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Effects of reduced irradiance on leaf morphology, photosynthetic capacity, and fruit yield in olive (*Olea europaea* L.)

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Abstract

One-year-old olive trees (cv. Koroneiki) were grown in plastic containers of 50 000 cm^3 under full daylight and 30, 60, and 90 % shade for two years. The effects of shade on leaf morphology and anatomy, including stomatal density and chloroplast structure, net photosynthetic rate (P_N), stomatal conductance (g_s), and fruit yield were studied. Shade reduced leaf thickness due to the presence of only 1–2 palisade layers and reduced the length of palisade cells and spongy parenchyma. The number of thylakoids in grana as well as in stroma increased as shade increased, while the number of plastoglobuli decreased in proportion to the reduced photosynthetically active radiation (PAR). The higher the level of shade, the lower the stomatal and trichome density, leaf mass per area (ALM), g_s, and P_N. Shade of 30, 60, and 90 % reduced stomatal density by 7, 16, and 27 %, respectively, while the corresponding reduction in P_N was 21, 35, and 67 %. In contrast, chlorophyll *a*+*b* per fresh mass, and leaf width, length, and particularly area increased under the same shade levels (by 16, 33, and 81 % in leaf area). P_N reduction was due both to a decrease in PAR and to the morphological changes in leaves. The effect of shade was more severe on fruit yield per tree (32, 67, and 84 %) than on P_N indicating an effect on bud differentiation and fruit set. The olive tree adapts well to shade compared with other fruit trees by a small reduction in stomatal and trichome density, palisade parenchyma, and a significant increase in leaf area.

Additional key words: areal leaf mass; chlorophyll; chloroplasts; fruit yield; leaf anatomy; net photosynthetic rate; shade; stomata; trichomes.

Introduction

The olive tree is one of the major crops in the Mediterranean region. Whilst its cultivation has spread to other regions around the world, olive production is of vital importance to the economy of Mediterranean countries.

Plant productivity is directly dependent on the photosynthetic capacity of the leaves, the dominant photosynthetic organs. Net photosynthetic rate (P_N) is greatly dependent on irradiance, absorption and utilisation of photon energy (Boardman 1977, Jackson 1980). Low irradiance affects P_N directly by reducing the utilization of photon energy, but this effect differs amongst plants and is dependent on their saturation irradiance. Although the olive is grown in regions of high sunlight, it has a low saturation irradiance compared to other fruit trees (Higgins *et al.* 1992, Bongi and Palliotti 1994). Shade reduced leaf P_N in olive (Tombesi and Cartechini 1986, Bongi *et al.* 1987, Proietti *et al.* 1988, Tombesi 1992). Long exposure of leaves to shade might also alter leaf morphology, anatomy, and other photosynthetic parameters, such as stomatal density and chlorophyll (Chl) contents, and thus might indirectly affect leaf P_N in several crops (Boardman 1977). Such changes in leaves can be permanent, particularly for those leaves that have emerged under shade (olive: Proietti *et al.* 1988).

The effect of shade on leaf morphology and anatomy has not been extensively studied. Long-term exposure to shade increased shoot length, internodal length, and leaf area, but decreased the number of flower buds and fruit set in three olive cultivars (Tombesi and Standardi

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1977, Tombesi and Cartechini 1986). Some differences were revealed between cultivars and their seasonal responses to shade (Tombesi and Cartechini 1986). Proietti *et al.* (1988) and Tombesi (1992) found that shaded leaves of olive had a larger area and smaller areal leaf mass (ALM) compared to those grown under full daylight. Proietti *et al.* (1988) also found that 30 % shaded leaves (cv. Leccino) had increased Chl contents and were thinner than those grown under natural irradiation, due to only having two layers of palisade cells compared to the three layers of the control leaves.

Stomata are important in leaf photosynthesis, with P_N being directly dependent on stomatal density and, importantly, the total area of stomatal pores. Olive leaves have stomata mainly on the lower surface and stomatal density varies amongst different olive cultivars (Leon and Bukovac 1978, Bongi *et al.* 1987). We found no references either to stomatal density or to chloroplast structure being influenced by shading in olive, although both play an important role in leaf photosynthesis.

Despite the similar adaptations of plants to various irradiances, differences have been found between various species, even amongst various clones of the same species (Björkman and Holmgren 1963, Boardman 1977). The olive tree, as an evergreen, has a permanent photosynthetic system that functions all year round and has a capacity for saccharide storage in the leaves late in winter (Priestley 1977). These characteristics make the leaves significant storage organs and therefore the effect of shade on leaf morphology, P_N, and saccharide storage is very important for tree growth and production. A reduced leaf P_N caused by shading might decrease saccharide contents in leaves and consequently reduce inflorescent bud initiation leading to a non-fruiting year ('off year') for olive, which is by nature a strongly alternate bearing tree (Proietti 2000).

In fruit tree production, precise information on the effects of various irradiances on leaf morphology and photosynthesis is needed to guide orchard management. A systematic study of the effects of increasing shade on leaf morphology as well as leaf P_N in relation to tree productivity has been performed in some tree crops such as peach (Kappel and Flore 1983), carambola (Marler *et al.* 1994), and hazelnut (Hampson *et al.* 1996) but no such knowledge exists for olive.

In traditional olive orchards, but more so in modern ones which tend to have a higher planting density, seasonal development of the tree canopy influences radiation distribution in the tree and may affect leaf morphology, physiology, and particularly leaf P_N. Reduced irradiance can also decrease flower initiation, fruit set, fruit quality and size, as well as fruit yield in most orchard crops (Jackson and Palmer 1977, Hampson *et al.* 1996, Proietti 2000).

