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Forest Ecology and Management 212 (2005) 291–301

Forest Ecology and Management

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Photosynthetic performances of *Quercus pannosa* vary with altitude in the Hengduan Mountains, southwest China

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Received 23 August 2004; received in revised form 20 March 2005; accepted 30 March 2005

Abstract

Quercus pannosa Hand.-Mazz. is an evergreen sclerophyllous oak that is an important component of montane forests at altitudes between 2500 and 4300 m in the Hengduan Mountains. To understand photosynthetic adaptability of *Q. pannosa* to alpine environments, photosynthetic performances and leaf traits were investigated along altitudinal gradients (at altitudes of 3240, 3650 and 4170 m, respectively). Observed trends with increasing altitude were: (1) progressively lower carboxylation efficiency (CE), light-saturated photosynthesis (P_{max}), daily average photosynthetic rate (P_{day}), photosynthetic pigments, leaf N content per unit area, optimum photosynthetic temperature (T_{opt}) and (2) progressively higher leaf mass per unit area (LMA) and ratio of intercellular CO_2 concentration (C_i/C_a). However, stomatal conductance (G_s) and water use efficiency (WUE) remained relatively constant along the altitudinal gradient. Lower photosynthetic rates in *Q. pannosa* at higher altitudes would be explained by combination of different factors: reducing CE, lower leaf N content and photosynthetic pigments content, higher LMA and C_i/C_a. In addition, *Q. pannosa* showed a significant mid-day depression in P_n, G_s, E and WUE due to high water vapor pressure deficits (VPD) and high temperatures. In general, the T_{opt} of *Q. pannosa* was significantly lower than those of Mediterranean oaks. This species could adapt well to low temperature, but high temperature, which indicated that cold-tolerance would be an important determinant of distribution of this oak in the Hengduan Mountains.

Keywords: *Quercus pannosa*; Leaf trait; Photosynthesis; Low temperature; Distribution

1. Introduction

Altitudinal limits on plant distribution has long been attributed to specific factors of the alpine environment, which were characterized by lower temperature, lower atmospheric pressure and partial CO_2 pressure, high light intensity, etc. (Friend and Woodward, 1990). These pressures had important effect on leaf morphology and physiology (Hovenden and Brodribb, 2000). Photosynthesis of plants was subjected to influences of environmental factors, therefore the plant distributed to adverse ecosystem environment showed different photosynthetic adaptability. Most studies showed that the photosynthetic characteristics and leaf traits of

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doi:10.1016/j.foreco.2005.03.031

alpine plants distinguished from those of lowland plants. In general, leaves decreased in area, therefore became thicker with altitude (Hultine and Marshall, 2000; Cordell et al., 1999; Körner, 2003). Meanwhile, the leaf N content per unit area and $\delta^{13}C$ value of alpine plants was higher than those of lowland plants (Körner and Diemer, 1987; Körner et al., 1988; Hultine and Marshall, 2000). There were also some evidences that plants from higher elevations had higher photosynthetic capacity (P_{max}) and carboxylation efficiency (CE) than those of lowland plants (Friend and Woodward, 1990; Körner, 2003). However, many reports on altitudinal trends in photosynthesis and leaf characteristics did not agree with the above-mentioned results (Rada et al., 1998; Cabrera et al., 1998). Körner and Diemer (1987) suggested that plant species, which had a wide altitudinal distribution, did not have high efficiency of CO_2 uptake at higher altitude. Would plants along higher altitudinal range (above 2500 m a.s.l.) in the Hengduan Mountains exhibit increase in photosynthesis with altitude?

Quercus sect. *Heterobalanus* (Oerst.) Mentis, is a group of oaks consisting of nine species (Zhou et al., 2003). They are distributed from Chiang Mai in Thailand to southwest China and Afghanistan. However, they are mainly concentrated in the Hengduan Mountains of southwest China (Zhou, 1993). The *Heterobalanus* group of oaks is dominant within the evergreen sclerophyllous oak forests in this area. These forests comprise an important part of the high mountain ecosystems, which form several watersheds of China's major river systems (Zhou and Coombes, 2001).

