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Short note

Environmental control of CO_2 assimilation rates and stomatal conductance in five oak species growing under field conditions in Greece

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Summary — The present study compared CO_2 assimilation rate and stomatal conductance of five oak species from the beginning of May through November 1993 under similar natural conditions. Gas exchange, leaf characteristics and water status were measured on 30-year-old *Quercus ilex*, *Q macrolepis*, *Q pedunculiflora*, *Q pubescens* and *Q trojana* growing in experimental plots in northern Greece. The seasonal pattern of assimilation rates was similar for all study species but differences occurred between the species. After initial leaf expansion, all species rapidly developed high photosynthetic rates. In addition, assimilation rates were high in all species in May and November after rain events. No significant differences in stomatal conductance were observed among species during the growing season. The relationships between assimilation rate and stomatal conductance displayed variation in the slopes among species and months.

***Quercus* sp / assimilation rate / stomatal conductance / seasonality / drought**

Résumé — Effets de l'environnement sur l'assimilation nette de CO_2 et la conductance stomatique de cinq espèces de chênes en plantation en Grèce. *Cette étude a permis la comparaison des niveaux d'assimilation nette de CO_2 et de conductance stomatique de cinq espèces de chênes en plantation comparative dans le nord de la Grèce, au cours d'une saison de végétation (mai–novembre 1993). Les échanges gazeux, les caractéristiques foliaires et l'état hydrique ont été déterminés sur des arbres de 30 ans de* Quercus ilex, Q macrolepis, Q pedunculiflora, Q pubescens *et* Q trojana. *La dynamique saisonnière d'assimilation nette était très semblable entre espèces. Des différences significatives sont apparues à certains moments. Après la phase d'expansion initiale des feuilles, toutes les espèces présentaient de fortes valeurs d'assimilation, de même que pendant les périodes pluvieuses en mai et en novembre. Peu de différences interspécifiques de conductance stomatiques ont été détectées au cours de la saison. Cependant, les relations entre conductance stomatique et assimilation nette ont fortement différé suivant les espèces et les périodes de mesure.*

Quercus ***/ assimilation nette de CO_2 / conductance stomatique / variations saisonnières / sécheresse***

INTRODUCTION

Oak forests in Greek cover about 23% of the total forest area. Oak species occurring in Greece are generally considered to be drought resistant and suitable for forest regeneration in the Mediterranean zone characterized by frequent summer droughts.

Drought adaptation has been shown to govern species distribution (Bahari et al, 1985; Dafis, 1986). Drought is the result of a combination of environmental factors including low precipitation, irregular rainfall distribution, low air humidity and high air temperature. Limited soil moisture, high evaporation demand and high temperature can have negative effects on gas exchange. The knowledge of how environmental conditions influence gas exchange may improve the understanding of oak species habitat within the Mediterranean forest complex. There is a lack of knowledge about the physiological features of drought adaptation in Greek oak species.

Eleven oak species occur in Greece. They grow in habitats widely differing in extent of drought. The following rating of drought tolerance based on species distribution has been suggested (Athanasiadis, 1986; Dafis, 1986): *Quercus pedunculiflora* (least tolerant) < *Q ilex* < *Q trojana* < *Q macrolepis* < *Q pubescens* (most tolerant). However, these apparent differences in drought tolerance have not yet been confirmed by comparative studies on trees growing in natural conditions.

The present study was undertaken to identify differences in drought adaptation between these species. Stomatal conductance and CO_2 assimilation behavior were compared.

MATERIALS AND METHODS

Study area

The experimental area is located near the Forest Research Institute of Thessaloniki (latitude 40°35"N , longitude 22°58"E, altitude 10 m). The area consists of experimental plots of the following species: *Quercus ilex* L, *Q macrolepis*, Kotschy, *Q pedunculiflora* K Koch, *Q pubescens* Willd and *Q trojana* Webb. Each plot occupies an area of 1 550 m^2 (45 x 30 m), the planting space was 3 x 3 m and had been planted 30 years before the study (table I). In each plot, nine dominant trees were selected for further measurements.