This is why the effect of long-term exposure to various shade levels on olive leaf morphology, the consequent effect on leaf P_N, and the relation of these changes to olive productivity were the purposes of this study.

Materials and methods

Plants and experimental design: Olive trees (1-year-old) grown from cuttings of a local olive tree cv. Koroneiki were planted in plastic containers of 50 000 cm^3 in March 1995 and placed in the field. The containers had a mixture of soil : peat : sand (1 : 1 : 1, v : v : v) of pH 7.4, 6 % organic matter, 8.5 % $CaCO_3$, and conductivity 1.4 dS m^{-1} at 25 °C. The containers were set at large distances from each other so that none of the trees was shaded by another. The experiment took place at the Agricultural Research Institute in Nicosia, Cyprus (33°23'E longitude, 35°11'N latitude).

The experimental design in the field was that of a completely randomized design of four replicates with five trees each. Green plastic netting (*Novatex Italia S.P.A.*), supported by iron stakes 2.5 m in height, was used to cover the young trees thus providing shading of 30, 60, and 90 %. The control plots were without netting (0 % shade, full daylight). Water (pH 7.94, conductivity 1.048 dS m^{-1} at 25 °C) was applied *via* a drop irrigation system. Fertilization was applied every 15 d from the beginning of March to the end of November during each year of the experiment. Fertilizing solution (1 000 cm^3) containing 35, 10, and 15 g m^{-3} of N, P, and K, respectively, was added to each tree. In addition, the micronutrients were added using 1 kg m^{-3} of a commercial product (*MICR-O-PLEX*, *Rhone-Poulenc*, U.K.) containing 4 % Fe (EDTA), 4 % Mn (EDTA), 1.5 % Cu (EDTA), 1.5 % Zn (EDTA), 5.43 % MgO, 0.50 % B, and 0.10 % Mo at a rate of 1 000 cm^3 per tree.

Leaf morphology and surface characteristics: Fifty leaves were randomly collected from each experimental tree (winter 1997) and the following parameters were measured: (*a*) leaf thickness, in the middle of the leaf area, using a digimatic electronic micrometer; (*b*) leaf area, (*c*) leaf length, and (*d*) leaf width using a leaf area meter (*CID*, U.S.A). ALM was estimated by dividing the dry mass of 25 leaves by the corresponding leaf area.

Trichome density was determined (winter 1997) using leaves from 4 trees of each replicate of each treatment. Leaf trichomes were removed by gently pressing a transparent self-adhesive tape onto the leaf surface. Trichome density was estimated using the method of Karabourniotis *et al.* (1992). The adhesive tape was weighed before application and again after removal from the leaf. The difference between these two measurements is the mass of trichomes.

Stomatal frequency was measured in leaf samples taken in the morning from two shoots from two trees of each replicate. Shoots were wrapped in plastic bags, put

in a polystyrene box with dry-ice, and transferred to the laboratory. Leaf hair was removed from the lower surface as described above. Measurements were made from three different sections of the lower surface of the leaf (base, middle, and tip). Three measurements were made on each section, the surface area of each being 0.16 mm^2; hence, nine measurements per leaf were taken. Measurements of stomatal density were performed in four different seasons in 1997 using a *Zeiss Axiolab* fluorescence microscope equipped with a *G-365* excitation filter and an *FT-395* chromatic beam splitter (Karabourniotis *et al.* 2001).

Chloroplast structure and characteristics: One-year-old olive leaves were taken from the middle of the shoots. For transmission electron microscopy (TEM), small areas of leaf laminae were taken and immediately fixed in 3 % glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.3 at 4 °C; during this stage, the air was removed using a vacuum pump. The specimens were post-fixed in 1 % OsO_4 for 2 h, washed in buffer, dehydrated in a series of ethanol, embedded in Spurr epoxy resin, and polymerised at 70 °C for 36 h. Ultra-thin sections were cut with a *Reichert OMU-3* ultramicrotome, stained with uranyl acetate and lead citrate, and examined and photographed with a *Zeiss 9S* TEM. The photographs were used in morphometry for the quantification of chloroplast organelle surfaces (such as thylakoids). Eight photographs for each treatment were used for the quantitative measurements of thylakoids, starch grains, and plastoglobuli in this study. The process was as follows: photographs of chloroplasts were covered by a gridded (0.5×0.5 cm) transparent film. Each organelle at a grid intersection was counted and its % as a total of intersections over the whole chloroplast surface was calculated (Toth 1982, Savidis *et al.* 1989).

Leaf anatomy: The same thin cross sections (0.5–1.0 μm width) that had been prepared (as previously described) for TEM observations were used for light microscopy. The specimens were fixed and stained with 0.5 % toluidine blue in 1 % borax. Observations were made using a *Zeiss III* and *Zeiss Axioplan* light microscope to study the structure of spongy mesophyll and palisade cells.

Chl contents: Seven leaves from two trees of each replicate were randomly selected in the morning and seven leaf discs (6.8 mm diameter) were taken from each leaf, a total area being 2.5 cm^2. Each leaf sample was homogenized in 20 cm^3 *N*,*N*-dimethylformamide and put in a dark fridge (5 °C) for a 48-h extraction. Chls *a* and *b* were determined according to the method of Olesinski *et al.* (1989) using a UV-visible spectrophotometer (*M350* Double Beam, *Campec*) at 647 and 664 nm using the equations proposed by Moran (1982). Measurements were taken on the same dates as those of photosynthesis (Table 1).

