Effect of Pruning on some Physiological and Anatomical Features in Olive Transplants (*Olea europaea* L.)

أثر التقليم على بعض الخواص الفسيولوجية والتشريحية لأشتال الزيتون

"Rezq Basheer" Salimia

"رزق بشير" سليمية

Department of Plant Production and Protection. Faculty of Agriculture. Hebron University. Hebron. Palestine.

E-mail: rezqbasheer@hebron.edu

Received: (14/7/2007). Accepted: (8/4/2008)

Abstract

The effect of pruning (cut-back at 5cm height) in comparison to no pruning (plants with central leader) was studied at eco-physiological and anatomical levels in olive trees. Photosynthetic rate, stomatal conductance, leaf water potential, leaf osmotic potential, turgor potential, relative water content, and different anatomical features were reported. The results indicate that, non-pruned plants presented higher value of photosynthetic rate and stomatal conductance compared to the pruned plants. In addition, they maintain higher turgor potential for the same values of relative water content (RWC). At anatomical level, non-pruned plants exhibited higher relative volume of spongy cells, spongy thickness and significantly higher number of vascular bundles per leaf section compared to the pruned plants. Palisade parenchyma thicknesses as well as non-glandular hair density (No./mm^2) were lower in pruned plants. Possible effect of pruning on juvenility is discussed.

Kew words: *Olea europaea*, pruning, gas exchange, water relations, leaf anatomy.

ملخص

في هذه الدراسه تم مقارنة فسيولوجية وتشريحية على اشتال الزيتون المقلمة على ارتفاع ٥سم من سطح التربة مع النباتات غير المقلمة (نباتات مرباة باستخدام القائد الوسطي) على المستويين الفسيولوجي والتشريحي، حيث تم قياس المعايير التالية: معدل التمثيل الضوئي، كفاءة التوصيل لدى خلايا الثغور، الضغط المائي، الضغط الأسموزي، الجهد المائي (Turgor Potential)، المحتوى المائي النسبي، وكذلك بعض الخصائص التشريحية لأوراق الزيتون. أظهرت النتائج أن الأشجار غير المقلمة (المرباة بطريقة القائد الوسطي) كانت ذات معدلات أعلى من حيث التمثيل الضوئي وكفاءة التوصيل لدى خلايا الثغور مقارنة مع الأشجار التي تم تقليمها. كما حافظت خلايا الأشجار غير المقلمة على جهد مائي عال للقيم نفسها من المحتوى المائي النسبي. أما على المستوى التشريحي، فقد كانت أوراق الأشجار غير المقلمة أكثر سماكة، وتميزت طبقة خلاياها الإسفنجية بكثافتها وسمكها. كما ولوحظ أيضاً زيادة في أعداد الحزم الوعائية، وزيادة سماكة الطبقة البرانشيمية، مع زيادة كثافة الشعيرات لكل مقطع من الورقة مقارنة مع الأشجار التي تم تقليمها.

Introduction

Juvenility is well known as the period during which a plant cannot be induced to flowering. In woody plants, the duration of the juvenile phase is quite variable and can be quite lengthy (Meilan, 1997). The length of the juvenile phase is inherited (Visser, 1965) although it can be influenced to varying degrees by environmental and genetic factors (Hackett, 1985). Additionally, maturity can be reversed by sexual or apodictic reproduction; adventitious bud/embryo formation and by various nutritional regimes, hormonal treatments or environmental conditions (Hackett, 1985).

There are progressive ecophysiological, morphological and anatomical changes associated with the passing from juvenility to maturity as well as many developmental differentiations concerning the form and the shape of leaf shoot orientation (Hackett and Murray, 1993; Gupta, et al, 1990; and Murray, et al, 1994). On the other hand, among the factors affecting the duration of juvenility phase pruning is one of the most prominent (Di Marko, et al, 1990; and Ferrara, et al, 1999). Nevertheless, which factor is involved in the process of the phase changes from juvenility to maturity stages and the physiological and/or metabolic mechanisms responsible for the exchange of the phases is still

remaining unknown (Lavee, et al, 1996). Thus, identified markers related to the phase changes would not only enable the evaluation of the plant's developmental stage but also it will help in understanding the mechanism involved in this process (Garcia, et al, 1999). Due to the fact that very few studies are available on the influence of pruning on the changes of growth phases (juvenility-maturity) in olive trees; more information is needed for evaluation of the long-term effects and the ecophysiological behavior of cultivars to pruning (Di Marko, et al, 1990; and Ferrara, et al, 1999). This may prove a useful technique in studying and overcoming the phenomena of juvenility and related problems of phase changes especially in olive breeding programs.

