Ecophysiology of seedlings of three Mediterranean pine species in contrasting light regimes

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Abstract

Seasonal dynamics of net photosynthesis (A_{net}) in 2-year-old seedlings of *Pinus brutia* Ten., *Pinus pinea* L. and *Pinus pinaster* Ait. were investigated. Seedlings were grown in the field in two light regimes: sun (ambient light) and shade (25% of photosynthetically active radiation (PAR)). Repeated measures analyses over a 12-month period showed that A_{net} varied significantly among species and from season to season. Maximum A_{net} in sun-acclimated seedlings was low in winter (yet remained positive) and peaked during summer. Maximum A_{net} was observed in June in *P. pinea* (12 µmol m^{-2} s^{-1}), July in *P. pinaster* (23 µmol m^{-2} s^{-1}) and August in *P. brutia* (20 µmol m^{-2} s^{-1}). Photosynthetic light response curves saturated at a PAR of 200–300 µmol m^{-2} s^{-1} in winter and in shade-acclimated seedlings in summer. Net photosynthesis in sun-acclimated seedlings did not saturate at PAR up to 1900 µmol m^{-2} s^{-1} in *P. brutia* and *P. pinaster*. Minimum air temperature of the preceding night was apparently one of the main factors controlling A_{net} during the day. In shade-acclimated seedlings, photosynthetic rates were reduced by 50% in *P. brutia* and *P. pinaster* and by 20% in *P. pinea* compared with those in sun-acclimated seedlings. Stomatal conductance was generally lower in shaded seedlings than in seedlings grown in the sun, except on days with a high vapor pressure deficit. Total chlorophyll concentration per unit leaf area, specific leaf area (SLA) and height significantly increased in *P. pinea* in response to shade, but not in *P. pinaster* or *P. brutia*. In response to shade, *P. brutia* showed a significant increase in total chlorophyll concentration but not SLA. Photosynthetic and growth data indicate that *P. pinaster* and *P. brutia* are more light-demanding than *P. pinea*.

Keywords: diurnal, gas exchange, Mediterranean region, *Pinus brutia, Pinus pinaster, Pinus pinea*, seasonal

Introduction

Pinus brutia Ten., *Pinus pinea* L. and *Pinus pinaster* Ait. cover more than 5 million ha in the Mediterranean region (Barbéro et al. 1998). These species are widely used in re-

forestation programs in the area, mainly to control erosion (Richardson 1998). *Pinus brutia* is found mainly in the warm eastern Mediterranean, whereas *P. pinea* and *P. pinaster* are usually restricted to the cooler western Mediterranean. These species form pure stands in arid and semi-arid areas; however, in more humid areas, they may comprise an intermediate stage in succession to broad-leaved trees.

With the exception of *P. pinaster*, few ecophysiological studies have been conducted on these Mediterranean pines. An understanding of their physiological responses to stress will help to determine how site conditions can be manipulated to improve survival and growth. The objectives of this study were to compare seasonal trends in photosynthetic variables in 2-year-old seedlings of *P. brutia*, *P. pinea* and *P. pinaster* in ambient light and in shaded environments and to determine the effects of environmental variables on photosynthesis.

Materials and methods

The study was conducted at the Forest Research Institute in Vassilika, 15 km east of Thessaloniki, Greece (10 m altitude, 40°45′ N and 22°50′ E). The climate is Mediterranean, the mean monthly temperature varies between 5 °C in January and 25 °C in July, and mean annual precipitation is 460 mm. Three widely spread Mediterranean pine species were chosen for the study: *P. brutia*, *P. pinea,* and *P. pinaster*. *Pinus brutia* is found mostly in the eastern Mediterranean basin at altitudes between 0 and 800 m. *Pinus pinea* is spread over low altitudes along the Mediterranean coast. *Pinus pinaster* is found mostly in the western Mediterranean basin at altitudes between 0 and 800 m. In September, 2-year-old seedlings (30 per species) were randomly selected from a nursery located near Thessaloniki. Seedlings were transplanted to 3-liter plastic pots filled with a 2:1 (v/v) mix of peat moss and sandy loam. Seedlings were placed in an open nursery and were watered and fertilized

to prevent stress. At the beginning of the growing season (March), shade frames and plastic shade cloth were used to reduce insolation to about 25% of full sunlight. One half of the seedlings from each species were transferred to the shade frames (shade treatment), whereas the other half were kept under ambient light conditions (sun treatment).

Gas exchange measurements

Seedling gas exchange measurements were made on all seedlings (n = 30) every 7–10 days over the course of a year (November through October) with a portable photosynthesis system (LI-6250, Li-Cor, Lincoln, NE). Measured variables were net photosynthetic rate (A_{net}; µmol m^{-2} s^{-1}), stomatal conductance (g_s; mol m^{-2} s^{-1}) and intercellular CO_2 concentration (C_i; ppm). Measurements were conducted between 1100 and 1300 h during winter, and between 1000 and 1200 h during the spring and summer months. Air and leaf temperatures in the chamber were maintained within 1 to 2 °C of ambient by varying the speed of the chamber fan. Photosynthetically active radiation (PAR; µmol m^{-2} s^{-1}), vapor pressure deficit (VPD; kPa) and CO_2 concentrations (350–360 µmol m^{-2} s^{-1}) were maintained near ambient. The diurnal course of net photosynthesis was followed once a month on clear days between April and September. Diurnal measurements were made hourly on three seedlings per species between 0600–0700 h and 1900– 2000 h. Light response curves were recorded for three seedlings per species before noon in the field in winter (January– February) and in summer (June–July).