P_N and stomatal conductance (g_s) were measured in the field using a closed portable infrared gas analysis system *LI-6200* (*LI-COR*) under cloudless conditions and taken in five consecutive seasons in 1997 and 1998 (Table 1). Leaf temperature, air temperature, photosynthetically active radiation (PAR), and relative humidity (RH) were also recorded.

In the spring of 1997, two leaves were randomly selected in the middle of the shoots from two trees of each replicate (8 trees, 16 leaves per treatment), and measurements were taken from each leaf on irrigated trees and on cloudless days in the morning from 08:00 to 11:00 h. The leaf was enclosed in a 250 cm^3 chamber connected to the IRGA and airflow into the system was approximately 200 μmol s^{-1}. P_N in trees grown under various shade levels was measured, then the same trees were transferred to full daylight, and P_N was measured again after 15 min. Two leaves of four trees per treatment were used and measurements were taken on 14 July 1997 from 07:30 to 10:30. P_N values were compared with those from trees grown constantly under full daylight.

In another experiment, on 21 July from 08:30 to 09:30, P_N was measured on two leaves of four trees growing under full daylight; then the same trees were transferred in sequence to 30, 60, and 90 % shade, where, after a period of 15 min, P_N was measured again on the same leaves.

Table 1. Seasons and dates at which measurements were taken.

Measurement	Season / date	Leaf age [d]
1st	Spring / 6 May 1997	60
2nd	Summer / 10 July 1997	105
3rd	Autumn / 22 October 1997	210
4th	Winter / 12 February 1998	320
5th	Spring / 19 May 1998	410

Fruit production: Bud differentiation was recorded on four shoots per tree. The mean % of inflorescent buds differentiated was equal to (no. flower buds per total no. buds)×100. The fruit number per tree, mean fruit dry mass, and total fruit yield (fresh and dry masses, FM and DM) per tree were also measured at harvest time.

Statistical analysis: The Statistical Analysis System (S.A.S. 1990) was used for the statistical analysis and Duncan's New Multiple Range Test (SAS 1990) for the comparisons of mean values.

Results and discussion

Leaf morphology: Long-term exposure of olive leaves to reduced PAR significantly affected all leaf parameters measured (Table 2). The strongest and most consistent effect of shade was that on leaf area, which is one of the most important factors for leaf photosynthesis. Similar shade effects on leaf area in olive were reported by Tombesi and Standardi (1977), Tombesi and Cartechini (1986), and Proietti *et al.* (1988) and also in peach (Kappel and Flore 1983, Nii and Kuroiwa 1988), kiwifruit (Chartzoulakis *et al.* 1993), and carambola (Marler *et al.* 1994). This is a common adaptation to low irradiance (Marler *et al.* 1994). However, some differences between olive and other fruit trees have been noted. Thus, while shade of 90 % caused an 81 % increase in the leaf area of olive, the increase was only 20–36 % in peach (Kappel and Flore 1983, Nii and Kuroiwa 1988), and 49 % in hazelnut (under 92 % shade, Hampson *et al.* 1996). The greater leaf size in shaded leaves may have been caused by an increase in contents of auxins and gibberellins within leaves under low irradiance (Salisbury and Ross 1978).

In contrast, leaf thickness was significantly reduced; the greater the shade, the smaller the leaf thickness. This was mainly due to the reduction of both palisade and spongy parenchyma (Fig. 1). The thickness of the palisade parenchyma layer was 176.4, 156.0, 148.3, and 124.9 μm and that of spongy parenchyma 246.6, 242.1, 210.7, and 220.1 μm for the control, 30, 60, and 90 % shade, respectively. Similar effects on leaf thickness have been reported for other fruit trees (in citrus, Syvertsen

Table 2. The effect of shade on dimensions, area, and number of one-year-old leaves and their chloroplast characteristics of olive trees (cv. Koroneiki) in winter 1997. Means followed by the same letters are not significantly different using Duncan's New Multiple Range Test; $n = 16$ (leaves) or 8 (chloroplasts), $p \leq 0.05$. *Leaf thickness including main vein. 50 leaves were measured from each tree for each measurement.

Treatment	Length [cm]	Width [cm]	Area [cm^2]	Thickness* [mm]	Leaves [per tree]	Total area [cm^2 $tree^{-1}$]	Thylakoids [% total chloroplast area]	Starch grains	Plastoglobuli
Full daylight	4.14 c	0.88 c	2.50 d	0.66 a	16765 a	42563 a	18.45	3.86	8.35
30 % shade	4.79 b	0.91 c	2.91 c	0.58 b	14869 b	42633 a	22.00	3.05	3.77
60 % shade	4.91 b	1.02 b	3.33 b	0.55 b	13089 b	41461 a	30.00	0	4.35
90 % shade	5.58 a	1.19 a	4.52 a	0.49 c	8843 c	35639 b	43.57	0	2.61