The area displays an Emberger coefficient of 50.8 and the climate is characterized as a semiarid Mediterranean bioclimate with dry and hot summers and cold winters (Mauromatis, 1980). The area belongs to the *Ostryo-carpinion* vegetation zone (Dafis, 1973). The study was conducted at a site where *Q pubescens* would occur naturally.

The mean annual precipitation is 409 mm (range 211–704 mm) for the 30 year period. The summer dry period begins in June and lasts until October. The driest months are August and September with a mean monthly precipitation of 22 and 21 mm, respectively. A weather station, located at the study site, was used to record hourly the average of the following parameters:

Table I. Main characteristics of the study stands.

	Q ilex	Q macrolepis	Q pendunculiflora	Q pubescens	Q trojana
Years	30	30	30	30	30
Mean height (m)	4.32 (0.50)	8.2 (0.72)	9.34 (0.80)	8.4 (0.60)	7.96 (0.55)
Stand density	70%	72%	75%	71%	73%

total incoming radiation, air temperature, relative humidity, precipitation, barometric pressure, wind speed and wind direction.

The soil belongs to soil brun Mediterranean type and consists of sand and clay with pH(H_2O) 7.7 in the surface layer and 8.0 in deeper layers. The texture varies between clay and loam.

Leaf water status

Leaf (xylem) water potential (Ψ) was measured with a pressure chamber on two or three leaves per sampled tree, harvested between 1130 and 1400 hours. The sample leaves were enclosed in plastic sheaths immediately before cutting (Turner, 1981).

Gas exchange

Gas exchange measurements were made using a portable closed-loop photosynthesis system (Li 6200, Li-Cor, Inc, Lincoln, NE, USA) with a 1/4 L leaf cuvette (Li-6200-13). The leaf was enclosed in the cuvette for 12–45 s with each measurement of gas exchange. Photosynthetically active radiation (PAR), air and leaf temperature, and relative air humidity were also recorded. Net CO_2 assimilation rates and stomatal conductance were estimated on a project area basis. Gas exchange measurements were made on two mid-canopy, south-facing branches on each of the nine selected trees per examined plot. The same branches were used throughout the growing season. Measurements were taken in three different positions on each selected branch on one or two fully expanded leaves from the upper canopy. Days with no clouds occurred; consequently, there were no values of PAR below 700 μmol m^{-2} s^{-1}. Measurements were made at seven different times during the growing period, which started in May and ended in November 1993.

Data analysis

Analysis of variance and the Duncan test were used. Average standard error was calculated from the residual mean square from the analysis of variance. The relationship between the assimilation rate (*A*) and stomatal conductance (g_s) was also subjected to regression analysis. Mean values per selected tree were used. Differences between slopes of regression lines were tested according to Yates (1982).

RESULTS AND DISCUSSION

Weather conditions during the measured period

Seasonal changes in daily air temperature (mean, minimum and maximum) are shown in figure 1a. Consistently high temperatures were recorded in July and August. Similar to temperature, relative humidity fluctuated during the experimental period; however, the highest values were noted in April and November (fig 1b). Rainfall occurred until mid-June, after which there was almost no rain until the end of October; during this period, soil moisture declined.

The weather conditions during the study period differed from the long-term average

Table II. Monthly rainfalls.

	May	*June*	*July*	*Aug*	*Sept*	*Oct*	*Nov*	*Total*
Mean average for last 30 years	40	37	30	22	21	38	64	252
for 1993	84	28	4	5	4	7	31	163

(table II). Except in May when rainfall was high, the other months were much drier than the average. The total rainfall between 1 July and 26 October 1993 was 13 mm while the normal rainfall for this period is 111 mm.