Gas exchange measurements were conducted by enclosing 14–20 current-year needles in the chamber of the photosynthesis system. The duration that needles were enclosed in the chamber was based on three changes of either 12 s or 3– 4 µmol mol^{-1} of CO_2. Results were expressed on a projected leaf area basis. Leaf area was measured with a portable leaf area meter (LI-300, Li-Cor).

Chlorophyll concentration, specific leaf area and seedling height

Total chlorophyll concentration on a leaf area basis (T_{chl}; µg cm^{-2}), specific leaf area (SLA; cm^2 g^{-1}) and height (cm) were determined for all seedlings acclimated to shade (n = 15) or sun (n = 15). Three needles per seedling were collected and cut to 1.5 cm length for chlorophyll measurements, and needle area was calculated (Whiteman and Koller 1964). Needles were subsequently placed in tubes containing 3 ml of N,N-dimethylformamide (NNF) and stored at 4 °C in the dark for 72 h. Absorbance of the NNF was measured at 647 and 664.7 nm (A_{647} and $A_{664.7}$, respectively) with a spectrophotometer (Moran and Porath 1980). Total chlorophyll concentration (µg ml^{-1}) was then calculated:

$$\text{Total chlorophyll} = 17.9\, A_{647} + 8.08\, A_{664.7}. \qquad (1)$$

Specific leaf area of several needles from each seedling was estimated. Needles were oven-dried for 72 h at 75 °C and SLA (leaf area/dry mass) was subsequently determined. Seedling height was measured periodically throughout the study.

Data analysis

Repeated measures analyses were used to evaluate seasonal trends in photosynthetic variables within each species and to study the effect of shade on physiological variables during the growing season. Linear or quadratic contrast analyses for species, light and species × light interactions were performed. All data were analyzed with the Mixed Model Procedure in the SAS statistical software package (SAS Institute, Cary, NC). Pairwise mean comparisons were performed using the probability of difference (Pdiff statement in SAS, P < 0.05) (Steel et al. 1996). Single or multiple regression analyses were used to predict photosynthetic rates and stomatal conductance as functions of environmental conditions.

Results

Seasonal variation in A_{net} of plants in the sun treatment

Photosynthetically active radiation, air temperature and VPD at the time of measurement are presented in Figure 1. Repeated measures analyses over the 12-month period for seedlings growing in the sun treatment showed that maximum A_{net} varied significantly among species (Table 1). The
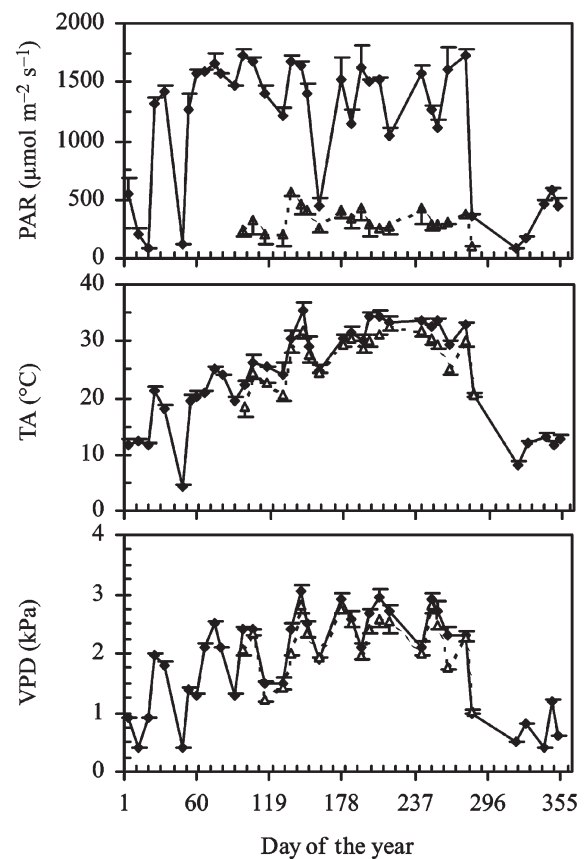

Figure 1. Seasonal course of photosynthetically active radiation (PAR), air temperature (TA) and vapor pressure deficit (VPD) with standard error bars. Values were taken at actual measurement times in the sun (Δ) and the shade (♦) ($n_{\text{growing season}}$ = 15, n_{winter} = 30).

Table 1. Analyses of variance and orthogonal contrasts of seasonal courses of net photosynthesis (A_{net}), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) during a 12-month period (November through October) in the sun and during the growing season (between April and October) in the sun or shade (sun versus shade) in three field-grown Mediterranean pine species (*Pinus brutia*, *P. pinaster,* and *P. pinea*).

Source	df	A_{net} ($\mu mol\ m^{-2}\ s^{-1}$)		g_s ($mol\ m^{-2}\ s^{-1}$)		C_i (ppm)	
		F	P	F	P	F	P
Sun							
Species	2	19.5	< 0.01	6.80	< 0.01	0.53	0.58
P. brutia versus *P. pinaster*	1	1.92	0.16	11.5	< 0.01	0.22	0.64
P. brutia versus *P. pinea*	1	141	< 0.01	51.6	< 0.01	6.28	< 0.01
P. pinaster versus *P. pinea*	1	146	< 0.01	19.7	< 0.01	14.3	0.01
P. brutia and *P. pinaster* versus *P. pinea*	1	36.9	< 0.01	2.23	0.13	0.86	0.35
Sun versus shade							
Light	1	246	< 0.01	3.98	0.05	98.9	< 0.01
Species	2	47.6	< 0.01	0.32	0.72	2.56	0.08
P. brutia versus *P. pinaster*	1	2.92	0.08	0.05	0.82	1.98	0.16
P. brutia versus *P. pinea*	1	56.2	< 0.01	0.30	0.58	0.74	0.39
P. pinaster versus *P. pinea*	1	82.8	< 0.01	0.57	0.44	5.03	0.24
P. brutia and *P. pinaster* versus *P. pinea*	1	92.6	< 0.01	0.59	0.44	3.21	0.07
Species × light	2	25.0	< 0.01	4.72	0.01	0.40	0.67
(*P. brutia* versus *P. pinaster*) × light	1	0.42	0.51	8.29	< 0.01	0.10	0.75
(*P. brutia* versus *P. pinea*) × light	1	41.9	< 0.01	5.37	0.02	0.77	0.38
(*P. pinaster* versus *P. pinea*) × light	1	33.2	0.01	0.3	0.25	0.31	0.57
(*P. brutia* and *P. pinaster* versus *P. pinea*) × light	1	49.4	< 0.01	1.06	0.30	0.68	0.40

