from 'Konservolea' olive trees had slightly lower TRDMP than the trunk from 'Kalamata' olive trees and this difference was found in all treatments (Table 21).

In 'Konservolea' olive trees, all treatments had similar TRDMP (Table 21). In 'Kalamata' olive trees, NW-treated trees had slightly lower TRDMP than the control trees. WW treatment slightly increased TRDMP compared to the control treatment and WN treatment did not affect TRDMP compared to the control treatment (Table 21).

3.13.6. Root dry matter percent (RDMP)

Both cultivars had similar RDMP in all treatments (Table 21).

In both cultivars, RDMP was not affected by any treatment (Table 21).

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Table 21. Dry matter percent in different tree parts from 'Konservolea' (Kon) and 'Kalamata' (Kal) olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) in 2008.

Treatment	New shoots (% DM)		New leaves (% DM)		Old shoots (% DM)	
	Kon	Kal	Kon	Kal	Kon	Kal
C	46.4	50.0	47.8	47.0	51.9	54.6
WN	45.4	45.8	43.2	45.0	51.0	56.3
WW	46.6	47.4	44.0	46.3	48.3	56.1
NW	46.3	51.2	46.6	47.2	49.7	59.2
Significance						
Treatment	***		**		NS	
Cultivar	***		NS		***	
LSD$_{0.05}$	5.1		2.7		3.4	

Treatment	Old leaves (% DM)		Trunk (% DM)		Root (% DM)	
	Kon	Kal	Kon	Kal	Kon	Kal
C	50.0	51.2	56.8	59.0	34.5	35.3
WN	48.7	47.3	56.2	58.3	34.9	34.3
WW	49.0	49.0	57.0	60.3	34.3	34.5
NW	48.5	51.0	55.8	57.0	34.5	35.2
Significance						
Treatment	NS		NS		NS	
Cultivar	NS		***		***	
LSD$_{0.05}$	3.0		2.3		0.96	

Significance levels: NS not significant, ** significant at < 0.01, *** significant at P< 0.001.

3.14. Dry matter content in different tree parts, 2008 data

3.14.1. Total tree dry matter (TTDM)

Both cultivars had similar TTDM in all treatments (Table 22).

WW and WN treatment decreased TTDM by 31% and 24% in 'Konservolea' and 'Kalamata' olive trees, respectively, compared to the control treatment. NW treatment did not affect TTDM compared to the control treatment (Table 22).

3.14.2. Shoot to root ratio for dry matter (SRRDM)

Overall, SRRDM of 'Konservolea' olive trees was 21% lower than SRRDM of 'Kalamata' olive trees and this difference was found in all treatments (Table 22).

WW and WN treatments increased SRRDM by around 26% and 29% in 'Konservolea' and 'Kalamata' olive trees, respectively, compared to the control treatment. NW treatment did not affect SRRDM compared to the control treatment (Table 22).

3.14.3. New leaves dry matter (NLDM)

NLDM from 'Konservolea' olive trees was 19% lower than NLDM from 'Kalamata' olive trees and this difference was found in all treatments (Table 22, Fig. 14A).

In 'Konservolea' olive trees, WN and WW treatments decreased NLDM by around 30% compared to the control treatment. NW treatment did not affect NLDM (Table 22, Fig. 15A). In 'Kalamata' olive trees, WN and WW treatment decreased NLDM by around 23% compared to the control treatment. NLDM from NW-treated trees was 12% higher than in the control trees (Table 22, Fig. 16A).

3.14.4. Old leaves dry matter (OLDM)

Overall, OLDM from 'Konservolea' olive trees was 31% lower than OLDM from 'Kalamata' olive trees and this difference was found in all treatments (Table 22, Fig. 14A).

In 'Konservolea' olive trees, WN and NW treatments decreased OLDM by 28% and 23%, respectively, compared to the control treatment. WW-treated trees had slightly lower OLDM than the control trees (Table 22, Fig. 15A). In 'Kalamata' olive trees, WN treatment slightly decreased OLDM compared to the control treatment. OLDM from WW-treated trees was slightly higher than the control trees. NW

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treatment did not affect OLDM compared to the control treatment (Table 22, Fig. 16A).

3.14.5. New shoot dry matter (NSDM)

Overall, NSDM from 'Konservolea' olive trees was 26% higher than 'Kalamata' olive trees and this difference was found in all treatments (Table 22, Fig. 14A).

In both cultivars, WN and WW treatments decreased NSDM by around 50% compared to the control treatment. NW treatment did not affect NSDM compared to the control treatment (Table 22, Figs. 15A, 16A).

3.14.6. Old shoot dry matter (OSDM)

OSDM from 'Konservolea' olive trees was 17% lower than OSDM from 'Kalamata' olive trees in WN and WW treatments. In the control treatment, both cultivars had similar OSDM. In NW treatment, 'Konservolea' olive trees had slightly lower OSDM than 'Kalamata' olive trees (Table 22, Fig. 14A).

In 'Konservolea' olive trees, WW treatment decreased OSDM by 24% compared to the control treatment. WN treatment slightly decreased OSDM compared to the control treatment. NW-treated trees had similar OSDM to the control trees (Table 22, Fig. 15A). In 'Kalamata' olive trees, all treatments had similar OSDM (Table 22, Fig. 16A).

3.14.7. Trunk dry matter (TDM)

'Konservolea' olive trees had slightly lower TDM than 'Kalamata' olive trees in all treatments except of the NW treatment, where both cultivars had similar TDM (Table 22, Fig. 14A).

In 'Konservolea' olive trees, WW and WN treatments slightly decreased TDM compared to the control treatment. NW treatment did not affect TDM compared to the control treatment (Table 22, Fig. 15A). In 'Kalamata' olive trees, all treatments had similar TDM (Table 22, Fig. 16A).

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3.14.8. Root dry matter (RDM)

Overall, RDM from 'Konservolea' olive trees was 14% higher than RDM from 'Kalamata' olive trees and this difference was found in all treatments (Table 22, Fig 14A).

In both cultivars, WW and WN treatments decreased RDM by around 40% compared to the control treatment. NW treatment did not affect RDM compared to the control treatment (Table 22, Figs. 15A, 16A).

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Table 22. Dry matter content of different tree parts from 'Konservolea' (Kon) and 'Kalamata' (Kal) olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) in 2008.

Treatment	New shoots DM (g)		New leaves DM (g)		Old shoots DM (g)		Old leaves DM (g)	
	Kon	Kal	Kon	Kal	Kon	Kal	Kon	Kal
C	28.2	22.0	42.8	47.4	33.9	34.9	11.6	14.8
WN	13.5	10.7	30.0	36.6	29.8	38.0	8.4	12.9
WW	14.5	11.2	28.6	38.6	25.9	38.0	10.6	15.4
NW	30.0	20.2	41.7	54.1	33.9	37.4	8.9	14.1
Significance								
Treatment	***		***		NS		**	
Cultivar	***		***		***		***	
LSD $_{0.05}$	2.6		5.1		6.8		2.2	

Treatment	Trunk DM (g)		Root DM (g)		Total tree DM (g)		Shoot /Root DM	
	Kon	Kal	Kon	Kal	Kon	Kal	Kon	Kal
C	45.0	49.3	104.2	90.5	265.8	257.5	1.6	1.9
WN	40.7	45.3	61.0	54.8	183.3	197.0	2.1	2.7
WW	40.0	44.9	61.4	52.2	181.0	200.4	2.3	2.9
NW	47.5	45.3	106.9	87.4	268.8	258.5	1.5	2.0
Significance								
Treatment	NS		***		***		***	
Cultivar	NS		***		NS		***	
LSD $_{0.05}$	7.8		11.4		25.7		0.44	