Plant water status

Figure 2a shows the seasonal progression of midday water potential (Ψ) of the five study species. There were no significant differences in Ψ between the species during

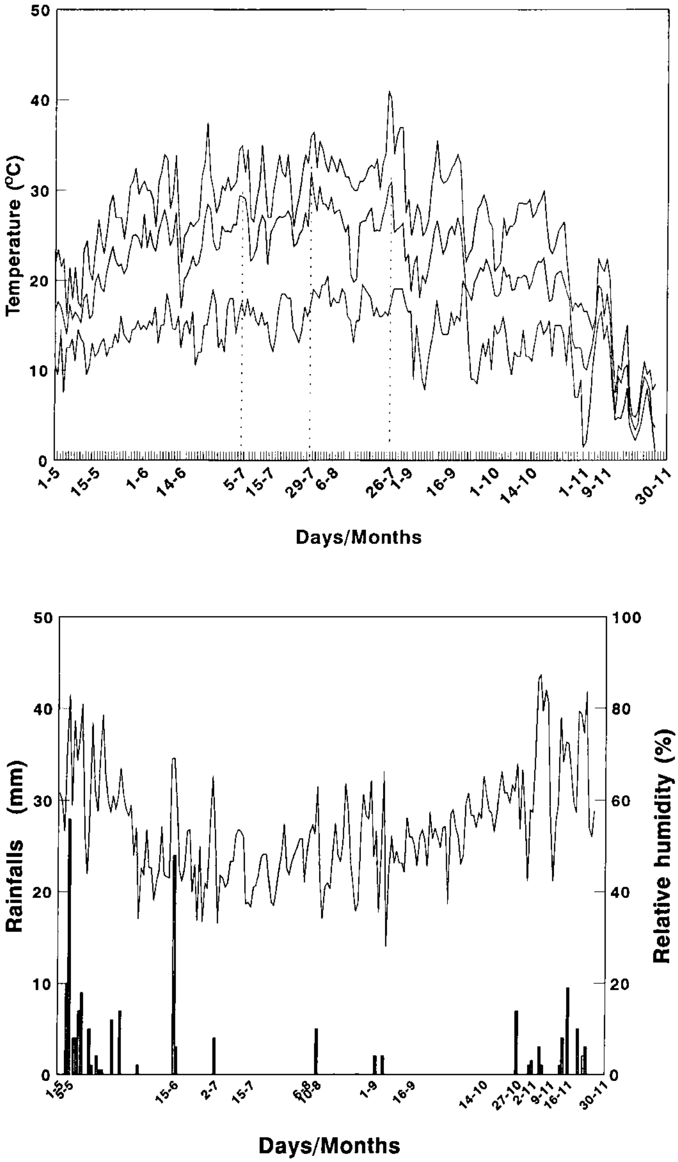

Fig 1. Seasonal changes in mean daily and maximum and minimum air temperature (**a**), in relative humidity and daily precipitation (**b**) represented by bars, recorded at climatic station which is located 300 m from the study experimental plots.

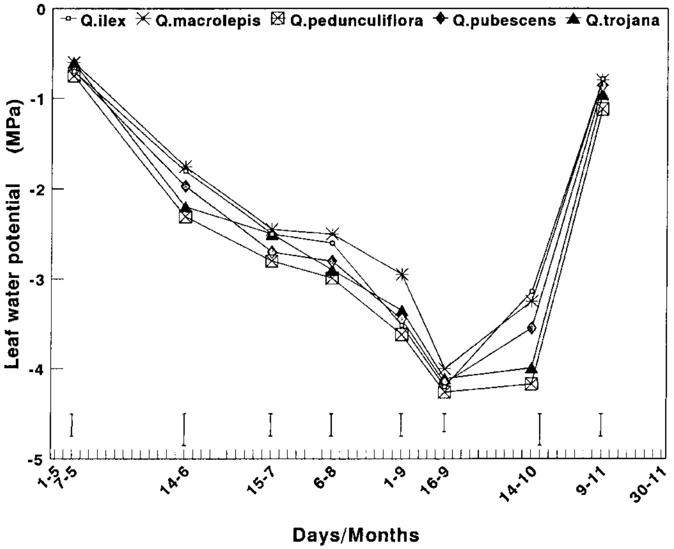

Fig 2. Seasonal variation in leaf water potential of five oak species. Vertical bars indicate ± SEM.