annual mean A_{net} of *P. pinea* was significantly lower than that of *P. brutia* and *P. pinaster* (5.17 ± 0.45, 8.06 ± 0.9, and 7.39 ± 1.08 µmol m^{-2} s^{-1}, respectively). Differences in maximum A_{net} among species were negligible during winter (November– February) (2.5 ± 0.6 for *P. brutia*, 2.35 ± 0.32 for *P. pinaster,* and 2.9 ± 0.39 for *P. pinea*). During the growing season (April–September), however, *P. pinea* exhibited a significantly lower mean maximum A_{net} in the sun treatment than the other species (Table 2).

There was significant seasonal variation in maximum A_{net}. Photosynthetic rates were minimal in January and February (Figure 2), but minimum air temperatures seldom dropped below 0 °C and A_{net} remained positive. An initial increase in maximum A_{net} was observed in March with the improvement in weather conditions. A second and major rise in photosynthetic capacity was noticeable in June in *P. pinea* (maximum A_{net} = 12.1 µmol m^{-2} s^{-1}), in July in *P. pinaster* (maximum A_{net} = 23.0 µmol m^{-2} s^{-1}) and in August in *P. brutia* (maximum A_{net} = 19.8 µmol m^{-2} s^{-1}) (Figure 2).

Light response curves were followed for all species in winter (January–February) and in summer (June–July) (Figure 3). At lower irradiances, A_{net} was higher and response curves were steeper in winter compared with summer, and in shade-treated compared with sun-acclimated seedlings. In addition, A_{net} in winter months and in the shade treatment saturated at about 200–300 µmol m^{-2} s^{-1}. In June and July, net photosynthesis did not saturate at measured PAR up to 1900 µmol m^{-2} s^{-1} in *P. brutia* and *P. pinaster*. Quantum yields and light compensation points derived from photosynthetic light response curves did not differ among species but differed with season and light regime. Quantum yields averaged 0.051 ± 0.003, 0.066 ± 0.002, and 0.038 ± 0.002, and light compensation points averaged 38.3 ± 2.8, 35.2 ± 2.3, and 43.0 ± 2.5 µmol m^{-2} s^{-1} in January–February, June–July (shade treatment), and June–July (sun treatment), respectively.

Regressing A_{net} as a function of environmental variables (Table 3) indicated that A_{net} was significantly dependent on daily air temperature and the minimum air temperature recorded on site during the night preceding sampling (Table 3, Figure 4).

Stomatal conductance and C_i showed substantial variability throughout the study (Figure 5). Both of these parameters were closely coupled to environmental condi-

Table 2. Growing season (April–September) means and standard errors (SE) of net photosynthesis (A_{net}), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) in sun and shade in three field-grown Mediterranean pine species. Means within columns followed by the same letters are not significantly different. An asterisk indicates a significant difference between shade- and sun-acclimated seedlings ($P < 0.05$).

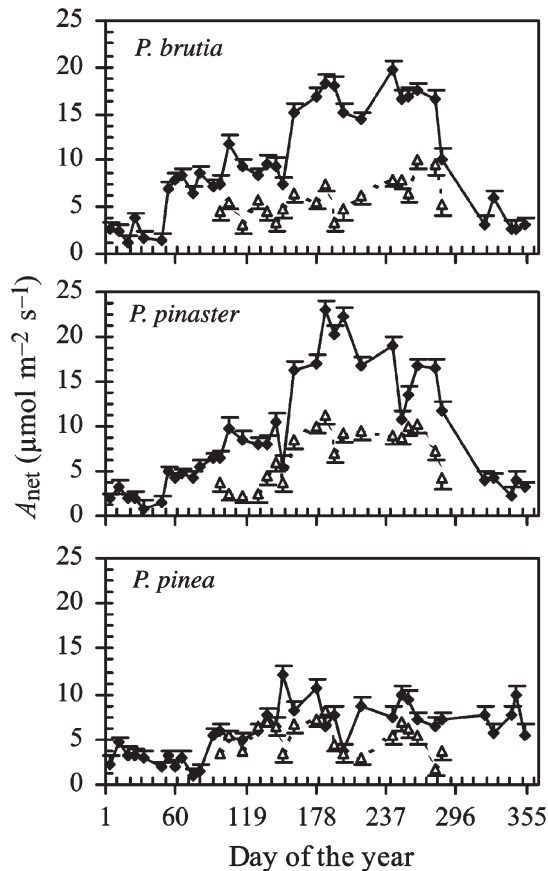| Species	A_{net} ± SE ($\mu mol\ m^{-2}\ s^{-1}$)		g_s ± SE ($mol\ m^{-2}\ s^{-1}$)		C_i ± SE (ppm)	
	Sun	Shade	Sun	Shade	Sun	Shade
P. brutia	13.4 ± 1.32 a	5.9 ± 0.45 ab*	0.34 ± 0.02 a	0.31 ± 0.02 a	279.7 ± 8.1 a	321.7 ± 5.6 a*
P. pinaster	13.9 ± 1.49 a	6.91 ± 0.69 a*	0.29 ± 0.02 b	0.35 ± 0.02 a*	274.6 ± 8.6 a	313.3 ± 8.6 a*
P. pinea	7.43 ± 0.45 b	5.43 ± 0.41 b*	0.31 ± 0.02 ab	0.35 ± 0.02 a*	287.8 ± 9.8 a	321.6 ± 9.3 a*