Significance levels: NS not significant, ** significant at < 0.01, *** significant at P< 0.001
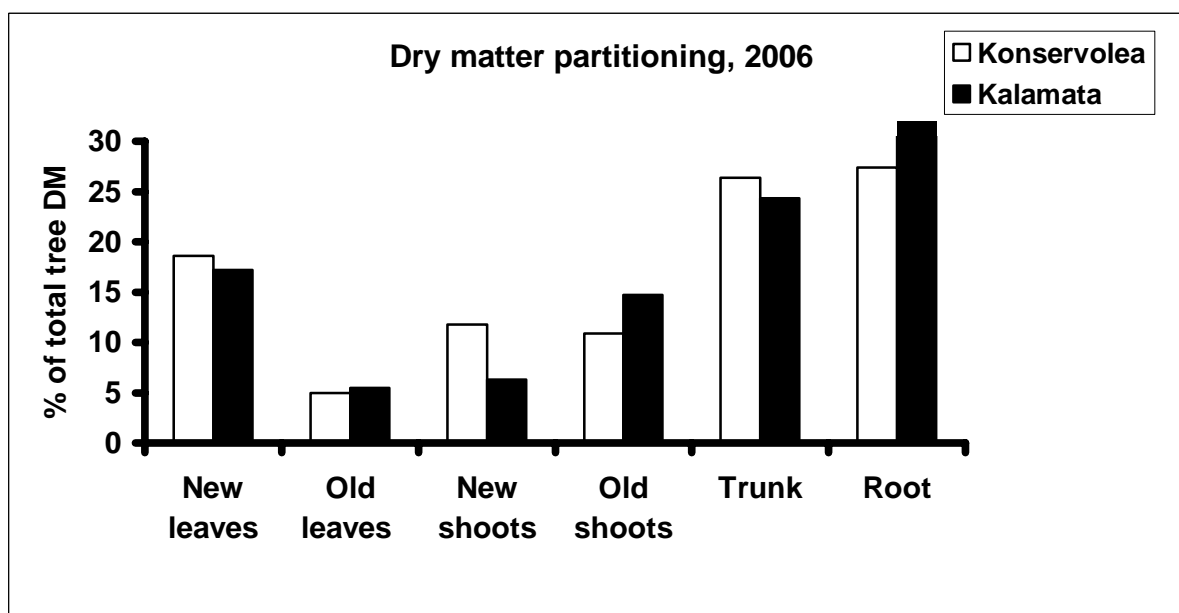
3.15. Dry matter partitioning, 2008 data

3.15.1. New leaves percent dry matter of the total tree dry matter (NLPC)

Overall, NLPC from 'Konservolea' olive trees was 18% lower than NLPC from 'Kalamata' olive trees with the difference being larger in the WW and NW treatments than in the control and WN treatments (Table 23, Fig. 14B).

In both cultivars, all treatments had similar NLPC (Table 23, Figs. 15B, 16B).

3.15.2. Old leaves percent dry matter of the total tree dry matter (OLPC)

Overall, OLPC from 'Konservolea' olive trees was 27% lower than OLPC from 'Kalamata' olive trees and this difference was found in all treatments (Table 23, Fig. 14B).

In 'Konservolea' olive trees, NW treatment decreased OLPC by 28% compared to the control treatment. WN treatment did not affect OLPC compared to the control treatment, while WW treatment increased OLPC by 21% compared to the control treatment (Table 23, Fig. 15B). In 'Kalamata' olive trees, NW treatment had similar OLPC to the control treatment, while WN treatment slightly increased OLPC and WW treatment increased OLPC by 26% compared to the control treatment (Table 23, Fig. 16B).

3.15.3. New shoot percent dry matter of the total tree dry matter (NSPC)

Overall, NSPC from 'Konservolea' olive trees was 26% higher than NSPC from 'Kalamata' olive trees and this difference was found in all treatments (Table 23, Fig. 14B).

In both cultivars, WW and WN treatments decreased NSPC by around 30% compared to the control treatment. NW treatment did not affect NSPC compared to the control treatment (Table 23, Figs. 15B, 16B).

3.15.4. Old shoot percent dry matter of the total tree dry matter (OSPC)

Overall, OSPC from 'Konservolea' olive trees was 16% lower than OSPC from 'Kalamata' olive trees in all treatments (Table 23, Fig. 14B).

In 'Konservolea' olive trees, WN treatment increased OSPC by 20% and WW treatment slightly increased OSPC compared to the control treatment. NW-treated trees had similar OSPC to the control trees (Table 23, Fig. 15B). In 'Kalamata' olive trees, WN and WW treatments increased OSPC by around 30% compared to the

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control treatment. NW treatment did not affect OSPC compared to the control treatment (Table 23, Fig. 16B).

3.15.5. Trunk percent dry matter of the total tree dry matter (TRPC)

Both cultivars had similar TRPC in all treatments (Table 23, Fig. 14B).

WN and WW treatments increased TRPC by around 24% in 'Konservolea' olive trees and by around 16% in 'Kalamata' olive trees compared to the control treatment. NW treatment did not affect TRPC compared to the control treatment in both cultivars (Table 23, Figs. 15B, 16B).

3.15.6. Root percent dry matter of the total tree dry matter (RTPC)

Overall, RTPC from 'Konservolea' olive trees was 16% lower than RTPC from 'Kalamata' olive trees and this difference was found in all treatments (Table 23, Fig. 14B).

WN and WW treatments decreased RTPC by around 15% in 'Konservolea' olive trees and by around 21% in 'Kalamata' olive trees compared to the control treatment. NW treatment did not affect RTPC compared to the control treatment in both cultivars (Table 23, Figs. 15B, 16B).

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Table 23. Dry matter partitioning (percent dry matter of total tree dry matter) from different tree parts from 'Konservolea' (Kon) and 'Kalamata' (Kal) olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) in 2008.

Treatment	New leaves (%)		Old leaves (%)		New shoots (%)	
	Kon	Kal	Kon	Kal	Kon	Kal
C	16.0	18.5	4.7	5.7	10.8	8.4
WN	16.3	18.7	4.6	6.6	7.4	5.4
WW	15.9	19.4	6.0	7.7	8.0	5.6
NW	15.6	21.0	3.4	5.5	11.0	7.8
Significance						
Treatment	NS		***		***	
Cultivar	***		***		***	
LSD$_{0.05}$	2.5		1.0		1.2	

Treatment	Old shoots (%)		Trunk (%)		Root (%)	
	Kon	Kal	Kon	Kal	Kon	Kal
C	12.7	13.3	16.7	19.0	39.0	34.9
WN	16.0	19.2	22.2	22.7	33.3	27.4
WW	14.3	18.9	22.0	22.4	33.8	25.9
NW	12.6	14.4	17.7	17.4	39.8	33.8
Significance						
Treatment	***		***		***	
Cultivar	***		NS		***	
LSD$_{0.05}$	2.2		2.5		3.2	