the measured period. Lowest values of Ψ were consistently recorded in *Q pedunculiflora*. Values of Ψ at the beginning and end of the study period were high (between −0.5 and −1.0 MPa) and these high values coincided with relatively high soil moisture. Values of Ψ declined rapidly from early June and reached values less than −4 MPa in mid-September. The minimum values of Ψ that appear in the five study species seem to be comparable to those of other *Quercus* sp growing under water stress. Values of −3.5, −2.8 and −3.4 MPa, respectively, were recorded for *Q suber*, *Q coccifera* and *Q petraea* (Hinckley et al, 1983; Tenhunen et al, 1984; Rhizopoulou and Mitrakos, 1990; Cochard et al, 1992). The Ψ values such as those reported here are easily found also in the literature for other trees of the Mediterranean ecosystem (Nunes et al, 1992). The capacity of many species to develop very low leaf water potential is well recognized (Sobrado, 1986) and all the studied oaks belong to these species.

Phenological data

According to table III, new leaves appeared approximately on 11 April. The leaves com-

Table III. Leaf area/leaf (cm^2).

	11 Apr	*27 Apr*	*6 May*	*27 May*	*16 June*	*22 June*	*15 July*
Q ilex	4.34	4.55	4.57	4.85	4.8	4.87	4.9
Q macrolepis	4.57	14.27	15.24	17.77	17.83	17.91	17.95
Q pendunculiflora	8.3	22.74	29.09	33.89	35.56	37.14	35.34
Q pubescens	1.09	15.93	11.54	18.82	19.38	21.33	22.05
Q trojana	1.74	8.78	10.1	10.73	11.84	11.84	11.95
SEM	0.41	0.91	0.73	0.75	0.8	0.79	0.76

pleted development around 22 June. On 7 May, the leaves were 88, 79, 51 and 88% of the full expanded size for *Q macrolepis*, *Q pedunculiflora*, *Q pubescens* and *Q trojana*, respectively. All species develop early and quickly the final leaf area.

Seasonal trends in assimilation rate

The seasonal pattern of net assimilation (*A*) was similar in all study species (fig 3a). During May under high soil moisture conditions, the assimilation rates at midday were high-