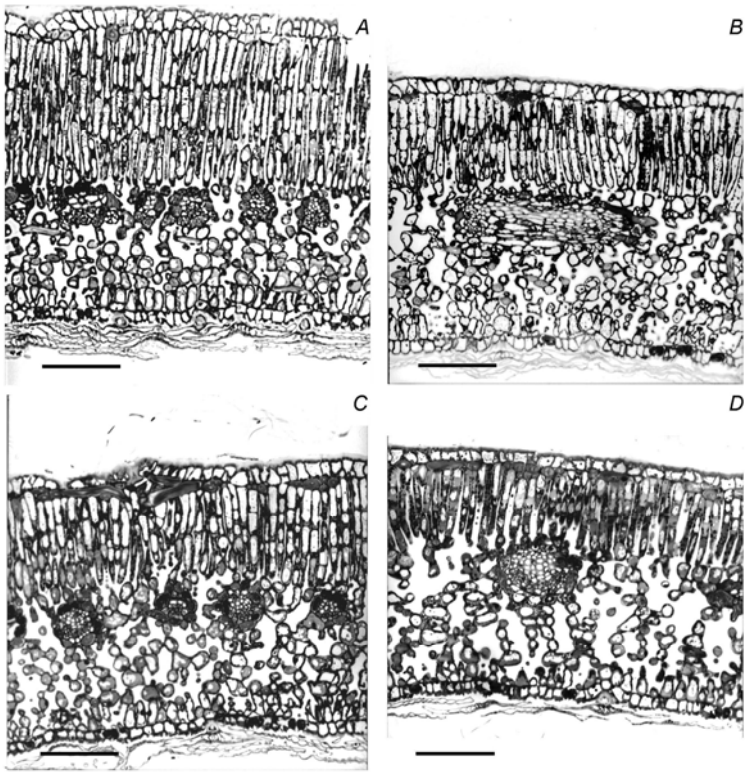

Fig. 1. Light micrographs showing the leaf structure and thickness of olive leaves (cv. Koroneiki) grown under full daylight (*A*), 30 % shade (*B*), 60 % shade (*C*), and 90 % shade (*D*) in 1997. *Bars* = 100 μm.

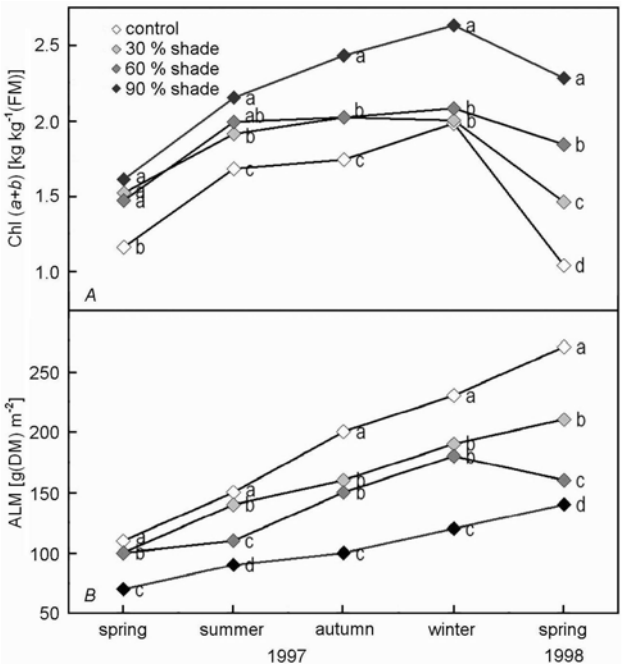

Fig. 2. The effect of irradiance on chlorophyll (Chl) (*a*+*b*) contents (*A*) and areal leaf mass (ALM) (*B*) in leaves of young olive trees (cv. Koroneiki) in 1997–1998. Means with the same letters for the same season are not significantly different using Duncan's New Multiple Range Test ($n = 8$, $p \leq 0.05$).

and Smith 1984; in peach, Nii and Kuroiwa 1988; in carambola, Marler *et al.* 1994). However, while at 90 % shade palisade tissue was reduced in peach by 51.7 % (Nii and Kuroiwa 1988), the reduction in olive was only by 29 %. In contrast, intercellular space was increased by shade and such a change might alter the CO_2 conductance from the substomatal cavities to the sites of carboxylation in chloroplasts, thus restricting the photosynthetic rate (Boardman 1977, Proietti *et al.* 1988, Syvertsen *et al.* 1995).

Chl and ALM: Chl content increased while ALM decreased with increasing shade in all seasons of this study (Fig. 2). Similar effects of shade on Chl and ALM have been found in peach (Kappel and Flore 1983, Nii and Kuroiwa 1988), carambola (Marler *et al.* 1994), hazelnut (Hampson *et al.* 1996), and kiwifruit (Chartzoulakis *et al.* 1993). The lower ALM of shaded leaves may be a result of the changes in leaf structure, leaf area, and some photosynthetic products stored in the leaves as indicated by the lower P_N and confirmed by the lower saccharide contents in shaded leaves (Vemmos *et al.*, unpublished). The increased Chl content in shaded leaves found in this study, in combination with increased number of thylakoids (Table 2), might increase the potential for absorption of photons by shaded leaves as has been suggested by Proietti *et al.* (1988). The relative increase in Chl *b* content (decreased Chl *a*/*b* ratio) in shaded leaves (data not shown) may also enhance their ability to capture and utilize photon energy. Chl (*a*+*b*) contents were not positively correlated with P_N with the exception of autumn (Table 5). This may be due to the changes in leaf morphology, chloroplast structure, or RuBP carboxylase activity, factors that might limit P_N in light-stressed leaves (Kappel and Flore 1983). The fact that Chl (*a*+*b*) contents per leaf area were similar in shaded and non-shaded leaves (data not shown) is probably another reason for the negative correlation of Chl with P_N.