Quercus sect. *Heterobalanus* originated in subtropical broad-leaf forests. After the uplifting of Hengduan Mountains where the climate became cold (Zhou, 1993), and such an environment was not favorable for most broad-leaf evergreen trees again. However, the oaks of sect. *Heterobalanus*, having obvious xerophytic characters such as dense hairs, thick cuticles, lignified epidermal cell walls and cuticles and low stomatal density (Zhou et al., 2003), were adapted to the environmental change, and therefore became dominant in this area (Zhou and Coombes, 2001). Although the physiological and ecological adaptation of evergreen sclerophyllous oak has long interested ecologists (He et al., 1994), the ecophysiology of alpine plants in the Hengduan Mountains has not been well studied (Terashima et al., 1993).

Morphological and AFLP evidence suggested that oaks of this section were very similar to Mediterranean oaks such as *Q. ilex* and *Q. suber* (Zhou et al., 2003) whose physiological traits have been widely studied (Tenhunen et al., 1981; Damesin et al., 1997; Mediavilla and Escudero, 2004). For those Mediterranean oaks, drought, high irradiance and high temperature were the most important factors limiting photosynthesis and growth (Garcia-Plazaola et al., 1997). However, the applicability of these Mediterranean studies to species from the monsoon climate in the Hengduan Mountains was limited because the climate in the Hengduan Mountains is characterized by temperate and rainy summers, cold and dry winters, which differs from Mediterranean region.

Previous studies suggested that the oaks of sect. *Heterobalanus* could adapt to alpine environment, and therefore became dominant in the Hengduan Mountains due to their xerophytic characters (Zhou, 1993; He et al., 1994; Zhou and Coombes, 2001; Zhou et al., 2003). However, the hypothesis has been lacking in support from physiological information. We studied the photosynthetic traits of *Q. pannosa* at three sites of different altitudes. The goals were to (1) understand the photosynthetic adaptability of sclerophyllous oaks to alpine environments and (2) explore the relationship between the physiological characteristics and distribution.

2. Materials and methods

2.1. Site and plant description

The research was carried out at three sites along an altitudinal gradient in the Hengduan Mountains, site A (E 99°38.797′, N 27°46.096′), B (E 99°42.461′, N 28°00.144′) and C (E 99°49.752′, N 28°32.568′) at altitudes of 3240, 3650 and 4170 m, respectively. Mean annual temperature and precipitation were 5.4 °C and 624.8 mm (30 year mean) at site A. Previous study in this region showed that the altitudinal lapse rate of air temperature was 0.71 °C/100 m, but there was no significant change in precipitation at altitudinal range of 3000–4500 m (Zhang, 1998). According to the altitudinal lapse rate of air temperature, the annual mean air temperature at sites B and C are 2.5 and −1.2 °C, respectively. In addition, the climate from

May to October is the rainy season with 87% annual rainfall, while dry season occurs from November to April. The soil types at three sites are brown soil with pH values of 6.2–6.9 and similar fertility.

Quercus pannosa Hand.-Mazz. is an endemic tree that occurs in the mountain oak forests or pine oak mixed forests at altitudes between 2500 and 4300 m in the southwest of China. It is an important component of the evergreen sclerophyllous broad-leaved forest. *Q. pannosa* flowers from May to June and acorns mature in two years (Zhou and Coombes, 2001). *Q. panosa* can grow up to height of 15 m, but the sampling trees are generally 4–5 m in height.

2.2. Gas exchange

Observations at three sites were conducted in May (flowering period) 2003. The trees of *Q. pannosa* chosen were approximately 4–5 m in height. Five trees were selected and labeled for measurements at each site.

Diurnal photosynthetic courses were measured on May 15, 21 and 25 in 2003 at sites A–C, respectively. Five fully expanded leaves per site were randomly selected from sampling trees for measurements hourly from 08:00 to 19:00 h. The photosynthetic rate (P_n), transpiration rate (E), stomatal conductance (G_s) and intercellular CO_2 concentration (C_i), leaf temperature (T_l) and photosynthetically active radiation (PAR) were measured using a portable infrared gas exchange system with a leaf chamber type PLC-B (CIRAS-1, PP Systems, UK) in open-system configuration. Meanwhile, a Li-188 quantum sensor, thermometer and hygrometer were used to record irradiance, air temperature and relative air humidity hourly, respectively. Leaf and air temperature and relative humidity were used to calculate vapor pressure deficit (VPD) between leaf and air. The water use efficiency (WUE) was calculated as the ratio between P_n and E according to Wang et al. (2000).