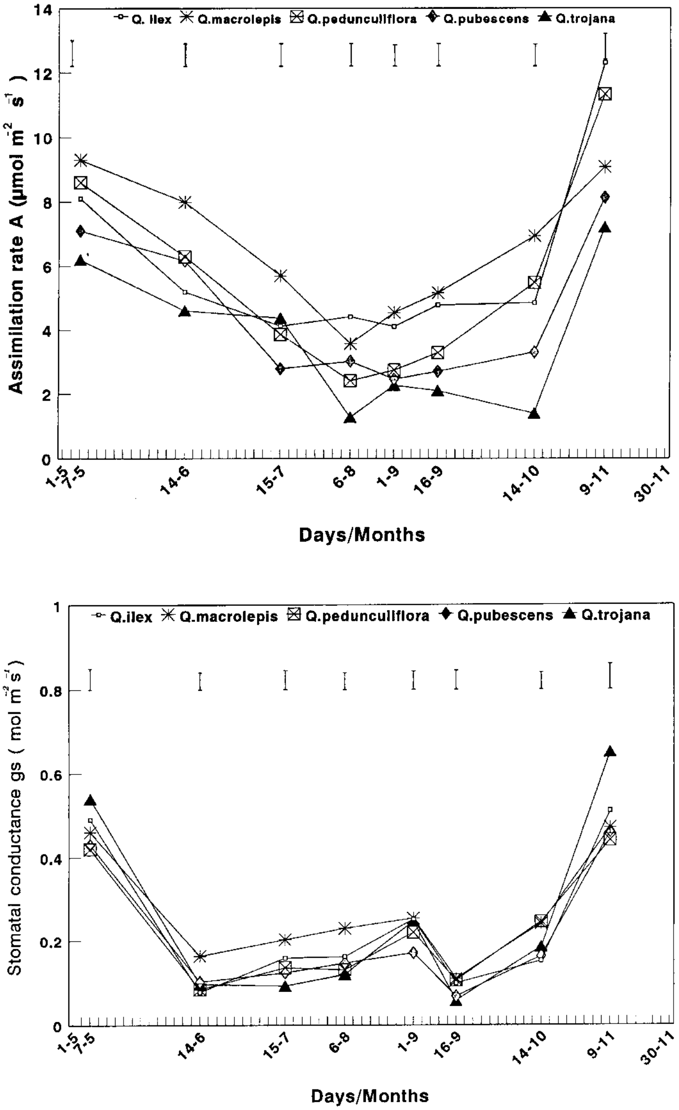

Fig 3. Seasonal variation in assimilation rate *(A)* (**a**) and in stomatal conductance (g_s) (**b**) of five oak species. Vertical bars indicate ± SEM. Photosynthetic active radiation ranged between 700 and 1 400 µmol m^{-2} s^{-1}, leaf temperature 26 to 43 a and relative humidity 30 to 60%.

est in all species and particularly for *Q macrolepis*. As the soil dried, the assimilation rate decreased in all species. As early as 14 June, when Ψ was around –2 MPa, photosynthesis had declined in all species. *Q macrolepis* had the highest assimilation rate and *Q trojana* the lowest. By 9 August, the lowest *A* values were either reached or approached. This was a month before the lowest Ψ values were observed. On 9 August, *Q ilex* had the highest *A* values and *Q trojana* the lowest.

Photosynthetic rates recovered in all species by early November after the drought had ended. *Q ilex*, *Q pedunculiflora* and *Q pubescens* had the highest values and *Q trojana* the lowest. In general, the highest values of *A* were observed during early November. Leaves were fully mature and the microclimate within the cuvette was less stressful in November versus early June (data not shown). As noted by Dougherty and Hinckley (1981), the maintenance of the high photosynthetic potential for most of an oak leaf's life is important. Consequently, the ability of oak species to keep a high photosynthetic rate in November is advantageous for tree growth.

All study species developed rapid high photosynthetic rates. On 7 May, the leaves were not fully enlarged, the assimilation rates of which were high. Others have noted that *A* in deciduous leaves approaches maximum values before leaves are fully expanded (Dougherty et al, 1979). The early development of photosynthetic capacity is especially advantageous for tree growth in a Mediterranean climate where the drought starts early. It was reported for *Robinia pseudocacia* that the maximum photosynthetic rate was achieved 20–39 days after leaf emergence (Medrahtu and Hanover, 1991). Early development of high photosynthetic rate was reported for conifers such as *Pinus taeda* (Radoglou and Teskey, 1993). This behavior also characterizes fast growing species and species growing in warm climates.

The recorded values were found to be in the range reported for oak species (Tenhunen et al, 1987a; Ceulemans and Saugier, 1991). The potential rates of net CO_2 assimilation of oak leaves were probably much higher than those reported here. Such values may not be reached under all conditions. High photosynthetic rates for deciduous and evergreen sclerophyllous oak species were also reported (Dreyer et al, 1992; Epron and Dreyer, 1993). It is well known that drought does not only decrease *A* and g_s but also changes the diurnal pattern of gas exchange (Tenhunen et al, 1987b). Higher rates of assimilation than those reported here may therefore be reached, particularly in the morning.

Seasonal trends in stomatal conductance

The seasonal variation of stomatal conductance was similar for all study species (fig 3b). There were no significant differences in g_s during the growing season between study species, except in November when *Q trojana* had high values. Maximum leaf conductance was reached in May and November under high air humidity and soil moisture conditions. Throughout the season, low values of g_s appeared. Values of recorded g_s were similar to those reported for leaves from other *Quercus* species (Ni and Pallardy, 1991) and for *Ceratonia siliqua* in the Mediterranean area (Nunes et al, 1992). This has been observed for five different oak species of the *robur* section (*Q robur*, *Q petraea*, *Q pubescens*, *Q pyrenaica* and *Q canariensis*), which submitted to drought in parallel; the decline in g_s was almost the same magnitude and precocity (Dreyer et al, 1993).