Table 3. The effect of shade on stomatal density [mm^{-2}] and trichome density [mg cm^{-2}] of olive leaves (cv. Koroneiki) in four different seasons in 1997. Mean values followed by the same letters are not significantly different using Duncan's New Multiple Range Test; $n = 24$ (stomata) or 16 (trichome density), $p \leq 0.05$.

Treatment	Number of stomata					Trichome density
	Spring	Summer	Autumn	Winter	Mean of 4 seasons	
Full daylight	406.0 a	442.2 a	432.0 a	445.3 a	431.4 a	0.85 a
30 % shade	370.1 b	399.5 b	422.8 a	412.1 a	401.1 b	0.77 b
60 % shade	343.4 c	354.6 c	382.0 b	365.2 b	361.3 c	0.66 c
90 % shade	307.0 d	302.3 d	326.1 c	327.6 b	315.8 d	0.42 d

Stomatal density increased from spring towards summer for the control and 30 % shaded leaves, while this occurred later in autumn for the leaves receiving 60 and 90 % shade (Table 3). Thus leaves exposed to high irradiance might mature earlier than those growing under low irradiance. Our results of stomatal density of leaves grown in daylight are similar to those for "Manzanillo" olive (Leon and Bukovac 1978), higher than those reported by Bongi *et al.* (1987) (246–300 stomata mm^{-2}) but lower than those found by Roselli *et al.* (1989) on leaves of four olive cultivars (486–713 stomata mm^{-2}). The various results of stomatal density in olive indicate a cultivar effect, as suggested by Bongi *et al.* (1987). However, the environment might also affect stomatal density (Roselli *et al.* 1989). Table 3 and Fig. 3 also show that the number of stomata per unit leaf area decreased significantly, but not proportionally, with increasing shade. Thus shaded olive leaves had a 7.0, 16.2, and 27.0 % reduction in stomatal density (30, 60, and 90 % shade, respectively) in relation to the control. In peach for instance, a greater reduction in stomatal density of 44.1% was found under 90 % shade (Nii and Kuroiwa 1988), but

only 14 % in carambola under 53 % shade (Marler *et al.* 1994) and 30 % in hazelnut under 92 % shade (Hampson *et al.* 1996). The smaller reduction in both stomatal density and thickness of palisade tissues, as well as the greater increase in leaf area under 90 % shade of olive than in peach and hazelnut, suggests a better adaptation of olive to low irradiance compared with these fruit trees. This may give the olive tree the advantage of maintaining a relatively higher photosynthetic capacity compared with the above fruit trees under the same low irradiance.

Trichomes, apart from their general protective role in leaves, are important in the gas diffusion pathway, and protect against UV-B radiation damage (Karabourniotis and Fasseas 1996). Table 3 shows that trichome density fell to 50 % under 90 % shade but this drop was much less compared to *Quercus ilex* where shaded leaves had 8 times lower trichome density and 11 times less UV-B absorbing capacity than those exposed to daylight (Liakoura *et al.* 1997). We suggest that olive leaves growing under low irradiance might possibly lose a significant part of their protection against UV-B radiation.

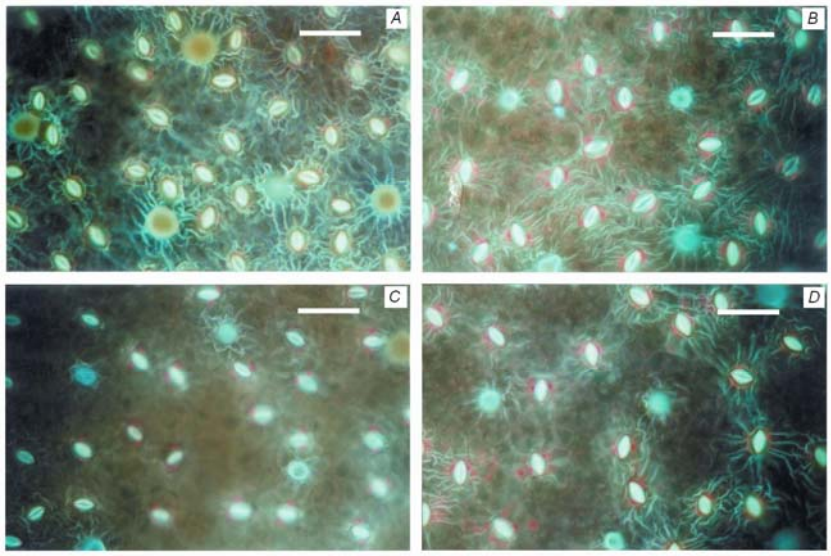

Fig. 3. Fluorescence micrographs showing the stomatal density in the adaxial surfaces of olive leaves (cv. Koroneiki) grown under full daylight (*A*), 30 % shade (*B*), 60 % shade (*C*), and 90 % shade (*D*) in 1997. *Bars* = 50 μm.

Chloroplast morphology: The structure of chloroplasts was also affected by shade as shown by the electron micrographs under the various shade treatments (Fig. 4, Table 3). Thus, the number of plastoglobuli decreased in proportion to the reduced irradiance, suggesting another means of photosynthesis limitation in shaded leaves. Plastoglobuli play an important role during the light reaction stage of photosynthesis. The lower starch accumulation in the chloroplasts of heavily shaded (60 and 90 %) trees is also an indication of the lower photosynthetic capacity of those leaves. Similar results were reported for other species (Boardman 1977, Nii and Kuroiwa 1988). The length of chloroplasts was not measured in this study; the electron micrographs, however, showed a possible chloroplast enlargement in heavily shaded leaves (Fig. 4) that is in agreement with similar shade effects in other species (Boardman 1977, Nii and Kuroiwa 1988).