Figure 2. Seasonal course of net photosynthesis (A_{net}) with standard error bars in sun ($\triangle$) and shade ($\blacklozenge$) treatments in three Mediterranean pine species ($n_{growing\ season}$ = 15, n_{winter} = 30).

tions. Mean annual g_s was lower in *P. pinaster* (0.24 mol m^{-2} s^{-1}) than in the other species, but did not differ significantly between *P. brutia* and *P. pinea* (0.29 mol m^{-2} s^{-1}). Mean g_s during the growing season showed a similar pattern (Table 2). Stomatal conductance was positively correlated with net photosynthesis (Figure 6). Regression analyses showed that relative humidity and VPD were the major factors influencing g_s in all species (Table 3). Relative humidity had a direct effect on g_s, particularly in winter. Intercellular CO_2 concentration did not differ among the three species.

Effect of shade on seasonal trends

Analyses of repeated measures indicated that light had a significant effect on A_{net} and C_i (Table 1). However, the effect varied among species. Net photosynthesis in the shade treatment was less than 50% of that in the sun treatment in *P. brutia* and *P. pinaster*, but shade reduced A_{net} by only 20% in *P. pinea*. Intercellular CO_2 concentration increased by 10 to 13% in all species in response to shade, whereas shade increased g_s in *Pinus pinaster* and *P. pinea*, especially on days with high VPD, but not in *P. brutia* (Table 2).

Chlorophyll concentration, specific leaf area and height

To evaluate morphological and physiological changes in needles in response to shade, total chlorophyll concentration
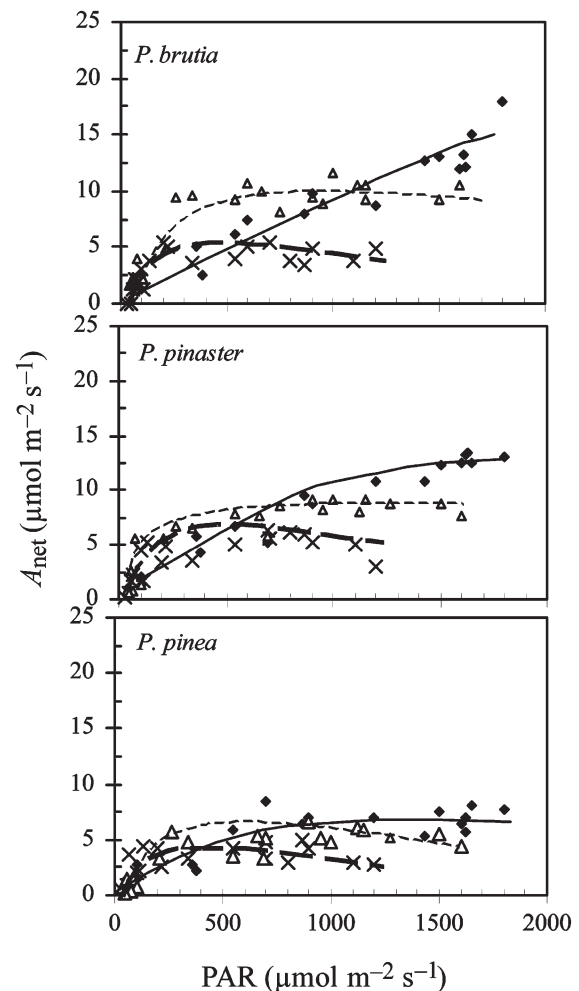

Figure 3. Photosynthetic light response curves for three Mediterranean pine species (n = 3). January–February ($\times$): mean air temperature = 10 °C and vapor pressure deficit = 0.86 kPa. June–July (sun: $\blacklozenge$, shade: $\triangle$): mean air temperature = 29 °C and vapor pressure deficit = 2.1 kPa.

per unit leaf area (T_{chl}) and SLA were measured in September after 5 months of shade acclimation (Table 4). In response to shade, both SLA and T_{chl} increased significantly in *P. pinea*. No significant changes were observed in *P. pinaster* needles, whereas needles of *P. brutia* showed a significant increase in T_{chl}, but not SLA.

Seedling height growth began in March (Figure 7), and was fastest in *P. pinaster*, followed by *P. brutia* and then *P. pinea*. Shade-acclimated seedlings of *P. pinaster* and *P. brutia* grew significantly more slowly than sun-acclimated seedlings, whereas seedlings of *P. pinea* were significantly taller in the shade treatment than in the sun treatment.

Diurnal pattern of net photosynthesis

Diurnal patterns of A_{net} during the growing season were generally sinusoidal (Figure 8). Daily A_{net} was lower in the shade treatment than in the sun treatment. Maximum A_{net} was generally reached before noon in sun-acclimated seedlings. In shade-acclimated seedlings, maximum A_{net} oc-

Table 3. Multiple regression analyses of seasonal courses of net photosynthesis and stomatal conductance of three Mediterranean pine species (*Pinus brutia*, *P. pinaster*, and *P. pinea*) in the sun treatment as a function of photosynthetically active radiation (PAR), vapor pressure deficit (VPD), air temperature at the time of measurement (TA), and minimum air temperature of the preceding night (TA$_{min}$).