The aim of this study was to detect the changes on anatomical and ecophysiological levels induced by pruning in olive tree.

Material and Methods

1. Plant Material

Fifteen to eighteen cm-long cuttings with four leaves at their distal end were taken in fall 2001 from water sprouts of olive bushes (*Olea europaea* L. var. Chondrolia Chalkidikis) grown at the farm university of the Aristotle University of Thessaloniki.

The cuttings were soaked in a fungicide solution (Benlate 3%) for 10 minute. After drying, the basis were refreshed and then dipped for 5 second in indole-3-butyric-acid (IBA) (4000 ppm). Then, the cuttings were placed in rooting trays (60×40×15cm) with peat-moss and perlite as a substrate (1:1 v). The cuttings were maintained under mist for a period of 6 weeks. The rooted cuttings were planted in black plastic bags (two liters volume), and transferred into the greenhouse for acclimatization.

At the beginning of the following growing season (spring 2002), the plants were transplanted into big pots of nine-liter volume. When the plants reached one meter height, two treatments were applied: a) pruning [cut-back at 5 cm height] and b) no pruning [plants with central leader]. All plants were maintained under the same green house meanwhile, the branches were left to grow freely without any further pruning.

At the beginning of the second growing season (spring 2003), the plants were transferred and distributed outdoors using a completely randomized design.

2. Leaf Sampling

All measurements were conducted bi-weekly and/or monthly, on fully-developed leaves taken from the middle region of annual shoots. The leave ages were seven to ten months old.

3. Gas Exchange Measurements

Ten leaves from each treatment were used for measuring the photosynthetic rate (Pn) and stomatal conductance (Gs) during the growing season using a portable IRGA (Li-Cor 6400) device. The measurements were conducted monthly at 8:00 and 14:00 o'clock in the same day.

4. Water Status Measurements

Leaf water potential (Ψ) as well as leaf osmotic potential (π) was measured during the growing season using scholander pressure chamber (Scholander, et al, 1964; and Turner, 1981) and psychrometric techniques (Koide, et al, 1991), respectively. The measurements were conducted twice a month at 8:00 and 14:00 o'clock in the same day. For leaf water potential (Ψ) measurement, 6 replicates were used. At the same time of measuring the leaf water potential, six leaves/treatment were collected, directly wrapped by Parafilm and stored at -20 ^{0}C. After 24 hours, leaf disks (0.38 cm^2) were used from the stored samples for leaf osmotic potential (π) measurements.

Turgor potential (P) was calculated by calculating the difference between leaf water and osmotic potentials ($P = \Psi - \Psi_\pi$).

Additionally, other six leaves/treatment were collected {at the same time of measuring the leaf water potential (Ψ)}. The leaves were weighed (FW) and floated on water for 12 h at a temperature of 4-6 ^{0}C in darkness. The leaves were blotted dry and turgid weight (TW) was

determined. The dry weight (DW) was measured after drying the leaves for 12 h at 80 ^{0}C. RWC was calculated by the formula:

$$RWC = \frac{FW - DW}{TW - DW} \times 100.$$

5. Leaf Morphology and Anatomy

Leaf pieces were fixed for 3 h in 5% glutaraldehyde buffered with 0.025 M sodium phosphate (pH 7.2). Samples were then washed in the respective buffer and post-fixed for 5 h in 1% osmium tetroxide similarly buffered. Tissue dehydration was carried out in an alcohol series followed by infiltration and embedment in Spurr's resin. Sections for light microscopy (1µm thick) were obtained in a Reichert Om U_2 ultramicrotome, stained with 1% toluidine blue O in borax, and examined with a Zeiss III photomicroscope.

Morphometric assessments: The morphometric assessments included thickness of upper epidermis, upper palisade parenchyma, spongy parenchyma, lower palisade parenchyma, lower epidermis, as well as total leaf thickness. In addition, the relative volumes (%) of the leaf histological components were estimated.

The density of non-glandular hairs (= trichomes) on leaf surfaces per leaf mm^2 was also determined.