***P*$_N$ and fruit yield**: All shade levels significantly reduced both g_s and P_N in all seasons examined with the exception of the 30 % shade which did not significantly affect P_N in summer 1997/spring 1998. The g_s was not significantly different between shade treatments in winter 1997 and spring 1998 (Fig. 5). Changes in P_N and g_s were similar during the seasons and the high correlation between g_s and P_N was linear (r^2 = 0.76–0.90, Table 5). Values of g_s and P_N increased from spring reaching a maximum in autumn. The low values in early spring might be due to leaves being immature, as indicated by the lower number of mature stomata and Chl contents (Table 3, Fig. 2). The higher g_s and P_N in autumn compared to summer were probably due to more favourable temperatures and RH conditions. The mean leaf temperature in summer was 36 °C while in autumn 27 °C; the corresponding RH values were 30 and 41 %. Bongi and Long (1987) and Tombesi (1992) found that in olive the most favourable temperature for photosynthesis was between 25 and 30 °C and that temperatures above 32 °C reduced P_N. As PAR was not significantly different over the seasons, the drop of P_N in winter might be attributed to the relatively low temperature (21–23 °C) but more likely to the older age of leaves. This is indicated by the fact that P_N did not increase the following spring, when temperature conditions (27–32 °C) were favourable for photosynthesis. Bongi *et al.* (1987) reported that low temperature reduced g_s and consequently P_N in olive. P_N in 90 % shaded leaves remained low and stable through all the seasons examined despite the changes in the environmental conditions; this was likely due to the serious changes in leaf morphology that had taken place.

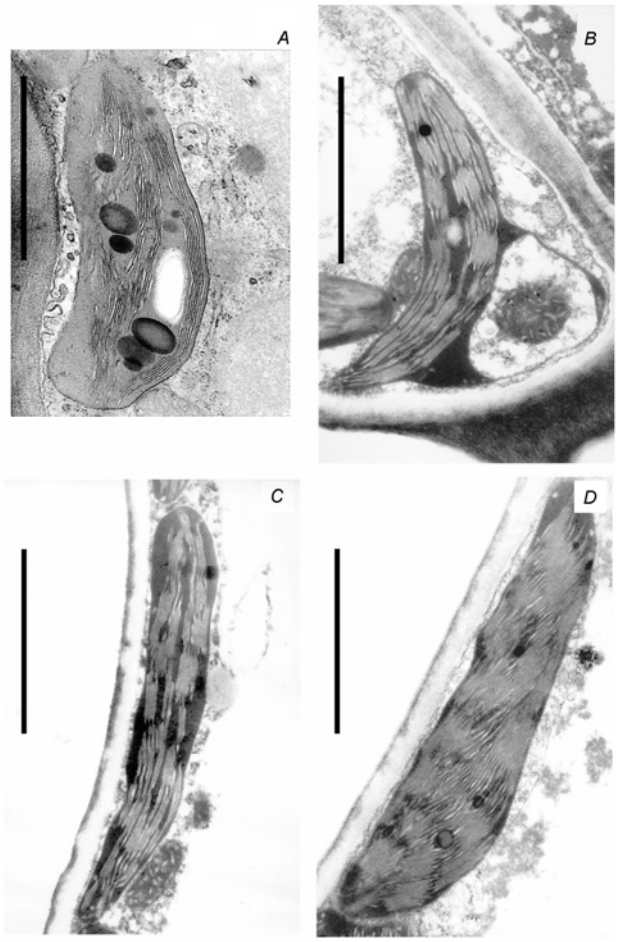

Fig. 4. Transmission electron micrographs showing chloroplast structure in olive leaves (cv. Koroneiki) grown under full daylight (*A*), 30 % shade (*B*), 60 % shade (*C*), and 90 % shade (*D*) in 1997. *Bars* = 7 μm.

P_N values in non-shaded leaves of the cv. Koroneiki were high compared with those reported for other cultivars (Bongi *et al.* 1987, Higgins *et al.* 1992, Bongi and Palliotti 1994) and in contrast to the reports that olive leaves have lower P_N than other fruit trees (Bongi *et al*. 1987, Higgins *et al.* 1992). Tombesi *et al.* (1984), however, reported high P_N for olive cv. Maurino similar to other fruit trees. It seems that the cultivar itself is one of the main factors affecting P_N (Bongi *et al.* 1987, Bongi and Palliotti 1994) and may account for these contradictory results. The high P_N of the cv. Koroneiki may be related to the relatively high stomatal density and might play an important role in the high productivity of this compared to other Greek olive cultivars.