Assimilation in relation to stomatal conductance

The ratio of CO_2 assimilation rate to stomatal conductance (A/g_s) is a major determinant of instaneous water use efficiency of plants. The A/g_s ratio has been shown to be under close physiological regulation and found to differ considerably among forest tree genotypes (Farquhar et al, 1989; Guehl et al, 1991). These differences may be associated with differential drought adaptation and consequently may have great ecological significance (Schulze, 1986).

Linear regressions applied to all the values reported, means for each selected tree for all study species and measured days. The relationship between A rates and g_s from June to November for *Q macrolepis*

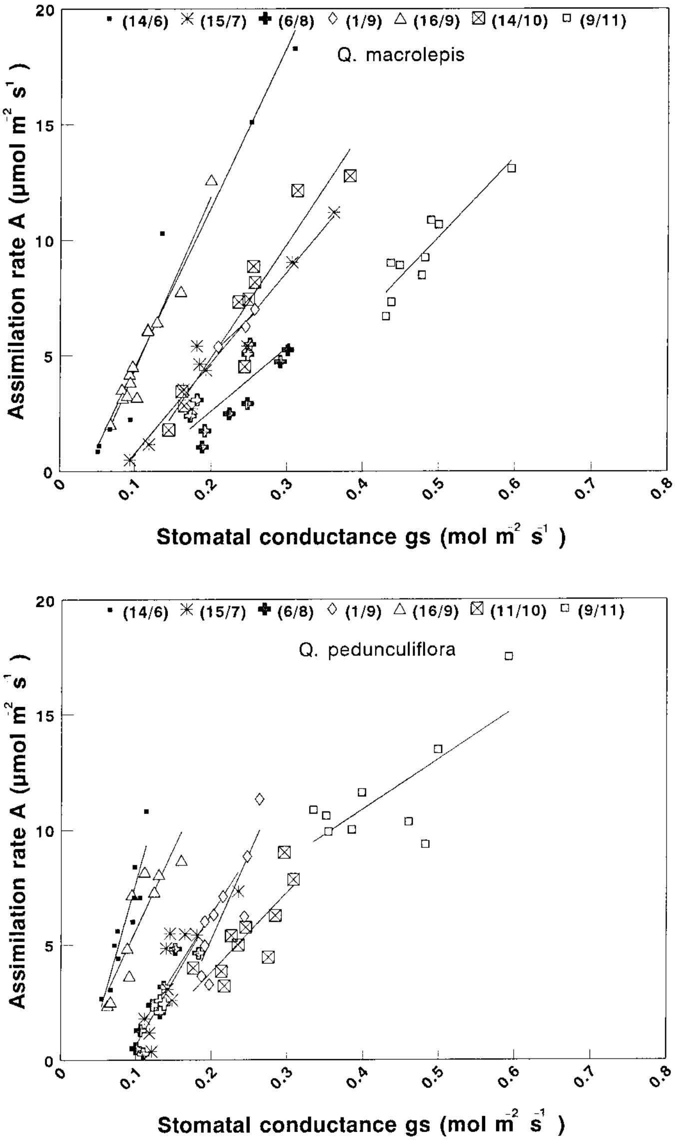

Fig 4. Relationship between stomatal conductance (g_s) and net photosynthesis (A) of *Q macrolepis* (**a**) and *Q pedunculiflora* (**b**) throughout the growing season between 1000 and 1300 hours under steady-state condition, with irradiance higher than 700 µmol m^{-2} s^{-1}. Data points represent different trees.

Table IV. Sensitivity of A to g_s (µmol m^{-2} s^{-1})/(mmol m^{-2} s^{-1}).

	14 June	*15 July*	*6 Aug*	*1 Sept*	*16 Sept*	*14 Oct*	*9 Nov*
Q ilex	68.653 (0.97)	37.065 (0.93)	31.772 (0.94)	30.652 (0.93)	106.93 (0.87)	35.69 (0.92)	21.428 (0.89)
Q macrolepsis	69.663 (0.98)	39.433 (0.97)	27.787 (0.79)	31.149 (0.97)	75.952 (0.97)	49.576 (0.94)	34.783 (0.91)
Q pendunculiflora	118.648 (0.92)	52.096 (0.84)	56.937 (0.91)	74.978 (0.85)	70.568 (0.88)	34.699 (0.80)	21.741 (0.73)
Q pubescens	100.53 (0.98)	64.817 (0.91)	81.55 (0.84)	31.673 (0.83)	92.306 (0.87)	52.545 (0.95)	30.86 (0.91)
Q trojana	65.872 (0.92)	32.808 (0.84)	60.14 (0.91)	34.049 (0.85)	99.89 (0.88)	30.173 (0.80)	15.974 (0.73)
SEM	5.9	5.1	4.3	4.1	7.3	5.2	3.6