In addition, leaf area (cm^2) was measured by image analysis, using an Olympus BX 10 TK 1280 E color video camera with a cosmica / pentax 8-48 mmTV zoom lens connected to a quantimet 500MC image processing and analyzing system with associated soft-ware (Leica Cambridge Ltd).

6. Data Analysis

In order to determine the significant differences between treatments, the SPSS statistical package (SPSS for windows, standard version, release 8), including the analysis of variance and *t*-tests were used.

Results

Non-pruned plants (central leader plants) presented lower values of water potential (Ψ) at the same value of relative water content (RWC) compared to pruned plants at 5cm height (Fig. 1). In addition, the non-pruned plants maintained higher turgor potential for the same values of RWC ranging between 0.8 - 0.90 (Fig. 2).

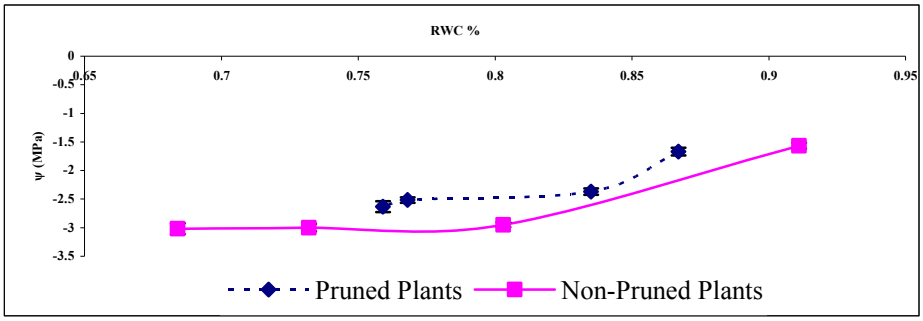

Figure (1): Changes of leaf water potential (Ψ) in relation to relative water content (RWC) in leaves of non-pruned and pruned plants. Bars represent the standard error of the mean of six replicates. (Figure with no visible error bars condenses very small standard error).

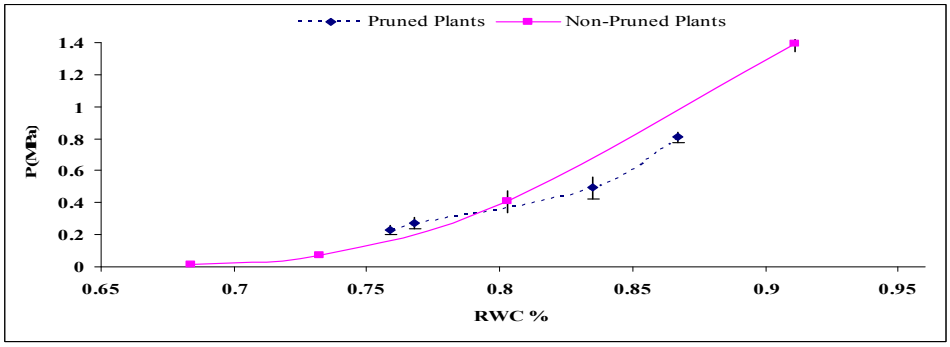

Figure (2): Relationship between leaf turgor potential (P) and relative water content (RWC) in leaves of non-pruned and pruned plants. Bars represent the standard error of the mean of six replicates. (Figure with no visible error bars condenses very small standard error).

The relation between the diurnal photosynthetic rate (Pn) and water potential indicated that, at lower values of Ψ, leaves of the non-pruned plants showed higher photosynthetic rate (Fig. 3) and higher values of stomatal conductance (Fig. 4) compared to those of the pruned plants.