	P. brutia		*P. pinaster*		*P. pinea*	
	Slope	P	Slope	P	Slope	P
Net photosynthesis ($\mu mol\ m^{-2}\ s^{-1}$)						
Intercept	–3.2	0.03	–3.8	0.03	–3.1	0.00
PAR	0.001	0.62	0.002	0.29	0.001	0.72
VPD	0.15	0.9	0.43	0.82	0.18	0.88
TA	0.46	0.02	0.47	0.04	0.02	0.98
TA$_{min}$	0.25	0.03	0.34	0.02	0.28	0.003
R^2	0.79		0.75		0.55	
Stomatal conductance ($mol\ m^{-2}\ s^{-1}$)						
Intercept	–0.037	0.77	–0.085	0.47	–0.102	0.40
PAR	0.002	0.76	0.003	0.57	0.0030	0.96
RH	0.006	0.05	0.007	0.01	0.008	0.01
VPD	0.230	0.04	0.231	0.04	0.301	0.01
TA	0.009	0.43	0.018	0.11	0.022	0.06
R^2	0.22		0.26		0.31	

curred mainly at noon or during the afternoon in *P. brutia* and *P. pinaster*, and before noon in *P. pinea*. Midday depression in A_{net} was observed during the summer months and was higher in the sun treatment than in the shade treatment.

Discussion

Seasonal oscillations in photosynthetic variables

Significant seasonal variation in maximum A_{net} was observed in *P. brutia* and *P. pinaster*, and to a lesser extent in *P. pinea* in the sun treatment. Seasonal variation in photosynthesis in pines is a result of genetically based endogenous rhythms as well as annual climate cycles (e.g., temperature) and radiation (Richardson 1998). A decrease in photosynthetic capacity in leaves was observed in the winter, but carbon balance remained positive. Photosynthesis in evergreen conifers is known to occur well beyond the growing season (Teskey et al. 1994). Troeng and Linder (1982) found

9 months of positive carbon balance in *P. sylvestris*, and Fry and Phillips (1977) found little reduction in photosynthetic capacity in conifers during mild winters.

The low winter photosynthetic rates observed in this study may be attributable, in part, to low air temperatures, periods of low solar irradiance, intermittent high solar irradiances causing photoinhibition or to a decrease in the T_{chl} (mean $6.5 \pm 0.5\ \mu g\ cm^{-2}$, data not shown). Temperatures seldom fell below 2–5 °C during measurements of photosynthesis, and at these temperatures A_{net} remained positive. Low net photosynthetic rates were also observed on clear days and days with low relative humidity or high VPD. Minimum air temperature during the preceding night was significantly correlated with photosynthetic capacity. A similar relationship was reported for *P. sylvestris* by Vogg et al. (1998), who suggested that a short-term drop in minimum temperature may have reduced photosynthetic capacity as a result of cold-triggered photoinhibition and damage to Photosystems I and II.

Maximum photosynthetic rate was highest in *P. pinaster* and *P. brutia* and lowest in *P. pinea*. Site adaptation with
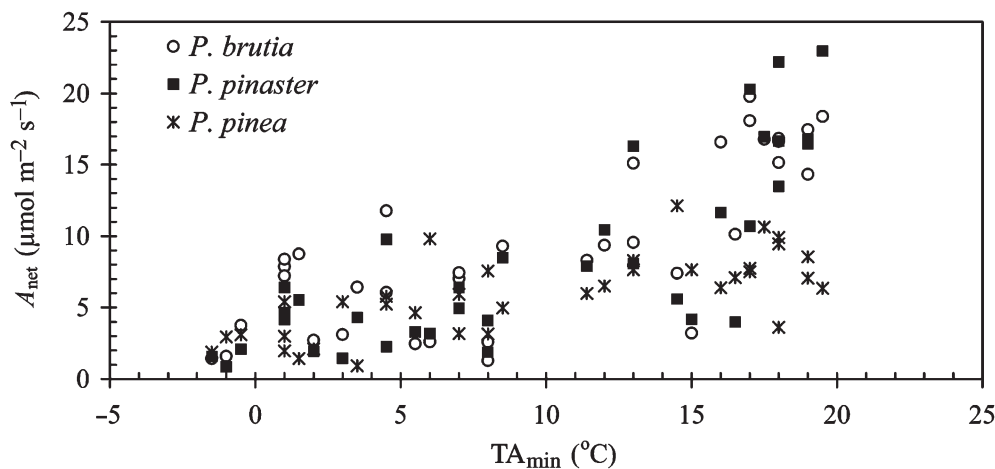