and *Q pedunculiflora* appears in figure 4a, b. Table IV shows the slopes of linear regressions of the relationship between A and g_s. The initial slope was different between species and during the growing season. Water use efficiency (WUE) increased as water stress was induced in the trees. *Q macrolepis* experienced the least amount of changes of the slope. Trees of *Q macrolepis* kept their stomata significantly open even at low soil water availability, low air humidity and low WUE. In other cases, the slope of *Q pedunculiflora* and *Q ilex* greatly increased and presented higher WUE. In all study species, the water use efficiency was not maintained constant during the growing period and the g_s was more affected by environmental changes than A.

The rating according to the maximum value of the slope was *Q pedunculiflora* < *Q ilex* < *Q trojana* < *Q pubescens* < *Q macrolepis.* The results were consistent with those reported for other *Quercus* species (Delucia and Heckathorn, 1989; Ni and Pallardy, 1991); for *Artemisia tridenteta*, a desert shrub; and for *Pinus ponterosa*, a more mesic species. High WUE and an inconstant state were also observed in xeric plants in other studies (Levitt, 1980; Schulze and Hall 1982; Field et al, 1983).

REFERENCES

Athanasiadis N (1986) *Forest Botany (Trees and Bushes of the Forest in Greece).* Univ of Thessaloniki, Thessaloniki, Greece, 70-91 [In Greek]

Bahari ZA, Pallardy SG, Parker WC (1985) Photosynthesis, water relations and drought adaptation in six woody species of oak-hickory forests in central Missouri. *For Sci* 31, 557-569

Ceulemans R, Saugier B (1991) Photosynthesis. In: *Physiology of Trees* (AS Raghavendra, ed), John Wiley & Sons, New York, 21-49

Cochard H, Bréda N, Granier A, Aussenac G (1992) Vulnerability to air embolism of tree European oak species (*Quercus petraea* (Matt) Liebl, *Q pubescens* Wild, *Q robur* L). *Ann Sci For* 49, 225-233

Dafis S (1973) Classification of forest vegetation in Greece. *Sci Ann School Agric For* 15, 57-91 [In Greek]

Dafis S (1986) *Forest Ecology.* Thessaloniki , Greece, 1-493 [In Greek]

Delucia EH, Heckathorn SA (1989) The effects of soil drought on water-use efficiency in a contrasting Great Basin and Sierran on Montane species. *Plant Cell Environ* 12, 935-940

Dougherty PM, Hinkley TM (1981) The influence of a severe drought on net photosynthesis of white oak *(Quercus alba). Can J Bot* 59, 335-341

Dougherty PM, Teskey RO, Phelps JE, Hinckley TN (1979) Net photosynthesis and early growth trends of a dominant white oak (*Quercus alba* L). *Plant Physiol* 64, 930-935

Dreyer E, Granier A, Bréda N, Cochard H, Epron D, Aussenac G (1992) Oak tress under drought constraints ecophysiological aspects. In: *Recent Advances in Studies of Oak Decline.* Proceedings of the International Congress, *Selva di Fasano, Italy,* 293-321

Guehl JM, Aussenac G, Bouachrine J, Zimmermann R, Pennes JM, Ferhi A, Grieu P (1991) Sensitivity of leaf gas exchange to atmospheric drought, soil drought, and water use efficiency in some Mediterranean Abies species. *Can J For Res* 21, 1505-1515

Epron D, Dreyer E (1990) Stomatal and non-stomatal limitation of photosynthesis by leaf water deficits in three oak species: a comparison of gas exchange and chlorophyll a fluorescence data. *Ann Sci For* 47, 435-450