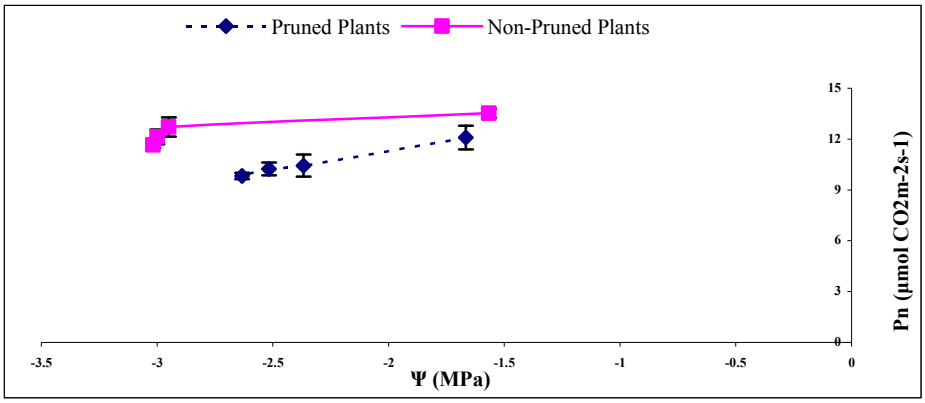

Figure (3): Changes of leaf water potential (Ψ) in relation to photosynthetic rate (Pn, µmol $CO_2m^{-2}s^{-1}$) in leaves of non-pruned and pruned plants. Bars represent the standard error of the mean of ten replicates.

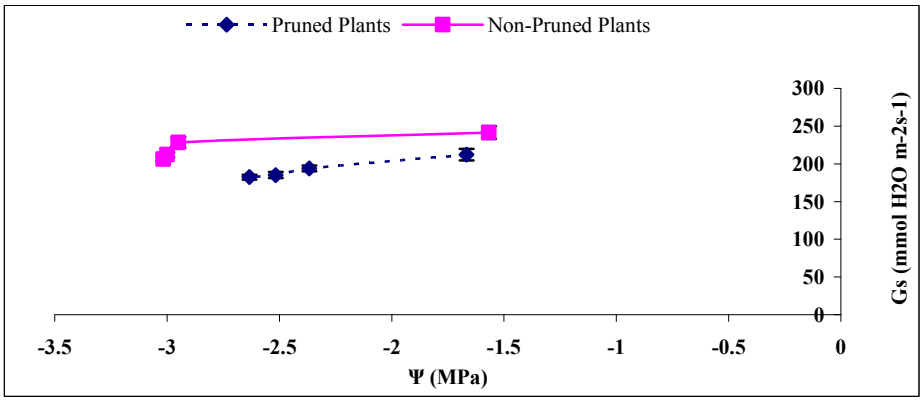

Figure (4): Changes of leaf water potential (Ψ) in relation to stomatal conductance (Gs, mmol $H_2O\ m^{-2}s^{-1}$) in leaves of non-pruned and pruned plants. Bars represent the standard error of the mean of ten replicates.

The seasonal changes of the photosynthetic rate and the stomatal conductance indicated higher values of non-pruned plants over all the growing season (Fig. 5 and 6).

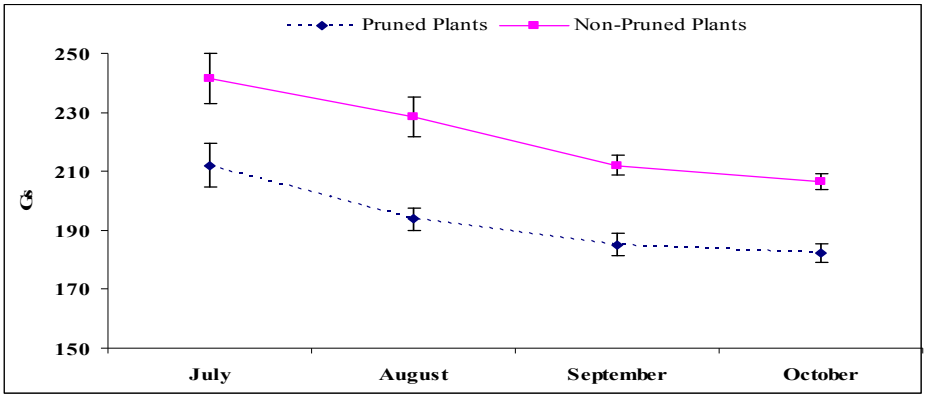

Figure (5): Seasonal changes in stomatal conductance (mmol $H_2O\ m^{-2}s^{-1}$) in leaves of non-pruned and pruned plants. Bars represent the standard error of the mean of 10 replicates.