Significance levels: NS not significant, *** significant at $P < 0.001$

A
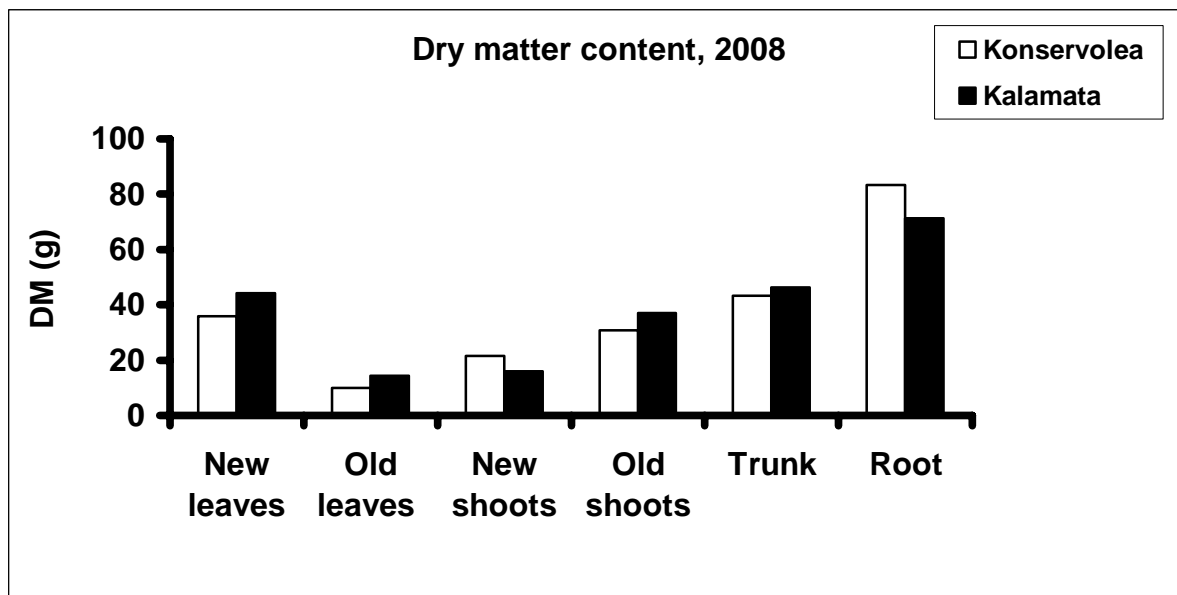
B
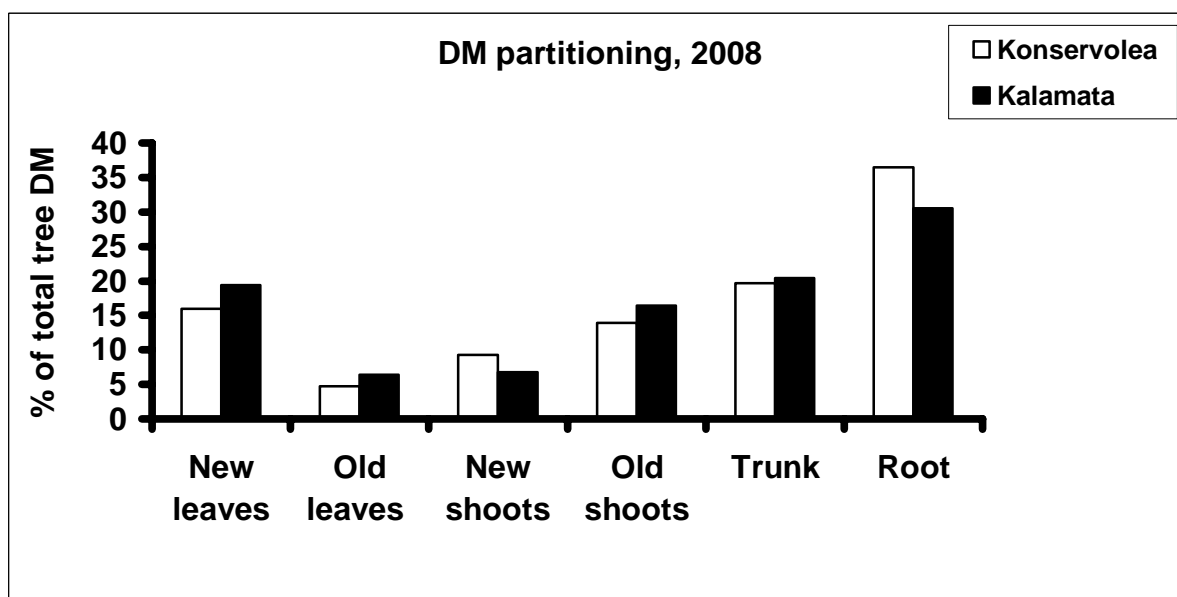
Fig. 14. Dry matter content (A) and dry matter partitioning (B) of different tree parts of 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock in 2008.

A
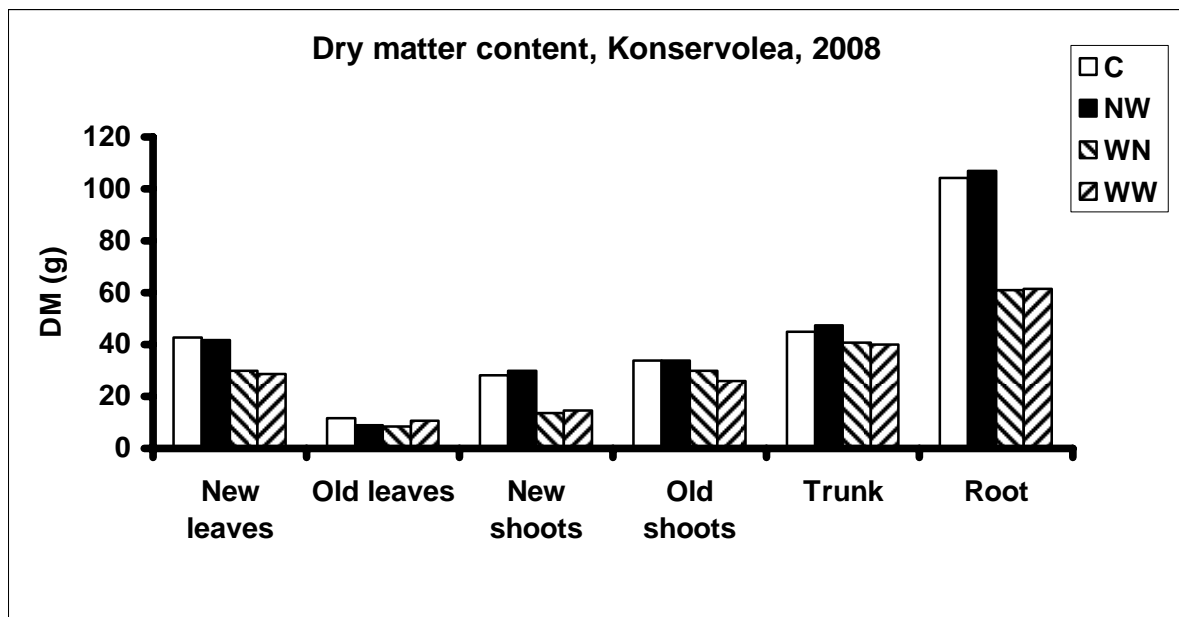
B
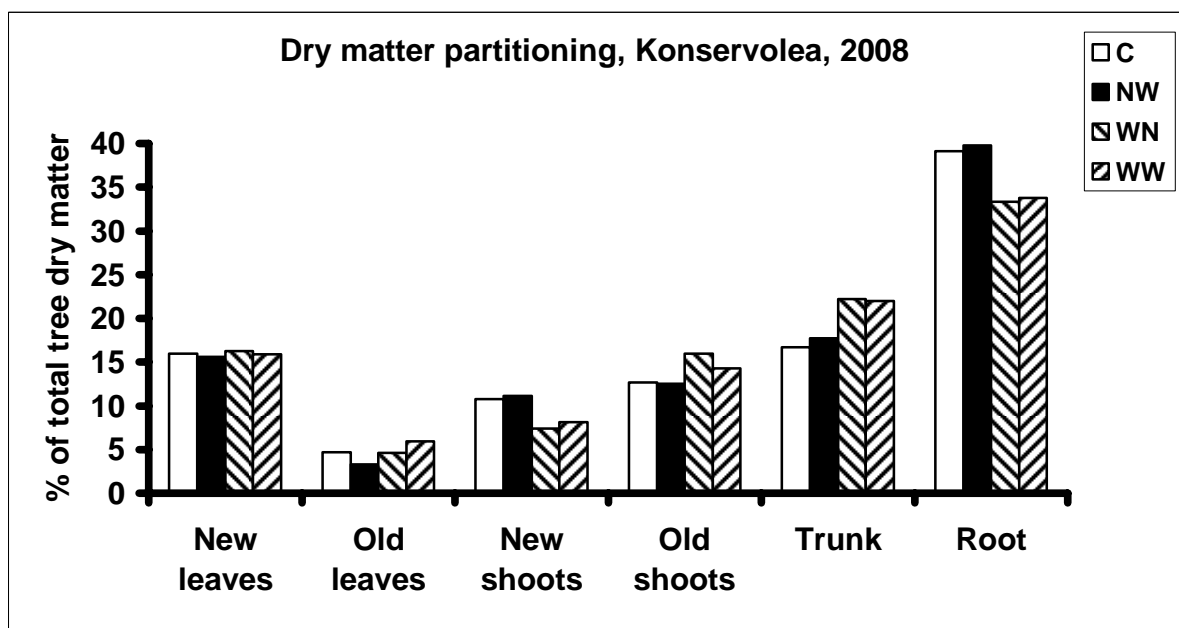
Fig. 15. Dry matter content (A) and dry matter partitioning (B) of different tree parts of 'Konservolea' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution and filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) in 2008.