Figure 4. Net photosynthesis (A_{net}) as a function of recorded minimum air temperature of the preceding night (TA$_{min}$) in three Mediterranean pine species growing in the sun.
Pinus brutia: $A_{net} = 3.2 + 0.63\ TA_{min}$, $P < 0.001$, $r^2 = 0.59$;
P. pinaster: $A_{net} = 1.48 + 0.72\ TA_{min}$, $P < 0.001$, $r^2 = 0.61$; and
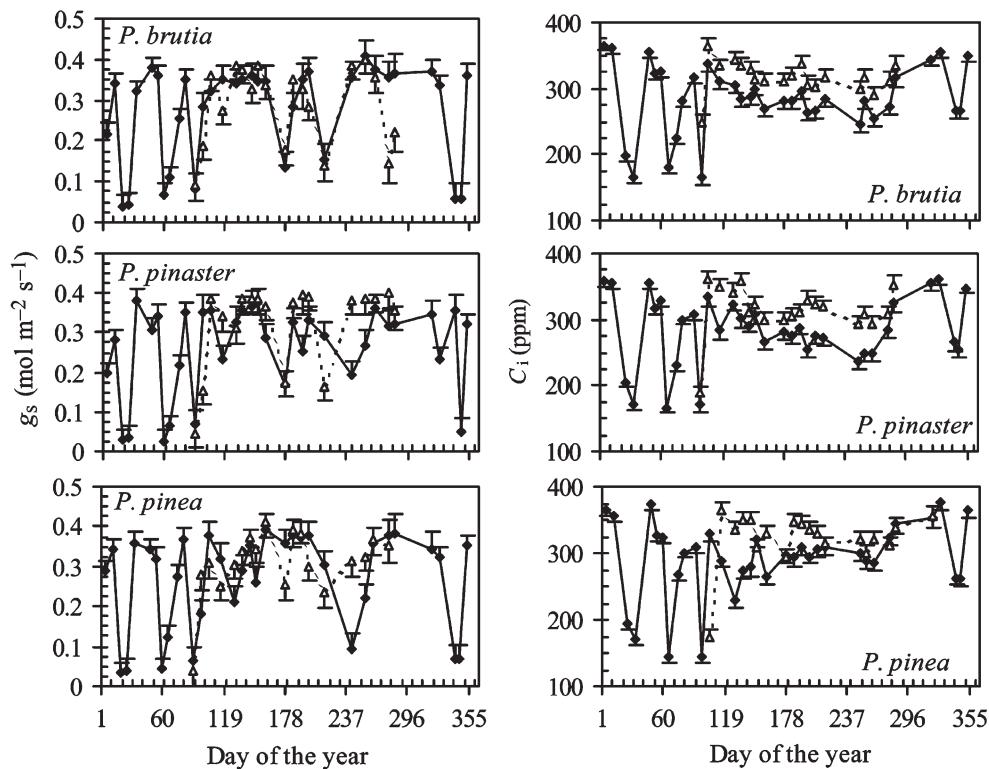*P. pinea*: $A_{net} = 3.13 + 0.28\ TA_{min}$, $P < 0.001$, $r^2 = 0.50$.

Figure 5. Seasonal courses of stomatal conductance (g_s) and intercellular CO_2 concentration (C_i), with standard error bars, in three Mediterranean pine species growing in the field in the sun (♦) and in the shade (Δ) (n_{winter} = 30, $n_{growing\ season}$ = 15).

respect to photosynthetic rates has been reported in the literature (e.g., Troeng and Linder 1982, Murthy et al. 1997, Law et al. 2001). *Pinus pinea* is found along the Mediterranean shore, and its needles are exposed to sea spray, which may increase cuticle permeability (Grossini et al. 1998). Sclerophyllous alterations and lignifications of the bundle sheath and stomata at an early stage of leaf development could be an adaptive strategy of this species to deal with salt spray (Grossini et al. 1998). These adaptations may explain the significantly lower photosynthetic rates observed in *P. pinea* in this study.

Net photosynthetic rate peaked in *P. pinea* in June and in *P. brutia* and *P. pinaster* later in the growing season. An explanation for this variation could be that *P. pinea*, which is usually found on sandy sites along the Mediterranean shore, experiences favorable conditions for photosynthesis earlier in the season than the other species.

Photosynthetic light response curves saturated at low irradiances in winter, whereas high irradiances caused a slight decline in photosynthesis, possibly as a result of photoinhibition. In contrast, photosynthetic light response curves did not appear to saturate during summer. This is reported to be characteristic of sun-adapted, early successional species (Bazzaz and Carlson 1982) such as *P. brutia* and *P. pinaster*.

The diurnal course of photosynthesis was dependent on available irradiance early in the morning and around sunset. During the course of the day, however, light, air temperature and VPD were the determinants of photosynthetic rates. Midday depression in photosynthesis was observed—a common phenomenon in the Mediterranean region—even though water was available. Similar observations have been reported for the Mediterranean shrub *Arbutus unedo* L. (Castell et al. 1994) and for *Quercus suber* L. (Faria et al. 1996).
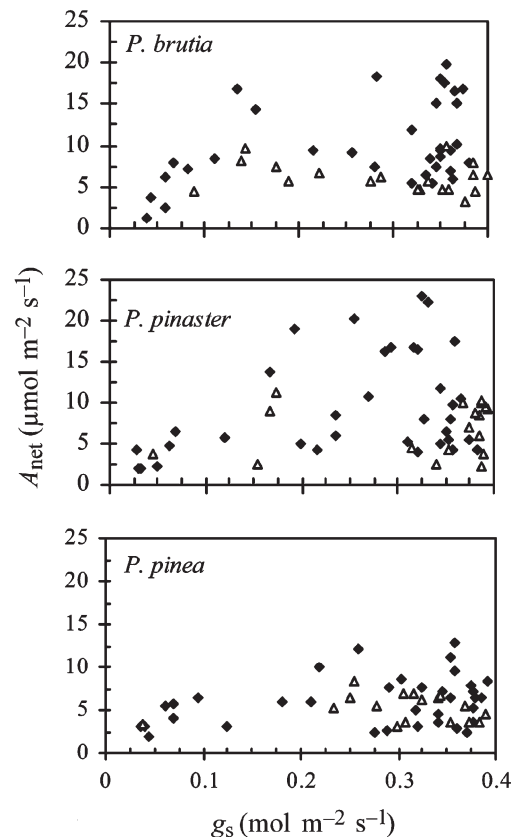

Figure 6. Net photosynthesis (A_{net}) as a function of stomatal conductance (g_s) in three Mediterranean pine species growing in the field. *Pinus brutia*: A_{net} = 4.4 + 17.8 g_s, r^2 = 0.20, P = 0.01; *P. pinaster*: A_{net} = 4.7 + 18.6 g_s, r^2 = 0.17, P = 0.04; and *P. pinea*: A_{net} = 4.0 + 7.32 g_s, r^2 = 0.12, P = 0.05.