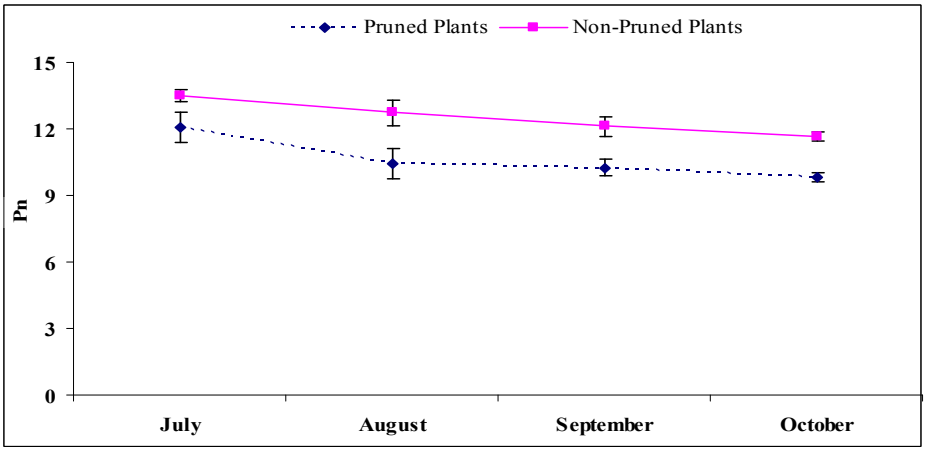

Figure (6): Seasonal changes in photosynthetic rate (µmol $CO_2m^{-2}s^{-1}$) in leaves of non-pruned and pruned plants. Bars represent the standard error of the mean of 10 replicates.

Cross section of leaves of non-pruned plants (Fig. 7A) showed that the palisade parenchyma of the mesophyll was composed of two parts, one being in contact with the upper epidermis and the other with the lower one. The upper palisade parenchyma was consisted of three to four layers relatively longer, thinner and more densely arranged cells. The lower palisade parenchyma had only one layer, short, not well distributed and with loosely arranged cells (Fig. 7A). The spongy parenchyma was located between the two palisade parenchymas and it contained large intercellular spaces. Between the upper palisade parenchyma and the spongy parenchyma, small vascular bundles occurred.

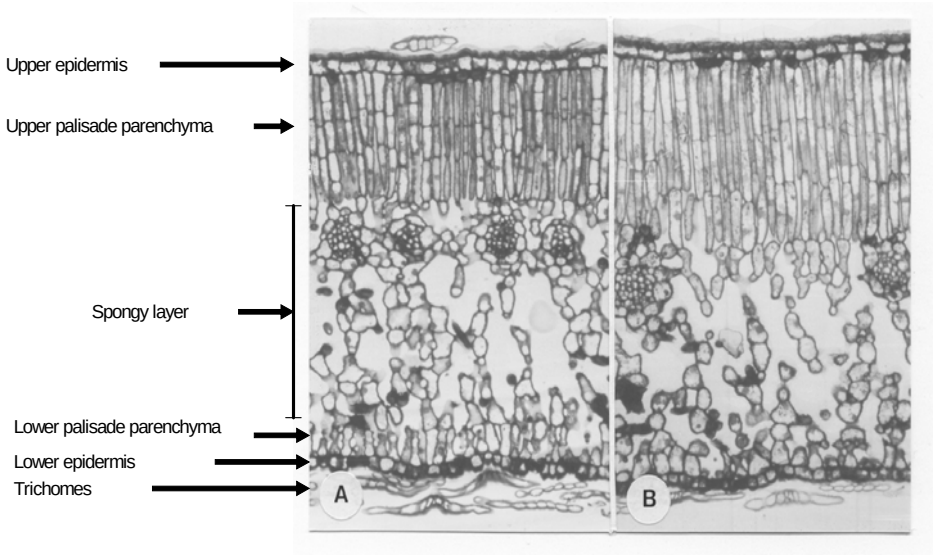

Figure (7): Comparative leaf anatomy in blade cross-sections of olive leaves.

A) Leaf of non-pruned plants (Central leader plants), and

B) Leaf of plants pruned at 5cm height. Magnification = X 170.

The leaf section of pruned plants showed that, the upper palisade parenchyma cells were larger and not densely arranged (Fig. 7B). And, its thickness as well as total leaf thickness was greater than that of non-pruned plants (Table 1).

Table (1): Thickness (μm), relative volume (%) and non-glandular hair density (No./mm^2) of the leaf histological components in two treatments of olive plants (n=9; ± SD, standard deviation; *, **, ***, significant differences at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively).