A

Dry matter content, Kalamata, 2008

(Bar chart: DM (g) on y-axis, ranging 0 to 100, for tree parts: New leaves, Old leaves, New shoots, Old shoots, Trunk, Root. Legend: C, NW, WN, WW.)

B

Dry matter partitioning, Kalamata, 2008

(Bar chart: % of total tree dry matter on y-axis, ranging 0 to 35, for tree parts: New leaves, Old leaves, New shoots, Old shoots, Trunk, Root. Legend: C, NW, WN, WW.)
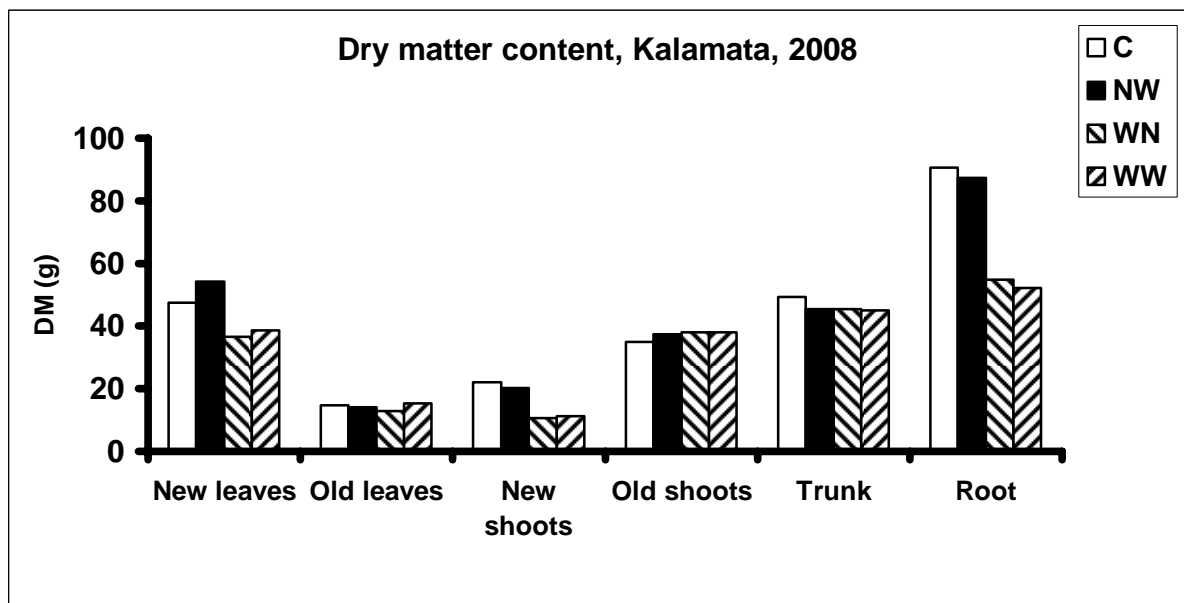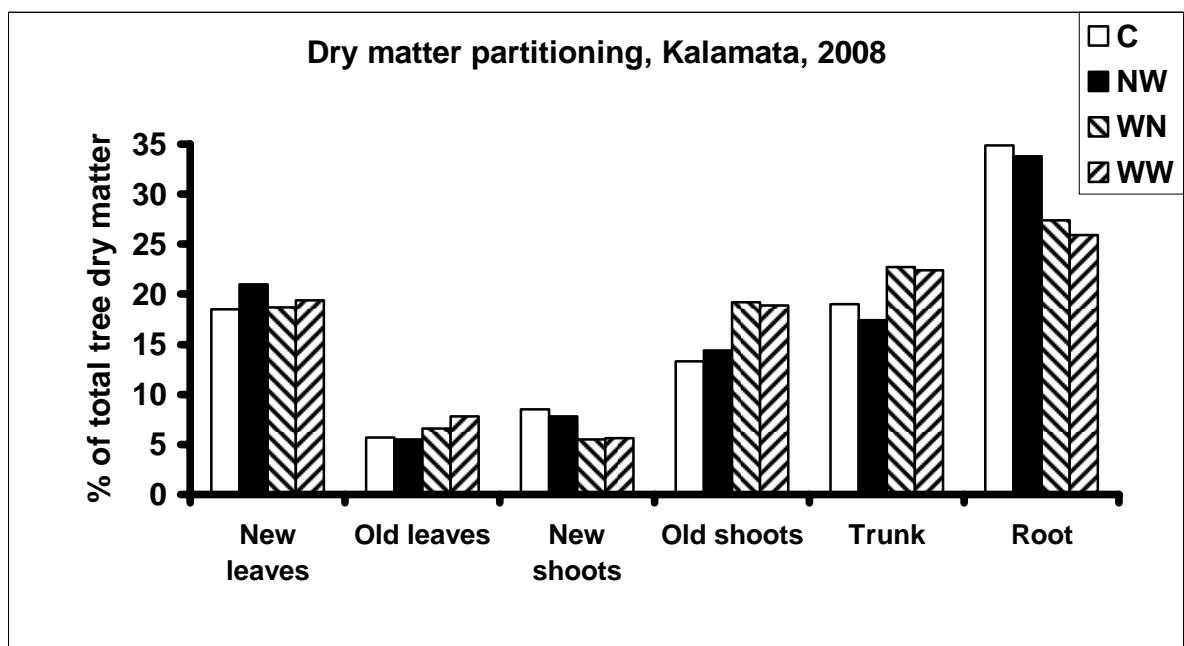
Fig. 16. Dry matter content (A) and dry matter partitioning (B) of different tree parts of 'Kalamata' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution and filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) in 2008.