Table 4. Means and standard errors (SE) of total chlorophyll concentration per unit leaf area (T_{chl}) and specific leaf area (SLA) in shade- and sunacclimated Mediterranean pine species. Means within columns followed by the same letter are not significantly different. An asterisk indicates a significant difference between sun- and shade-acclimated seedlings ($P < 0.05$).

Species	$T_{chl} \pm SE$ ($\mu g\ cm^{-2}$)		SLA $\pm$ SE ($cm^2\ mg^{-1}$)	
	Sun	Shade	Sun	Shade
Pinus brutia	13.3 ± 0.52 a	17.0 ± 0.54 a*	16.2 ± 1.5 a	15.1 ± 1.25 b
Pinus pinaster	8.55 ± 0.34 b	8.96 ± 0.36 b	21.3 ± 2.1 a	20.1 ± 2.0 ab
Pinus pinea	14.9 ± 0.94 a	17.8 ± 0.63 a*	17.4 ± 2.0 a	25.4 ± 2.14 a*

Effect of shade on photosynthetic variables

Net photosynthesis was significantly lower in shade-acclimated seedlings (25% PAR) than in seedlings growing in the sun. Differences in photosynthesis between light regimes were minimal on overcast days. In addition, there was no dieback in seedlings in the shade treatment. Parker and Mohammed (2000) found that seedling mortality increased at irradiances below 15% of full sun light in *Pinus resinosa* Ait., a species adapted to high irradiances. The results of this study suggest that seedlings in the shade contributed significantly to carbon assimilation of the tree canopy, in agreement with Schulze et al. (1977), who suggested that shaded leaves might contribute up to 40% of carbon assimilated by a tree canopy. Exposing shaded seedlings of *P. pinea*, *P. pinaster,* and *P. brutia* to full light resulted in an increase in photosynthesis similar to that reported in seedlings growing in the sun treatment (data not shown). The plasticity of these seedlings is consistent with trends reported for early successional species (Bazzaz and Carlson 1982). Stomatal conductance was greater in the shade than in the sun on days with high VPD and air temperature. This trait was more apparent in *P. pinea* and *P. pinaster* than in *P. brutia*. *Pinus brutia* was found to be a drought-resistant species occupying the driest sites around the Mediterranean Basin. On the other hand, *P. pinea* has been reported to experience significant depression in photosynthetic capacity in response to hot summer days (Manes et al. 1997) and *P. pinaster* has been classified as a drought-avoiding species with sensitive stomata (Picon et al. 1996).

Acclimation to shade induced a significant increase in T_{chl} and SLA in *P. pinea*, but not in *P. pinaster*. *Pinus brutia* responded to shade with an increase in T_{chl}. Bazzaz (1996) suggested that acclimation to light depends on the morphological and physiological adjustment of the plants to increased light interception. Specific leaf area and T_{chl} may provide a measure of acclimation to light. Based on our results, *P. pinaster* did not respond to low irradiances (25% of full light), an observation that contrasts with those of Porté and Lousteau (1998), who reported a significant increase in SLA of *P. pinaster* from top to bottom branches. Parker and Mohammed (2000) found that *P. resinosa* was plastic, i.e., low irradiances induced morphological and physiological changes typical of those observed in shade-adapted species.

Pinus brutia and *P. pinaster* performed better in the sun than in the shade. In contrast, *P. pinea* performed well in both light regimes, experiencing the least reduction in A_{net} and the highest increase in T_{chl}, SLA, and height in the shade, a response pattern characteristic of shade-tolerant and mid-successional plants (Bazzaz and Carlson 1982, Givnish 1988). *Pinus pinea* can survive in various light regimes at low altitudes, whereas *P. brutia* and *P. pinaster* are shade-intolerant, early successional species that invade sites after disturbance and have the ability to grow fast, competing with other plant forms (Richardson 1998). Rouget et al. (2001) reported that *P. halepensis*, which is a close relative of *P. brutia* (*P. halepensis* replaces *P. brutia* in western Europe, and in Greece these two species interbreed) is restricted to
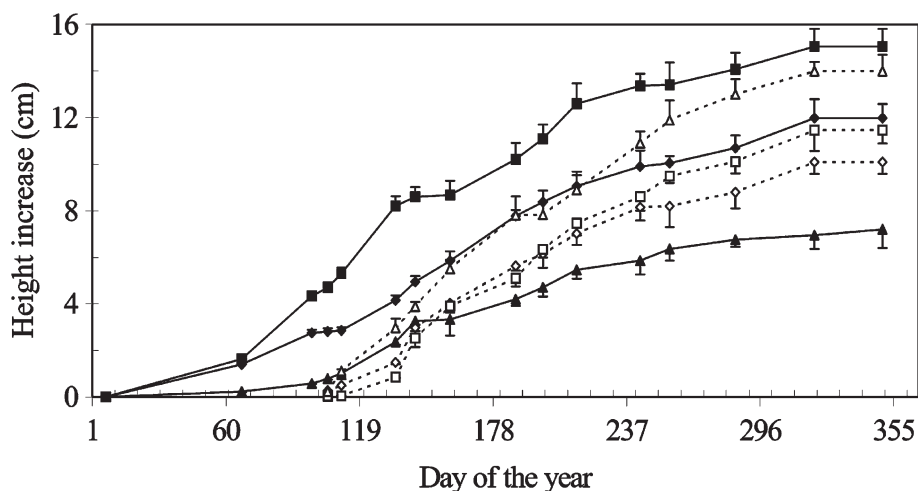