Leaf anatomical parameters	Treatments	
	Non-pruned plants (Central leader Plants)	Pruned plants (at 5 cm height)
1. Thickness:		
Upper epidermis	20.87 ± 0.92	18.53 ± 1.13***
Upper palisade parenchyma	141.38 ± 4.14	206.70 ± 7.01***
Spongy parenchyma	245.05 ± 4.08	216.45 ± 9.59***
Lower palisade parenchyma	30.94 ± 4.94	22.43 ± 5.84**
Lower epidermis	15.99 ± 1.34	16.51 ± 2.46
Total thickness (without hairs)	454.22 ± 3.68	480.61 ± 6.68***
2. Relative volume:		
Upper epidermis	4.02 ± 0.62	4.19 ± 0.74
Upper palisade parenchyma	31.30 ± 1.31	41.51 ± 0.86***
Spongy parenchyma	52.54 ± 1.75	45.17 ± 1.15***
Lower palisade parenchyma	8.02 ± 1.37	5.42 ± 1.64**
Lower epidermis	4.11 ± 0.72	3.70 ± 0.70
3. Non-glandular hair density.	229.87 ± 25.47	97.40 ± 22.63***

The cells of the spongy parenchyma and lower palisade parenchyma are much thicker in leaves of the non-pruned plants compared to those of pruned plants.

The vascular bundles of the non-pruned plants are small, uniform in size and more densely arranged (8 bundles/leaf section) compared to those of the pruned plants (3 bundles/leaf section). (Fig. 7A and B respectively).

The morphometric assessments of the relative volumes (%) and air space sizes showed that the total volume of the spongy layer was increased by 52.54% in the leaves of the non-pruned plants compared with 45.17% in the leaves of pruned plants. In addition, lower palisade parenchyma cells were also increased by 8.02% in the leaves of the non-pruned plants compared to 5.42% in the leaves of pruned plants (Table 1).

With respect to hairs, it was observed that the non-glandular hair density (No/mm^2) in the lower leaf surface was significantly higher in the non-pruned plants (Fig. 7A). Thus, the leaves of non-pruned plants became much more hairy than those of the pruned plants (Table 1).

Morphologically, the non-pruned plants appeared to have significantly larger leaf surface (6.26cm^2) compared to pruned plants exhibited (4.50 cm^2).

Discussion

The lower values of leaf water potential (Ψ) at the same values of the relative water content (RWC) in the non-pruned plants compared to those pruned plants would be apparently attributed to the lower values of osmotic potential. Similar results concerning the ability of olive tree for osmotic adjustment was also reported by Xiloyiannis, et al, 1988 and Chartzoulakis, et al, 1999. This osmotic adjustment suggests that turgor potential of non-pruned plants should be higher compared with those of pruned plants a fact that indeed is occurred.

This decrease of osmotic potential in response to water loss is a well-known mechanism by which many plants adjust to drought condition (Dichio, et al, 1997). If it is accepted, the osmotic adjustment in olive trees implies the accumulation of the net solutes especially from organic solutes that produced in photosynthesis (Chartzoulakis, et al, 2002). Thus, the higher ability of the non-pruned plants for osmotic adjustment

might explain the higher values of photosynthetic rate and stomatal conductance exhibited in the non-pruned plants at the same values of Ψ compared to the pruned plants.

The differences in photosynthetic rate between the two treatments could be attributed either to the stomatal conductance and/or to the differences in the leaf morphological and anatomical characteristics. Indeed the higher stomatal conductance observed in the non-pruned plants during the growing season could probably explain the higher photosynthetic rate compared to the pruned plants. This is in agreement with the findings of Chartzoulakis, et al, 2002.

On the other hand, it was expected that the non-pruned plants would exhibit higher relative volume of palisade parenchyma and palisade thickness where the photosynthetic takes place, explaining its higher rates. However, this was not evident in our results where the pruned plants presented higher values of the palisade parenchyma in terms of relative volumes and thickness. Therefore, it is assumed that the higher photosynthetic rate in the non-pruned plants might be probably attributed to the higher relative volume of spongy cells, spongy thickness and a significant higher number of vascular bundles per section, which could facilitate the CO_2 diffusion from the stomatal cavity into the carboxylation sites in the chloroplasts on other hand (Evans, et al, 1994; Syvertsen, et al, 1995; and Chartzoulakis, et al, 1999).