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3.16. New shoot length per tree (NSL) 2006

NSL of 'Konservolea' olive trees was 35% higher than NSL of 'Kalamata' olive trees and this difference was found in all treatments (Table 24).

In 'Konservolea' olive trees, WN and WW treatments decreased NSL by around 21% compared to the control treatment. NW treatment did not affect NSL compared to the control treatment (Table 24). In 'Kalamata' olive trees, WN and WW treatments decreased NSL by around 44% compared to the control treatment. NW treatment did not affect NSL compared to the control treatment (Table 24).

3.17. New Shoot length per tree (NSL) 2008

Overall, NSL from 'Konservolea' olive trees was 33% higher than NSL from 'Kalamata' olive trees and this difference was found in all treatments (Table 24).

In 'Konservolea' olive trees, WN and WW treatments decreased NSL by 34% compared to the control treatment. NW treatment did not affect NSL compared to the control treatment (Table 24). In 'Kalamata' olive trees, WN and WW treatments decreased NSL by 45% compared to the control treatment. NW treatment did not affect NSL compared to the control treatment (Table 24).

3.18. New leaf area per tree (LA), 2006

Overall, LA form 'Konservolea' olive trees was 19% lower than LA from 'Kalamata' olive trees and this difference was found in all treatments (Table 24).

In 'Konservolea' olive trees, WN and WW treatments decreased LA by around 38% compared to the control treatment. NW treatment did not affect LA compared to the control treatment (Table 24).

In 'Kalamata' olive trees, WN and WW treatments decreased LA by around 35% compared to the control treatment. NW treatment did not affect LA compared to the control treatment (Table 24).

3.19. New leaf area per tree (LA), 2008

Both cultivars had similar LA in all treatments (Table 24).

In 'Konservolea' olive trees, WN and WW treatments decreased LA by 35% compared to the control treatment. NW treatment did not affect LA compared to the control treatment (Table 24).

In 'Kalamata' olive trees, WN and WW treatments decreased LA by 33% compared to the control treatment. NW treatment did not affect LA compared to the control treatment (Table 24).

3.20. Leaf potassium, 2008

Leaves from both cultivars had similar leaf potassium content in all treatments (Table 25).

In both cultivars, WN and WW treatments decreased leaf potassium content compared to the control treatment. NW treatment did not affect leaf potassium content compared to the control treatment (Table 25).

3.21. Leaf sodium, 2008

Leaves from both cultivars had similar leaf sodium content in all treatments (Table 25).

In both cultivars, WN and WW treatments significantly increased leaf sodium content by almost 800% compared to the control treatment. NW treatment did not affect leaf sodium content compared to the control treatment (Table 25).

The reduction in K and the increase in Na content were not analogous as high salinity decreased leaf K content by 7 g kg^{-1} DW and increased leaf sodium content by only 1 g kg^{-1} DW, which can be a sign of olive tree tolerance to salinity.

Table 24. Leaf area and new shoot length of (Kon) 'Konservolea' and (Kal) 'Kalamata' olive trees grafted onto seedling rootstock and growing with half-strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) in 2006 and 2008.

Treatment	2006				2008			
	Leaf area (cm^2)		New shoot length (cm)		Leaf area (cm^2)		New shoot length (cm)	
	Kons	Kal	Kons	Kal	Kons	Kal	Kons	Kal
C	2725	3189	75	60.56	2419	2493	77.6	56.7
WN	1751	2223	56	32.12	1576	1668	51.3	29.6
WW	1624	1886	62.75	36.00	1526	1656	54.3	33
NW	2587	3458	88.2	55.1	2415	2514	76.5	54.3
Significance								
Treatment	***		***		***		***	
Cultivar	***		***		NS		***	
LSD$_{0.05}$	501.5		12.9		255		6.5	

Significance levels: NS not significant, *** significant at $P < 0.001$

Table 25. Sodium and potassium content of leaves from 'Konservolea' and 'Kalamata' olive trees irrigated with half strength Hoagland's solution with charcoal-filtered air (C) or ambient O_3 (NW) or with half-strength Hoagland's solution containing 100 mM NaCl with ambient O_3 (WW) or with charcoal-filtered air (WN) in 2008.

Treatment	K (g kg^{-1} DW)		Na (g kg^{-1} DW)	
	Konservolea	Kalamata	Konservolea	Kalamata
C	28.1	27.6	0.192	0.189
WN	21.0	21.25	1.140	1.126
WW	20.97	20.7	1.178	1.161
NW	27.45	27.07	0.192	0.189
Significance				
Treatment	***		***	
Cultivar	NS		NS	
LSD$_{0.05}$	2.76		0.09	

Significance levels: NS not significant, *** significant at $P < 0.001$.

Chapter 4. Discussion

4.1. Ozone concentrations

Ozone and its precursors can be transported to rural areas up to several hundred kilometers from emission sources depending on weather conditions and transport mechanisms. O_3 concentrations will often be higher in rural areas that are downwind of urban areas than in the urban area itself. This is because as the polluted body of air moves away from the emission sources, O_3 continues to form and concentrations continue to rise as fewer scavenging mechanisms are operating than in the urban atmosphere (Comrie, 1994).

The ambient ozone concentrations measured in the rural area of Velestino could damage sensitive crops and have been found before for Volos, a nearby urban area (Nelly et al. 2005). Even though olive is considered an ozone-resistant as a sclerophyllus evergreen species (Bussotti and Gerosa, 2002), differences in ozone sensitivity between two olive cultivars for ozone concentrations slightly below the ones we measured were previously found to affect some physiological parameters in olive (Minnocci et al., 1999).

4.2. Leaf Physiological characteristics

4.2.1 Differences between the two cultivars

'Kalamata' leaves are macroscopically darker than 'Konservolea' leaves. In both experimental years, this was measured as increased Chl a and total Chl content in 'Kalamata' leaves, as the increased Chl content gives leaves their dark-green color (Lambers et al., 2000). These leaves also had higher percent DM and SLM in 2006, but similar percent DM and SLM in 2008 to leaves from 'Konservolea'. This could conclude that 'Kalamata' leaves have similar or higher productivity than 'Konservolea' leaves. In our study, this was found only in June based on the Pn measurements, before the stressful high summer temperatures, when leaves from both cultivars had similar leaf Pn over the summer. In another study, which compared physiological functions of six own-rooted cultivars (Hagidimitriou and Pontikis 2005), 'Kalamata' leaves also had higher total Chl content than 'Konservolea' leaves, but lower SLM and Pn over the growing period. Also, Chartzoulakis et al. (2002) found that 'Kalamata' leaves had significantly lower Pn rate than 'Konservolea'

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leaves. These differences show that leaves from young 'Kalamata' trees even under the best growing conditions are as productive as 'Konservolea' leaves or less productive even though they spent more energy to develop the light-harvesting photosystems. This could be direct effect of high temperature stress experienced from 'Kalamata' leaves during the summer or the high temperatures experienced over the summer in the areas the cited studies were performed. In addition, we used plants of the two cultivars grafted onto olive seedling rootstock, which is typical for the commercially used plants of these two cultivars in Greece. The researchers in the other cited studies (Chartzoulakis et al., 2002; Hagidimitriou and Pontikis, 2005) used own-rooted plants from the same cultivars. Thus, the differences we measured could also be due to the seedling rootstock, as rootstocks have been found to affect scion leaf Pn and Gs (During, 1994; Jones et al., 1985). Based on the Pn results, we can propose that the seedling rootstock may have supported higher leaf productivity in 'Kalamata' trees than found in own-rooted plants.