Epron D, Dreyer E (1993) Photosynthesis of oak leaves under water stress: maintenance of high photochemical efficiency of photosystem II and occurrence of non-uniform CO_2 assimilation. *Tree Physiol* 13, 107-117

Farquhar GD, Erleringer JR, Tand KT, Hubick K (1989) Carbon isotope discrimination and photosynthesis. *Ann Rev Plant Physiol Mol Biol* 40, 503-537

Field C, Merino J, Mooney HA (1983) Compromises between water use efficiency and nitrogen use efficiency in five species of California evergreens. *Oecologia* 60, 384-389

Hinckley TM, Duhme F, Hinckley AR, Richter H (1983). Drought relation of shrub species assessment of mechanism of drought resistance. *Oecologia* 59, 344-350

Levitt J (1980) *Response of Plants to Environmental stress, Vol 2, Water, Radiation, Salt and Other Stress.* Academic Press, New York, 606 p

Mauromatis G (1980) The bioclimate of Greece. Relationship of climatic and natural vegetation. Bioclimatic maps. *For Res* vol 1, 1-63 [in Greek]

Medrahtu T, Hanover J (1991) Leaf age effects on photosynthesis and stomatal conductance of Black locust seedling. *Photosynthesis* 14, 90-127

Ni BR, Pallardy SG (1991) Response of gas exchange to water stress in seedlings of woody angiosperms. *Tree Physiol* 8, 1-9

Nunes MA, Ramalho J, Pijo P (1992) Seasonal changes in some photosynthetic properties of Ceratonia natural conditions. *Plant Physiol* 86, 381-387

Radoglou K, Teskey R (1993) Development of patterns in forest stands of lobolly pine (*Pinus taeda* L). In: *Abstract collection of the international workshop on Ecophysiology and Genetics of Trees and Forest in a Change Environment,* Viterbo, Italy, 183

Rhizopoulou S, Mitrakos K (1990) Water of evergreen sclerophylls. I. Seasonal changes in the water relation of 11 species from the same environment. *Ann Bot* 65, 171-178

Schulze ED (1986) Carbon dioxide and water vapour exchange in response to drought in the atmosphere and in soil. *Ann Rev Plant Physiol* 37, 247-274

Schulze ED, Hall AE (1982) Stomatal responses, water loss and CO_2 assimilation rates of plants in contrasting environments. In: *Physiological Plant Ecology II Water Relations and Carbon Assimilation* (OL Lange, PS Nobel, CB Osmond, H Ziegler, eds), Springer-Verlag, Berlin, Heidelberg, New York, 181-230

Sobrado M (1986) Aspects of tissue water relations and seasonal changes of leaf water potential components of evergreen and deciduous species coexisting in tropical dry forest. *Oecologia* 68, 413-416

Tenhunen JP, Lange OL, Gebel J, Beyschlag W, Weber JA (1984) Changes in photosynthetic capacity carboxylation efficiency and CO_2 compensation point associated with midday stomatal closure and midday depression of net CO_2 exchange of leaves of *Quercus suber. Planta* 162, 193-203

Tenhunen JP, Beyschlag W, Lange OL, Harley PC (1987a) Changes during summer drought in leaf CO_2 uptake rates in macchia shrubs growing in Portugal: limitation due to photosynthetic capacity carboxylation efficiency and stomatal conductance. In: *Plant Response to Stress.* NATO ASI series, vol 5 (JD Tenhunen, FM Catarino, O Lange, WC Oechel, eds), Springer-Verlag, Heidelberg, 305-328

Tenhunen JP, Pearcy RW, Lange OL (1987b) Diurnal variations in leaf conductance and gas exchange in natural environments. In: *Stomatal Function* (E Zeiger, GD Farquhar, IR Cowan, eds), Stanford University Press, Stanford, CA, 323-351

Turner NC (1981) Technique and experimental approaches for measurements of plant water status. *Plant Sci* 58, 339-366

Yates F (1982) Regression models for repeated measurements (the consultant's forum Reader reaction). *Biometrics* 38, 38850-38853