The high number of the vascular bundles observed in the leaves of non-pruned plants indicated a more efficient translocation system for the photosynthetic products from the leaves that might increase thereby the photosynthetic rate. The lower number of the vascular bundles in the pruned plants might lead to organic solutes accumulation around the bundles resulting in blocking the pathways and thus decreasing the photosynthetic rate according to the theory of Hay and Walker, 1989.

Furthermore, leaves of the non-pruned plants tend to produce several and denser layers of palisade parenchyma thereby the number of the chloroplast where the actual photosynthesis takes place increases. This could also facilitate the isotropic distribution of collimated light into the

leaf and also distribute light energy evenly throughout the mesophyll allowing in consequence more uniform rate of photosynthesis (Dunahue, 1991; Vogelmann, 1994; and Klich, 2000). Additionally, the denser layers of the palisade parenchyma implied greater chlorenchymatic biomass and thus more efficient photosynthesis (Kofidis, et al, 2003). On the other hand, the elongated cells of the palisade parenchyma and the higher leaf thickness in the leaves of pruned plants could be explained by the increase of the cell division at the palisade and/or cells elongation accompanied by decreased cell division and/or expansion of the epidermal cells (Pemberton, 1989).

The increasing of non-glandular hair density on the lower leaf surface of non-pruned plants (external blocking of water vapour movement) and the reduction of the mesophyll intercellular air space volume (internal blocking of water vapour movement) could also be considered as important anatomical features developed by olive leaves which could probably explain the lower values of transpiration for the same values of stomatal conductance observed in non-pruned plants (Bosabalidis and Kofidis, 2002).

Furthermore, the higher values of the leaf area observed in the leaves of non-pruned plants seems to be well associated with the need for larger surface area for gas exchange (for absorption of light and CO_2) (Boeger and Poulson, 2003). The smaller and thicker leaves in the pruned plants could be recognized as a mechanism of water conservation (Taiz and Zeiger, 1998). These differences in the anatomical characteristics in the non-pruned plants compared to the pruned plants were considered as structural mechanisms that increase photosynthesis rate.

In conclusion, it seems that pruning is causing the production of smaller but thicker leaves with lower hair density, promoting cell expansion particularly leaf palisade parenchyma cells and exhibiting lower photosynthetic rate compared to non-pruned plants. In contrary, non-pruned plants presented higher values of photosynthetic rate, which could probably be attributed to the contribution of both mechanisms; stomatal conductance and differences in anatomical features.

Furthermore, the relative contribution of the above mentioned mechanisms are needed to be clarified.

References

- Boeger, M.R. & Poulson, M.E. (2003). "Morphological adaptation and photosynthetic rates of amphibious *Veronica anagallis-aquatica* L. (Scrophulariaceae) under different flow regimes". Aquatic Botany. (75). 123-135.
- Bosabalidis, A.M. & Kofidis, G. (2002). "Comparative effects of drought stress on leaf anatomy of two olive cultivars". Plant Science. (163). 375-379.
- Chartzoulakis, K. Patakas, A. & Bosabalidis, A.M. (1999). "Changes in water relations, photosynthesis and leaf anatomy induced by intermittent drought in two olive cultivars". Environmental and Experimental Botany. (42). 113-120.
- Chartzoulakis, K. Patakas, A. Kofidis, G. Bosabalidis, A.M. & Natsou, A. (2002). "Water stress affects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars". Scientia Horticulturae. (95). 39-50.
- Dichio, B. Nuzzo, V. Xiloyiannis, C. & Angelopulos, K. (1997). "Drought stress-induced variation of pressure-volume relationships in *Olea europaea* L. cv. Coratina". Acta Horticulturae. (449). 401-409.
- Di marko, L. Giovannini. D. Marra, F.P. & Viglianisi, G. (1990). "Reproductive and vegetative behavior of four table-olive cvs". Acta Horticulturae. (187). 187-190.
- Dunahue, R.A. (1991). "Interactions of ambient light directionality with leaf anatomy and effects on photosynthesis of Sun and Shade leaf of *Thermopsis Montana"*. Unpublished Ph.D. thesis. University of Wyoming. Laramie. USA.
- Evans, J.R. Von Caemmerrer, S. Setchell, B.A. & Hudson, GS. (1994). "The relationship between CO2 transfer conductance and leaf

anatomy in transgenic tobacco with a reduced content of Rubisco". Australian Journal of Plant Physiology. (21). 4754-4795.