Also in our study, although leaves from both cultivars received similar PAR (and supposedly total light), 'Kalamata' leaves could have absorbed more light, due to deeper green color. This must have increased Gs and E, but did not increase Pn and thus decreased leaf WUE (synonymous to transpiration efficiency). The increased Gs means that more CO_2 could enter the mesophyll in 'Kalamata' leaves. Thus, Pn did not increase probably due to limitations in either mesophyll CO_2 conductance or dark reaction center capacity. This is still not explained from the fluorescence measurements, where leaf F_v/F_m was similar in the two cultivars, so similar light was sequestered and similar stress was experienced in the two cultivars. Interestingly, SWP was similar in the two cultivars, but E was higher in 'Kalamata' leaves. This is probably due to larger xylem conductance in 'Kalamata' trees, a point that needs further research as it could have implications to the cultivar's drought resistance (it is considered sensitive to dry climate) and freeze damage potential.

4.2.2. Changes during the growing period

In August, leaf Chl a content decreased and leaf Chl b content increased leaving total Chl content unchanged or slightly increasing. This suggests that Chl a is less stable than Chl b under stress conditions due to hot summer weather or prolonged water shortage, as at midday, leaves are experiencing lack of enough water for transpiration due to limited stem water conductance, even when they are well watered.

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This is supported from the decreased SWP values measured. The decrease in the ratio of Chl a / Chl b content has also been related to shading, but, in our young plants and under the experimental conditions, it is very unlikely. Other researchers have found a reduction in olive leaf total Chl content in late summer (Hagidimitriou and Pontikis, 2005; Proietti, 2000).

Olive leaves reached their final DM content early in the summer, as SLM did not change after June. This shows that olive leaves mature very soon after their development in spring, as olive is a drought-resistant plant growing well in dry summer conditions.

Leaf functioning of mature leaves gradually decreased with summer time as Gs and E and Pn rates decreased until August at least, even though the plants were properly fertilized and irrigated. Similar results were obtained by Hagidimitriou and Pontikis (2005) and Ben Ahmed et al. (2008) for irrigated young olive trees. This could be due to reductions in sink strength as shoot growth had ceased since early summer or due to reductions in leaf capacity from the summer stress as leaf Chl a content decreased as well, but not total leaf Chl content in our study. Furthermore, T_{leaf}, E and Gs decreased and Pn only slightly decreased in September, as the weather cooled down and sink strength had diminished.

4.2.3. Salinity and ozone effects

The decrease in Chl content in NaCl-stressed olive leaves has been found before (Melgar et al., 2008; Mousavi et al., 2008). This reduction in Chl due to NaCl is caused by the destruction of the chloroplast structure and the instability of pigment protein complexes and Chl degradation by chlorophyllase (Singh and Dubey, 1995).

We found that salinity in the absence or presence of ambient ozone significantly decreased leaf functioning parameters compared to trees growing with ambient or low ozone levels without salinity stress. This was partially due to decreased Chl content, but factors like Gs, mesophyll CO_2 conductance and SWP may have negatively affected each other and finally leaf Pn and dry matter productivity, as described by Loreto et al., (2003). We also found that incident PAR on the leaves in the NaCl-treated trees was slightly lower than in the control trees. This could be due to increased light reflectance of NaCl-stressed leaves with the reduced Chl content and Pn. As a result from this possible increased light reflectance, T_{leaf} was not affected by salinity stress despite the fact that E was reduced.

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The SLM was not affected by salinity as DM accumulates for balancing the very negative root osmotic potential, because the roots mainly accumulate the Na^+ and Cl^- ions in this type of glycophytes.

Salinity of irrigation water at 80 mM NaCl of irrigation water was previously found to be the limit for damage to olive (Therios and Misopolinos, 1988). Bernstein (1965) set the initiation of losses in olive productivity due to salinity when soil electrical conductivity (EC) exceeds 4 dS m^{-1}. We irrigated with 100 mM NaCl solution and substrate EC exceeded 13 dS m^{-1}. This resulted in substantial productivity losses accounting for more than 35% of leaf Pn in our plants.

Salinity sensitivity of the two cultivars used in our work was studied before with young own-rooted plants (Chartzoulakis et al., 2002). They concluded that 'Kalamata' plants were more tolerant to similar NaCl levels as their Pn rate was lower due to lower Gs than 'Konservolea' plants. In our study, with the two cultivars grafted on seedling rootstock, there were no differences in NaCl tolerance and Pn rate between the two cultivars. This was probably the result of the use of seedling rootstock, which seemed to affect the behavior of the two cultivars increasing the leaf productivity of 'Kalamata' trees and improving the NaCl tolerance of 'Konservolea' trees. It is expected and is well documented that roots play a substantial regulating role in Na^+ and Cl^- ion uptake and transport to leaves (Chartzoulakis et al., 2002; Levy and Syvetsen, 2004; Tattini et al., 1994).

Ozone alone (in the absence of salinity stress) did not affect leaf functioning in any case in our study. Ozone has been found to reduce Pn rate in various tree species (Pye, 1988). Olive is expected to be relatively tolerant to high O_3 levels due to some leaf traits including the position and size of stomata and the low stomatal and mesophyll conductance as a sclerophyllus evergreen drought-resistant species (Ribas et al., 2005). Thus, at least the two olive cultivars studied herein (grafted on seedling rootstock) are relatively resistant to high AOT40 exceeding 54000 nmol mol^{-1} h over the summer period. In other words, the high ambient ozone concentrations present today in various rural or not Mediterranean areas are high enough to damage many plant species (WHO, 200) but did not affect the basic olive leaf productivity for the two cultivars studied herein. Based on the results from an Italian group working with O_3 effects on cultivated olive, Gs was substantially reduced in both cultivars studied (own-rooted young plants) and Pn was reduced in only one of them after exposure to 100 nL L^{-1} O_3 for 5 h per day for 120 days reaching AOT40 around 36000 nmol mol^{-1}

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h (Minnocci et al., 1999). No conclusive results were also found on the effects from exposure to similar to our study's ozone AOT40 values to wild olive seedling plant productivity in Spain (Inclan et al., 1999, Ribas et al., 2005). Thus, damage from O_3 in olive seems to be genotype-dependent and may be affected from the rootstock and ozone concentration more than the duration of exposure to ozone, an estimation of which is AOT40. Nevertheless, olive has all leaf characteristics including low stomatal conductance and Pn (Bussotti and Gerosa, 2002) that make it resistant to O_3. In addition, olive is grown in dry climates where, during the summer months, transpirational demand during the hot daylight hours is high and stomatal conductance is very low. This coincides with the hours when O_3 concentration is also highest. Thus, O_3 can not significantly enter and accumulate in the leaves in levels high enough to cause damage (Bussotti and Gerosa, 2002).