Figure 7. Seasonal increase in height and corresponding standard error bars in sun- (closed symbols) and shade-acclimated (open symbols) seedlings of *P. brutia* (♦, ◊), *P. pinaster* (■, □), and *P. pinea* (▲, Δ) ($n_{winter} = 30$, $n_{growing\ season} = 15$).
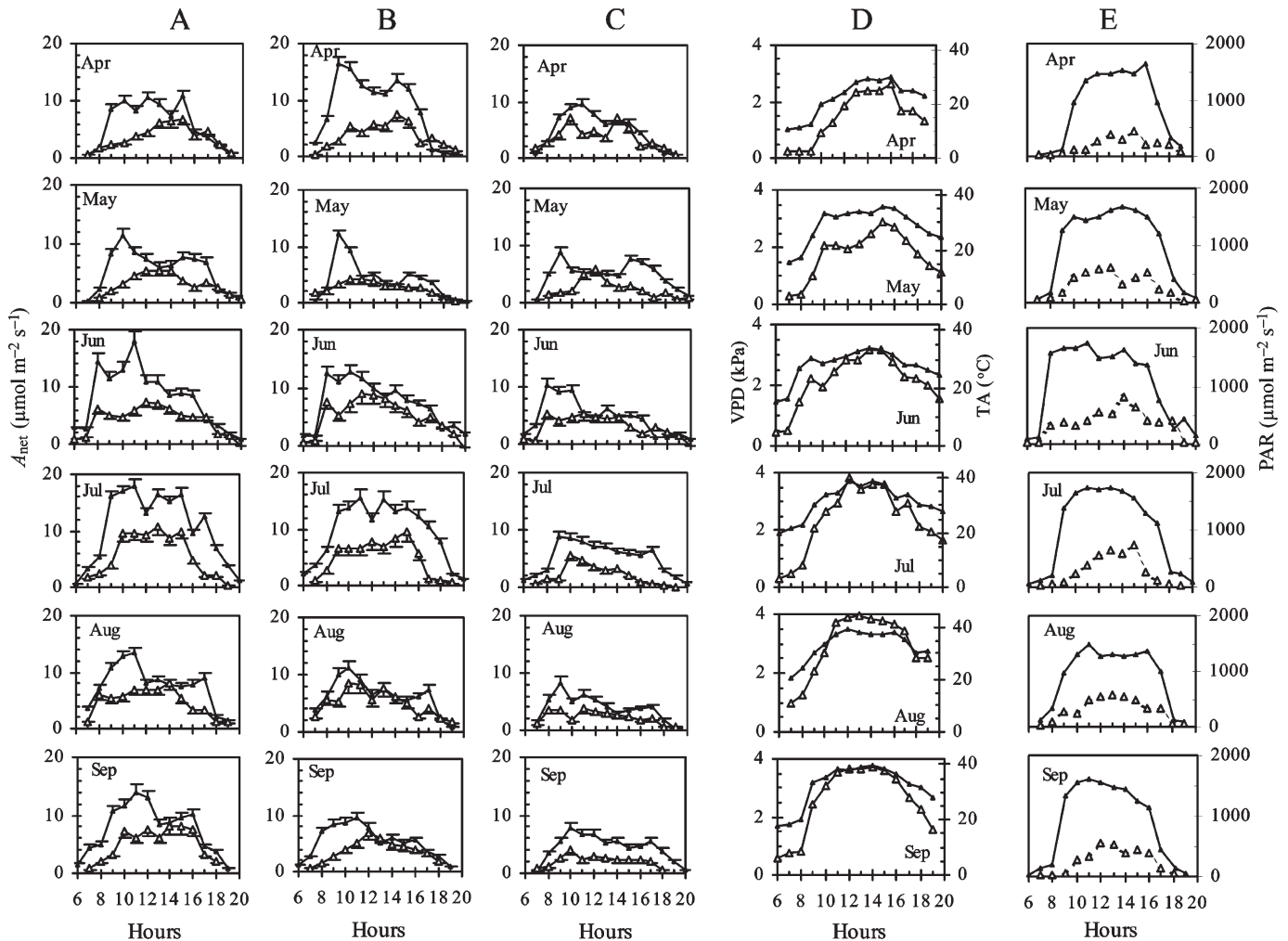
Figure 8. Diurnal curve of net photosynthesis (A_{net}) between April and September for *Pinus brutia* (**A**), *P. pinaster* (**B**), and *P. pinea* (**C**), measured on clear days in the field in the sun (▲) and in the shade (25% PAR; Δ). (**D**) Diurnal course of vapor pressure deficit (VPD; Δ) and air temperature (TA; ▲). (**E**) Diurnal curve of photosynthetically active radiation in the sun (▲) and in the shade (Δ) ($n = 3$).

dry areas, is unable to reproduce under its own shade and does not occur in areas dominated by *Quercus* species or other evergreen species in Catalonia, Spain.

Assessment of seasonal and diurnal variations in net photosynthesis provides a means to evaluate the environmental constraints to carbon assimilation. The positive carbon balance recorded during winter and summer months can contribute substantially to the carbon sequestered annually in the Mediterranean region. Our results, when combined with growth data, can be used for selection and breeding.

Acknowledgments

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