We found that the greater the shade, the greater the reduction in g_s and P_N. The reduced P_N was partly due to the lower PAR and partly to the morphological changes in leaves (stomatal density, leaf area and width, palisade cells, ALM, and chloroplasts) as measured in this study. This is also indicated by the strong linear correlation of P_N with stomatal density, and ALM (Table 5). The fact

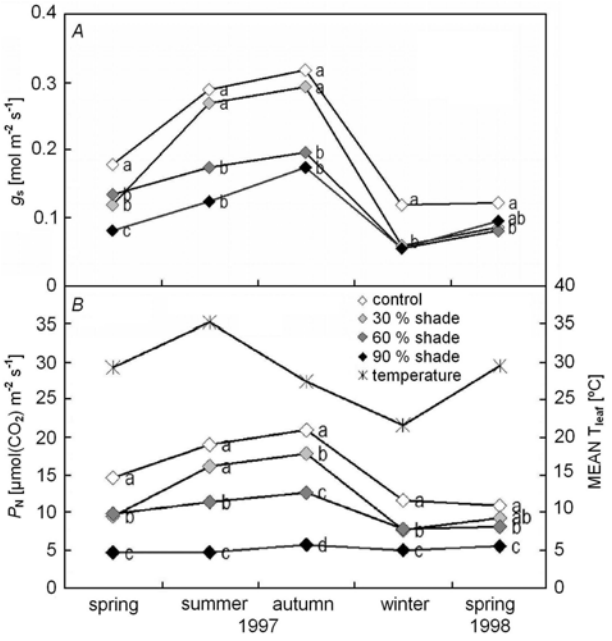

Fig. 5. The effect of irradiance on stomatal conductance (g_s) (*A*) and leaf temperature (T_{leaf}) and net photosynthetic rate (P_N) (*B*) in leaves of young olive trees (cv. Koroneiki) in 1997–1998. Means with the same letters for the same season are not significantly different using Duncan's New Multiple Range Test ($n = 8$, $p \leq 0.05$).

that the reduction in P_N in trees grown under full daylight after they were transferred to the various shade levels was smaller than that corresponding to the trees grown under the same levels of shade (Fig. 5*B*, Table 4) is another indication that the reduction in P_N is partly due to the morphological changes in leaves. The same conclusion comes from the small recovery in P_N in trees grown for two years under shade conditions when transferred to full daylight (Table 4). Similar effects on the photosynthetic capacity of olive leaves were found by Proietti *et al.* (1988) in plants grown for one year under shade. They concluded that such changes in the morphological characteristics of leaves were not completely reversible. In addition, our results showed that the higher the level of shade, the more severe the effect on leaf morphology and the greater the reduction in P_N.

The relatively small reduction in P_N in leaves growing under 30 and 60 % shade (21 and 35 %, respectively), with PAR reduced by 48 and 67 %, respectively (Fig. 6), may be due to the low saturation irradiance (900–1 000 μmol m^{-2} s^{-1}) and to the low compensation irradiance (53 μmol m^{-2} s^{-1}) for olive (Higgins *et al.* 1992).

The effect of reduced PAR was greater on the fruit yield per tree (Table 6) than on P_N (32 % reduction in fruit yield under 30 % shade and only 21 % reduction in P_N, Fig. 6).

The regression analysis of fruit yield [g $tree^{-1}$] with PAR also showed a higher correlation ($r^2 = 0.96$, Fig. 6) than that of P_N to PAR ($r^2 = 0.67$–0.88, Table 5). These

Table 4. Differences of net photosynthetic rate (P_N), photosynthetically active radiation (PAR), air and leaf temperatures (T_{air}, T_{leaf}), relative air humidity (RH), and stomatal conductance (g_s) in olive trees (cv. Koroneiki) grown (summer 1997) under full daylight when transferred to different shade levels (D→S) or grown under different shade levels when transferred to full daylight (S→D). *Absolute values for the control trees under full daylight are given; all other values are given as differences from the control. Means followed by the same letters are not significantly different using Duncan's New Multiple Range Test; $n = 8$, $p \leq 0.05$.

	Treatment	P_N [μmol(CO_2) m^{-2} s^{-1}]	PAR [μmol m^{-2} s^{-1}]	T_{air} [ºC]	T_{leaf} [ºC]	RH [%]	g_s [mol m^{-2} s^{-1}]
D→S	D*	(19.20)	(1890.00)	(35.50)	(36.80)	(39.50)	(0.285)
	D	0.00 c	0.00 d	0.00 b	0.00 d	0.00 b	0.00 b
	30 % S	–4.37 b	–780.10 c	–0.76 ab	–1.31 c	–2.89 a	–0.075 a
	60 % S	–5.32 b	–1091.60 b	–1.06 a	–2.19 b	–3.47 a	–0.062 a
	90 % S	–8.38 a	–1549.60 a	–1.46 a	–2.94 a	–2.96 a	–0.059 a
S→D	D	0.00 c	0.00 d	0.00 b	0.00 d	0.00 b	0.00 b
	30 % S	0.39 b	883.30 c	1.78 a	2.74 c	–0.48 ab	0.005 a
	60 % S	1.87 ab	1171.30 b	1.92 a	3.81 b	–1.48 b	–0.032 b
	90 % S	3.77 a	1633.70 a	1.88 a	4.75 a	–1.19 ab	–0.007 ab

Table 5. Correlation of net photosynthetic rate (P_N) with areal leaf mass (ALM), chlorophyll (Chl) content, stomatal density, photosynthetically active radiation (PAR), stomatal conductance (g_s), relative humidity (RH), and air and leaf temperature (T_{air} and T_{leaf}) in leaves of olive trees (cv. Koroneiki) for various seasons in 1997. $^{***}p \leq 0.001$, $^{**}p \leq 0.01$, $^{*}p \leq 0.05$, ^{NS} = no significant difference. Correlation coefficient $\alpha = r^2$.