Salinity similarly affected in the presence or absence of ambient O_3 levels the measured olive leaf physiological parameters in most cases. So, high O_3 present in many rural areas in the Mediterranean region did not seem to positively or negatively affect the stress experienced by olive leaves from 100 mM NaCl. As Gs is the driving force for O_3 entrance to mesophyll and its subsequent damage to the photosynthetic apparatus, and Gs was reduced due to salinity; the absence of damage due to O_3 in the combined stress treatment was expected. But nevertheless, ambient O_3 alone without salinity did not cause any measurable stress to olive plants in our study. Thus, the olive plants used in our study were resistant to O_3 concentrations found today in many Mediterranean areas and to exposure duration for up to one summer growing season and no interaction with salinity can be deduced for olive.

4.3. Leaf antioxidant enzymes activities
4.3.1. Differences between the two cultivars

Based on the two years' results, leaves from 'Konservolea' trees often had higher enzyme activity than leaves from 'Kalamata' trees with differences especially found in leaves from this year's growth and late in the season. This probably means that the main antioxidant mechanism in 'Konservolea' trees is more active and this cultivar is more tolerant to oxidative stress, in particular salinity stress herein, than 'Kalamata' olive, although actual differences were often small.

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4.3.2. Differences between new and old leaves

This year's (new) leaves always had higher enzymatic activity than last year's (old) leaves, as new leaves are more photosynthetically active and are exposed in the sun more intensely and often over the day. On the other hand, old leaves are closer to senescence and their reduced antioxidant enzyme activities may be interconnected to senescence and their sensitivity to oxidative damage due to age and shade (Prochazkova and Wilhelmova, 2007).

4.3.3. Changes during the growing period

Leaf SOD activity had a maximum in September, a trend that was not found with the other two analyzed enzymes over the measurement period. This could be a sign that SOD is a better stress indicator especially to high temperature and salinity stresses.

4.3.4. Salinity and ozone effects

The salinity treatment significantly increased enzyme activities over the summer and September period and strongly reduced enzyme activities in October compared to control leaves. This reduction late in the season could be due to prolonged stress or due to leaf functioning failure (Goreta et al., 2007). Oxidative stress occurs when there is a serious imbalance between the production of ROS and antioxidative defence (Ahmad et al., 2008). The increased enzyme activity due to salinity is a major metabolic reaction to salinity stress found herein in olives and, generally, is correlated to tolerance to salinity stress in plants (Allen, 1995).

The ambient O_3 levels did not have any effects on enzyme activity compared to control. This means that these two olive cultivars are relatively resistant to prolonged exposure to high ambient ozone levels exceeding 60 nL L^{-1}. Sebastiani et al. (2002) showed that APX activity in olive leaves was not affected by O_3 treatments in cv. Frantoio, while it increased in cv. Moraiolo exposed to 50 nL L^{-1} O_3.

4.4. Dry matter partitioning
4.4.1. Differences between the two cultivars

In 2006, 'Konservolea' plants had lower percent dry matter (%DM) and dry matter content in roots than 'Kalamata'plants. In 2008, 'Konservolea' plants had similar %DM and higher dry matter content and percent of total tree dry matter in

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roots than 'Kalamata' plants. These data mean that 'Konservolea' plants had smaller root system in 2006 and larger root system in 2008 than 'Kalamata' plants. This difference did not affect trunk and new and old shoot dry matter content or partitioning. Thus, in both years, 'Konservolea' trees had lower %DM in the trunk and new shoots than the 'Kalamata' trees and similar old shoot %DM. Nevertheless, in 2006 'Konservolea' plants with the smaller root system had lower total plant dry bmatter than 'Kalamata' plants.

Interestingly, salinity resulted in similar %DM in both cultivars in the new shoots and lower %DM in old shoots in 'Konservolea' compared to 'Kalamata' old shoots. This means either that 'Konservolea' plants reacted strongly to salinity reducing the dry matter accumulated to old shoots and increasing the dry matter accumulated to new shoots or 'Kalamata' plants reacted to salinity by increasing the dry matter accumulated to old shoots and decreased the dry matter accumulated to new shoots. These changes due to salinity although found in both years, are not followed by the absolute values of matter accumulated in each plant part. This means that, irrespective of treatment and in both years, 'Konservolea' trees had lower matter (expressed as g dry matter) accumulated in the trunk and old shoots and higher dry matter accumulated in the new shoots than 'Kalamata' trees. This also means that 'Konservolea' plants have larger capacity to produce new shoots even though the energy invested to develop leaf surface was similar to 'Kalamata' plants. This difference is also shown from the dry matter partitioning data, where 'Konservolea' plants had smaller % of total tree dry matter in the trunk and old shoots and higher % of total dry matter in the new shoots than 'Kalamata' plants. These data mean that 'Konservolea' plants produced more new shoots than 'Kalamata' plants. From our shoot length measurements, this was actually the case for all treatments.

Furthermore, even though the %DM of new and old leaves was similar in the two cultivars studied, 'Konservolea' plants invested less dry matter in new and old leaves than 'Kalamata' plants. These data together mean that 'Konservolea' plants produced less leaf surface than 'Kalamata' plants. Our data on total plant leaf surface confirm the above as 'Konservolea' plants were found to have partially lower total plant leaf surface than the 'Kalamata' plants.

Putting the data together, 'Konservolea' plants showed larger potential for new shoot growth even if supported from a smaller root and old shoot system, but had lower leaf dry matter production than 'Kalamata' plants.

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Finally, there is an interesting observation on % of total tree dry matter partitioning. In 2006, when 'Konservolea' roots were smaller than 'Kalamata' roots, 'Konservolea' trees under salinity stress had more dry matter (as % of total) invested in leaves than 'Kalamata' trees, while under no stress (control), 'Konservolea' trees had less dry matter invested in leaves than 'Kalamata' trees. To an extend the opposite was true in 2008, when salinity decreased the % of total dry matter invested in new leaves of 'Konservolea' plants compared to 'Kalamata' plants.

4.4.2. Salinity and ozone effects

During both years of the study, salinity did not affect the %DM of old shoots, trunk and root, as these organs had developed since the previous years or, in the case of root, their dry matter accumulation is a requirement to counteract salinity stress and a result of salinity stress. In addition, root growth of glycophytes is generally affected less by salinity than vegetative shoot growth (Maas and Nieman, 1978). The results of salinity on %DM of the new shoots and old and new leaves were different depending on the cultivar. In 'Konservolea' plants, salinity decreased %DM in new leaves but did not affect %DM in old leaves and new shoots. Again, 'Konservolea' plants seem to have a large capacity for new shoot growth despite the low investment in root dry matter, but, as expected, new leaf dry matter accumulation was negatively affected from salinity. Actually, from out data, shoot length and leaf surface per tree were reduced due to salinity. Salt-treated olive trees are usually characterized by smaller size, smaller leaves, shorter internodes, decreased number of shoots and leaves, and decreased leaf area than plants grown without saline stress (Chartzoulakis et al., 2002; Perica et al., 2008; Therios and Misopolinos, 1988). In 'Kalamata' plants, %DM was reduced in new and old leaves and in new shoots (except in new leaves in 2008, when it remained unchanged).

From the quantities of DM accumulated to each plant part, we can conclude that salinity decreased total tree dry matter due to decreased dry matter accumulated to new leaves, new shoots and roots. So, as salinity negatively affected photosynthetic rate and thus biomass production, it reduced the dry matter accumulated in the major tree parts growing over the period when salinity stress was imposed (Lazof and Bernstein, 1999). Old shoots and leaves, and trunk DM content did not change as this biomass had accumulated over the previous seasons of the tree growth. Munns et al.