- Ferrara, E. Lorusso, G. & Lamparelli, F. (1999). "A study of floral biology and the technological features of seven olive cvs of different origins". Acta Horticulturae. (474). 279-284.
- Garcia, J.L. Avidan, N. Troncoso, A. Sarmiento, R. & Lavee, S. (1999). "Possible juvenile-related proteins in olive tree tissues". Scientia Horticulturae. (85). 271-284.
- Gupta, P.K. Timmis, R. & Mascarenhas, A.F. (1990). "Field performance of micro-propagated forestry species". In vitro Cellular and Developmental Biology-Plant. (27). 159-164.
- Hackett, W.P. (1985). "Juvenility, maturation and rejuvenation in woody plants". Horticultural Review. (7). 109-155.
- Hackett, W.P. & Murray, J.R. (1993). "Maturation and rejuvenation in woody species". In: Ahuja M.R. (ed.). Micropropagation in woody plants. Kluwer Academic Publishers. Dordrecht, Netherlands. pp. 93-105.
- Hay, R. & Walker, A. (1989). "Dry mater partitioning". In: Wiley J. & Sons (eds.). An introduction to the physiology of crop yield. Longman group. New York. USA. pp. 107-153.
- Klich, M.G. (2000). "Leaf variation in *Elaeagnus angustifolia* related to environmental heterogeneity". Environmental and Experimental Botany. (44). 171-183.
- Kofidis, G. Bosabalidis, A.M. & Moustakas, M. (2003). "Contemporary seasonal and altitudinal variations of leaf structural features in oregano *(Origanum vulgare* L.)". Annals of Botany. (92). 635-645.
- Koide, R.T. Robichaux, R.H. Morse, S.R. and Smith, C.M. (1991). "Plant water status hydraulic resistance and capacitance". In: Pearcy, R.W. Ehleringer, J. Moony, H.A. Pandel, P.W. (eds.). Physiological Ecology. Chapman and Hall. London. UK. pp. 161-187.

- Lavee, S. Avidan, N. Haskal, A. & Ordovich, A. (1996). "Juvenility period reduction in olive seedlings. A tool for enhancement of breeding". Olivae. (60). 33-41.
- Meilan, R. (1997). "Floral induction in woody angiosperms". New Forestry. (14). 179-202.
- Murray, J.R. Smith, A.G. & Hackett, W.P. (1994). "Differential dihydroflavonol reductase transcription and anthocyanin pigmentation in the juvenile and mature phases of ivy (*Hedera helix* L.)". Planta. (194). 102-109.
- Pemberton, H.B. Starman, T.W. & Kelly, T.W. (1989). "Influence of gibberellin GA_3 and ancymidol on sunflower leaf anatomy". Canadian Journal of Botany. (68). 159-162
- Scholander, P.F. Hammel, H.T. Hemmingsen, E.A. & Bradstreet, (Eds.) (1964). "Hydrostatic pressure and osmotic potentials in leaves of mangroves and some other plants". In: Proceedings of the National Academy of Sciences. USA. (55). 119-125.
- Syversten, J.F. Lioyd, J. McConchie, C. Kriedemann, P.E. & Farquhar, G.D. (1995). "On the relationship between leaf anatomy and CO_2 diffusion through the mesophyll of hypostomatous leaf". Plant Cell Environment. (18). 149-157.
- Taiz, L. & Zeiger, E. (1998). "Plant physiology". Sinauer Associaties. Sunderland. USA.
- Turner, N.C. (1981). "Techniques and experimental approaches for the measurements of plant water status". Plant Soil. (58). 339-366.
- Visser, T. (1965). "On the inheritance of the juvenile period in apple". Euphytica. (14). 125-134.
- Vogelmann, T.C. (1994). "Light within the plant". In: Kendrick, R.E, Kronenberg G.H.M. (eds.). Photomorphogenesis in plants. Kluwer Academic Publishers. Doredrecht. Netherlands.
- Xiloyiannis, C. Pezzaroza, B. Jorba, J. & Angelini, P. (1988). "Effect of soil water content on gas exchange in olive stress". Advances in Horticultural Science. (2). 58-63.