	Spring	Summer	Autumn	Winter
ALM [g cm^{-2}]	0.729^{***}	0.840^{***}	0.801^{***}	0.697^{***}
Chl (*a*+*b*) [g kg^{-1}]	-0.479^{**}	-0.605^{***}	-0.663^{***}	-0.357^{*}
Chl (*a*+*b*) [g kg^{-1}]	-0.304^{NS}	-0.106^{NS}	0.388^{*}	0.200^{NS}
Chl *a*/*b*	0.390^{*}	0.706^{***}	0.288^{NS}	0.172^{NS}
Stomatal density [no. mm^{-2}]	0.757^{***}	0.803^{***}	0.785^{***}	0.416^{*}
PAR [μmol m^{-2} s^{-1}]	0.882^{***}	0.825^{***}	0.870^{***}	0.671^{***}
T_{air} [°C]	0.359^{*}	0.114^{NS}	0.495^{**}	0.031^{NS}
T_{leaf} [°C]	0.549^{***}	-0.047^{NS}	0.699^{***}	0.295^{NS}
RH [%]	-0.097^{NS}	0.430^{**}	-0.378^{*}	0.280^{NS}
g_s [mol m^{-2} s^{-1}]	0.885^{***}	0.904^{***}	0.760^{***}	0.856^{***}

Table 6. The effect of shade on fruit mass, fruit number, and fruit yield of young olive trees (cv. Koroneiki) in 1997. Means followed by the same letters are not significantly different using Duncan's New Multiple Range Test; $n = 16$, $p \leq 0.05$. *% inflorescent bud = (no. flower buds/total no. of buds) × 100.

	% inflorescent buds*	Fruit DM [mg]	Fruit number per tree	Total fruit FM [g $tree^{-1}$]	DM [g $tree^{-1}$]
Full daylight	73.6 a	57.7 a	2130 a	3189.6 a	1263.0 a
30 % shade	68.8 a	54.0 ab	1664 b	2265.6 b	857.9 b
60 % shade	54.7 b	43.5 bc	966 c	1360.8 c	422.7 c
90 % shade	33.5 c	40.1 c	513 d	661.5 d	202.6 d

results indicate that while the reduction of fruit yield is partly due to reduced P_N, other factors may also play a role. Since the total photosynthetic area of the tree was not affected by the 30 % shade (Table 2), this effect might be due to some morphogenetic factors, such as total number of inflorescent buds per tree, inflorescent bud differentiation, and fruit set. Table 6 shows that while the % of inflorescent buds at 30 % shade was lower, although not significantly, than that in the trees grown in full daylight, the fruit number was also significantly lower. Proietti (2000) suggested that reduced P_N might decrease the saccharide contents in olive leaves and consequently flower initiation. Lower saccharide content in shaded olive leaves has been found by Vemmos *et al.* (unpublished). In contrast, Stutte and Martin (1986) did not find any relationship between various irradiances and flowering in olive.

However, a similar, though stronger, effect of shade on fruit yield was found in hazelnut (Hampson *et al.* 1996), a 45 % reduction at 30 % shade. These authors

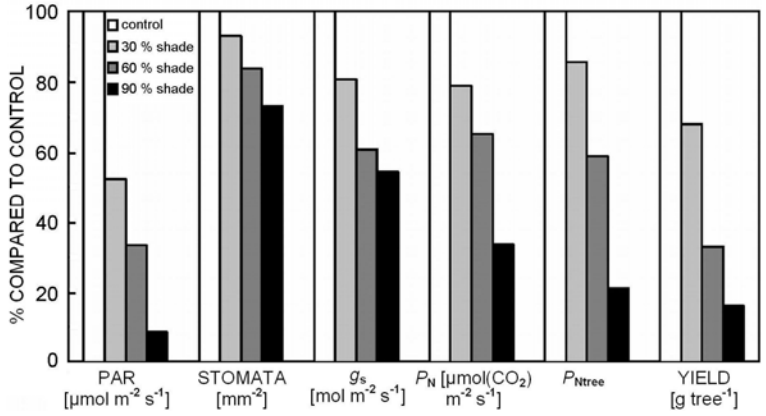

Fig. 6. Percent reduction in photosynthetically active radiation (PAR), stomatal density, stomatal conductance (g_s), net photosynthetic rate per area and per tree (P_N, P_{Ntree}), and fruit yield in relation to irradiance (means of all five seasons, 1997–1998).

suggested that fruit yield was more sensitive than flowering to low PAR.

We found that a long-term exposure of olive leaves to various PAR levels caused a number of serious anatomical and morphological changes in olive leaves that might be permanent. Thus, the reduction of P_N was due both to the reduced irradiance and to the morphological leaf changes brought about. This was also indicated by the small recovery in leaf P_N when trees grown under shade were transferred to full daylight. The reduction of P_N, however, was not proportional to the reduced irradiance. Thus, a 66.6 and 91.5 % reduction in PAR, for instance, caused only a 35.0 and 66.5 % reduction in P_N, respectively (Fig. 6). This might be due to the mechanisms of olive leaf adaptation to low irradiance as shown by a relatively small reduction in stomatal density and length of palisade cells, and an increase in leaf area, Chl content, and ALM.

Our results suggest that canopy management in olive should take into serious consideration that leaves must be exposed to irradiance greater than 1 000 µmol m^{-2} s^{-1} and that long-term exposure of leaves to low PAR may permanently reduce their photosynthetic capacity. The effect of shade on fruit yield, seeming not to be an effect of reduced P_N alone, indicates that other factors such as flower initiation, fruit set, or other morphogenetic characteristics warrant further investigation.

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