(1982) reported that shoot growth reduction caused by salinity originates in growing tissues, not in mature photosynthetic tissues.

Finally, from the total tree dry matter partitioning data, salinity again resulted in smaller % partitioning of the total tree dry matter in the root and new shoot and higher % partitioning of the total tree dry matter in the trunk, old shoot and old leaves. This was the result of the smaller investment of dry matter to new shoots and roots as described above due to salinity. Overall, the % of the total dry matter invested to new leaves was not finally affected by salinity due to its small share of the total tree dry matter.

There was not a single case that ambient O_3 was shown to affect dry matter accumulation to any of the plant parts of the young olive trees of both cultivars studied. Even though the concentration of ambient O_3 and its accumulated potential for phytotoxicity were high, there were neither macroscopic damages observed nor any changes in the partitioning of dry matter to all, new and old, plant parts studied.

Finally, the combination treatment of salinity stress and ambient O_3 concentrations in the Velestino area did not, in any case, affect the partitioning of dry matter to different plant parts of young olive plants in any significantly different way than the effects of salinity stress alone encountered in this study as described above.

4.5. Stored sugar metabolism in different olive plant parts
4.5.1. Differences between the two cultivars

The concentration of neutral sugars and starch in leaves, new shoots, old shoots and roots was also studied to further understand the differences present between the two cultivars and due to the treatments. The only differences found between the two cultivars were the following: 'Konservolea' plants had higher starch concentration in the old shoots, lower neutral sugars concentration in new shoots and roots and lower starch concentration in new leaves than 'Kalamata' plants.

4.5.2. Salinity and ozone effects

Salinity increased neutral sugar concentration in new shoots and leaves and decreased neutral sugar concentration in roots. The opposite was very often found for starch concentrations, which decreased mainly in leaves and, to a lesser extend, in new and old shoots and increased in roots. These changes in the above ground parts are the result of the increased requirements for more negative water potential to

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continue growth and plant functions in the presence of salinity in the soil as neutral sugars are osmotically active and starch is not. Salinity decreased the amounts of starch stored in leaves, but increased the levels of reducing sugars. Starch decreased under salinity stress, probably reflecting the lower rate of photosynthesis in the salt stressed leaves (Downton, 1977). The opposite is true in roots, where reducing sugars tended to decrease while starch tended to increase (Ackerson and Youngner, 1975; Rathert, 1983). Several authors reported that olive leaves accumulated glucose and mannitol during the period of salinity stress, which played a leading role in osmoprotection, osmotic adjustment, carbon storage, and radical scavenging, while starch content was decreased (Chartzoulakis et al., 2004; Mousavi et al., 2008; Tattini et al., 1996).

Ambient O_3 did not also seem to affect stored sugar metabolism in young olive plants as ambient O_3 did not affect photosynthetic rate in olive leaves.

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Chapter 5. General conclusions and perspective future research

This chapter presents the major research contributions - conclusions of the thesis and indicates some directions for future research. The basic idea of this work was that two major stresses are present in the Mediterranean region, where most of the world cultivation of olive exists and is one of the most important economic crops. Irrigation with saline water had been studied before in mature and young olive trees, but grafted olive plants had not been studied before. Furthermore, the combined effects of salinity and ozone had not been studied before in any woody species besides a work with red maple.

5.1. General conclusions

Even though the two table olive cultivars have been considered of different salinity tolerance in the literature, the plants of these two cultivars in our study behaved similarly to salinity stress. This must have been due to the same rootstock used in the experimental plants, which seemed to regulate the salinity response of young olive plants.

Over the summer period, olive plants showed signs of high temperature stress, which was combined with salinity stress wherever it was applied. This stress was able to be followed using many of the parameters studied including leaf gas exchange and water relations, antioxidant enzyme activities, etc.

Irrigation with 100 mM NaCl solution over the summer negatively affected most leaf gas exchange and water relations parameters measured resulting in reduced accumulation of dry matter in all the new grown tissues including shoots and leaves, but also in roots. The increased antioxidant enzyme activities and reducing sugar concentrations due to salinity are the result of stress and show that the olive plants reacted to the stress and may have partly counteracted the negative consequences. Nevertheless, practically speaking the olive plants showed a significant reduction in growth due to 100 mM NaCl salinity stress, which would affect tree growth and productivity, but did not seem to affect plant survival.

Ambient ozone was high enough to damage most plants, but olive was not negatively affected based on all plant parameters we examined. This shows that olive, and especially these two cultivars in our study grafted on seedling rootstock, must be considered a resistant to ozone species to levels exceeding 60 nL L^{-1}.

Furthermore, no interaction between salinity and ambient ozone was found on olive plant metabolism and our plants under the combined stress of salinity and ambient ozone behaved similarly to salinity alone stressed plants.

5.2. Perspective future research

This thesis provided background information from which several ideas for future research could be developed. Potential future research directions are:

Salt tolerance in olive cultivars that could be used as potential rootstocks should attract more attention in future research

Salt tolerance in olives is associated with the ability to exclude Na^+ or Cl^- ions at the root level. Olive trees are considered moderately tolerant to salinity, though certain cultivars are more tolerant to salinity than others. However, their responses may differ depending on the rootstock and especially those of wild olive. Unfortunately, the relative responses of olive rootstocks or the response of scions on different olive rootstocks are not known.

Improving salt tolerance in olives by using supplemental nutrients such as Ca^{+2}, K^+ and N

Calcium is a key element in limiting the toxic effects of Na^+, increasing the Ca^{+2}: Na^+ ratio in the external solution, which alleviates the toxic symptoms of NaCl and maintains plasma membrane selectivity of K^+ over Na^+. It should be possible to determine which nutrients are the limiting factor and the interaction between these nutrients with the toxic effects of Na^+. This research is of particular practical interest to possibly alleviate salinity stress and make brackish water more acceptable for olive irrigation.

Examine Sclerophylly and leaf anatomical traits of 'Kalamata' and 'Konservolea' under drought conditions

Because the leaf is the most flexible organ in its response to environmental conditions its structure reflects, more clearly than that of the stem and roots, the effects of drought stress. Olive cultivars are different in their leaf sclerophylly and anatomical traits. We speculated that 'Kalamata' plants are more prone to drought stress due to their leaf characteristics. Thus, differences among olive cultivars in their ability to adapt to drought stress should be examined.

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Molecular biology and biochemical studies

Plant genomics and proteomics are radically improving our understanding of plant response to salinity. The characterization of genes that contribute to salt tolerance and the underlying changes in cellular activities in response to salt stress include various biochemical alterations which could lead to the identification of specific molecular markers for the salt tolerance in olives and the effect of rootstock on olive scion tolerance and productivity. Using the gene transformation technologies for olives, the way is open to manipulate olive salt tolerance by insertion of specific resistance genes.

Screening of olive cultivars for high ozone tolerance

Further work should be done for screening the different olive cultivars for high ozone concentrations (above 100 nL L^{-1}) to define the critical levels of olive sensitivity to ozone. Then, the combination of salinity and ozone stresses should be further addressed.

Ethylene blockers and ozone sensitivity

Ethylene accelerates senescence and lowers photosynthetic rate, stomatal conductance and many other plant functions in response to stresses including chronic ozone fumigation. The availability of ethylene synthesis and action inhibitors nowdays could be a useful tool to study the role of ethylene to salinity and ozone stress and damage, but also its manipulation as a way to alleviate these stresses and reduce their negative consequences.

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