Light-saturated rate of photosynthesis (P_{max}) was measured at ambient CO_2 concentration, PAR of 1200 μmol m^{-2} s^{-1} and temperature 20 °C. Preliminary measurements showed that this light intensity was above the light-saturation point for these leaves. Light-saturated rates of photosynthesis were measured between 09:00 and 11:00 h. Ten fully expanded leaves were measured at any given site.

The photosynthetic response curves to CO_2, light and temperature at sites A–C were measured on May 18, 19 and 23 in 2003. Photosynthetic responses to light were measured at 14 light levels on leaves at each site using a CIRAS-1 infrared gas analyzer. The CO_2 concentration in the leaf chamber was set to 350 μmol mol^{-1} and temperature 20 °C. Sampled leaves were allowed to acclimate to the PAR of 1200 μmol m^{-2} s^{-1} before measurements. After the initial measurement at 0 μmol m^{-2} s^{-1}, light intensity was increased to produce 13 subsequent light levels at which photosynthetic rates were recorded. The CO_2 responses of photosynthesis were determined with a range of CO_2 concentrations (0–1600 μmol mol^{-1}) at a light intensity of 1200 μmol m^{-2} s^{-1} and temperature of 20 °C. After the initial measurements at 1600 μmol mol^{-1}, CO_2 concentration was reduced to produce other subsequent levels at which photosynthesis were recorded. Three leaves were measured at each site. Curves fitting software (Sigmaplot 8.0) was used to analyze both the P_n–C_i and P_n–PAR responses with a three component exponential function of form (Watling et al., 2000).

$$P_n = a(1 - e^{-bx}) + C \qquad (1)$$

where P_n = photosynthetic rate and $x = C_i$ or PAR. Using this equation, the apparent quantum yield was calculated as the initial slope of P_n–PAR curves in the range of 0–400 μmol m^{-2} s^{-1} following Swanborough et al. (1997). The carboxylation efficiency was estimated as the initial slope of the P_n–C_i curves (0–200 μmol mol^{-1}).

The dependencies of net photosynthesis on temperature were examined with fully expanded leaves using an infrared gas analyzer between 08:00 and 11:00 h to avoid high temperatures at mid-day. During the measurement, the CO_2 concentration in the reference chamber was kept at 350 μmol mol^{-1} and PAR 1200 μmol m^{-2} s^{-1}. Measurements began at 10 °C and proceeded at 1 °C increments until 35 °C. The second-order polynomial equation was used to fit the optimum photosynthetic temperature.

2.3. Leaf traits

After measuring photosynthetic responses, the leaves were harvested from the sampling trees, and sealed in a bag with silica gel and dry-stored. In the laboratory, leaf areas were measured using a leaf area meter (Li-3000A, USA). The dry mass was determined after drying for 48 h in an oven at 70 °C. Then, these leaves were ground to powder and leaf N concentrations were analyzed using an N analyzer (Leco FP-428, USA). LMA was calculated as leaf mass per unit leaf area (g m^{-2}).

Pigment contents were extracted from 20 leaf disk (0.38 cm^2 per disk) of five sampling trees with 3 ml *N*,*N*-dimethylformamide and stored in the dark at 4 °C. Pigment contents were analyzed with a spectrophotometer (Shimadzu-2501, Japan) and calculated by the method of Wang et al. (2000). Three replicates were made for each measurement.

Chlorophyll fluorescence was determined using a pulse amplitude modulated fluorometer (FMS 2, Hanstech, UK). After adaptation for 25–30 min in the dark, the fluorescence parameters of *Q. pannosa* of leaf were measured. The F_v/F_m ratio ($F_v/F_m = 1 - F_0/F_m$) was used to estimate the photoinhibition of PS II and a loss in F_v/F_m represented the aggravation of photoinhibition (Genty et al., 1989).

2.4. Statistical analysis

Statistical analysis was performed using SPSS Version 10.0 (SPSS Inc., Chicago, IL, USA). Normality of distribution and homogeneity of variance were checked before any further statistical analysis. Differences in leaf morphological and physiological variables were determined using analysis of variance (ANOVA) and LSD test for multiple comparisons. We addressed relationships between photosynthetic parameters and leaf traits using regression analysis.

3. Results

3.1. Diurnal course of environmental factors and leaf gas exchange

At all sites, the maximum PAR, temperature and VPD were observed from 13:00 to 15:00 h when the air humidity was the lowest (Fig. 1). The daily PAR averaged 1392.5 ± 214.4, 1546.3 ± 156.9 and

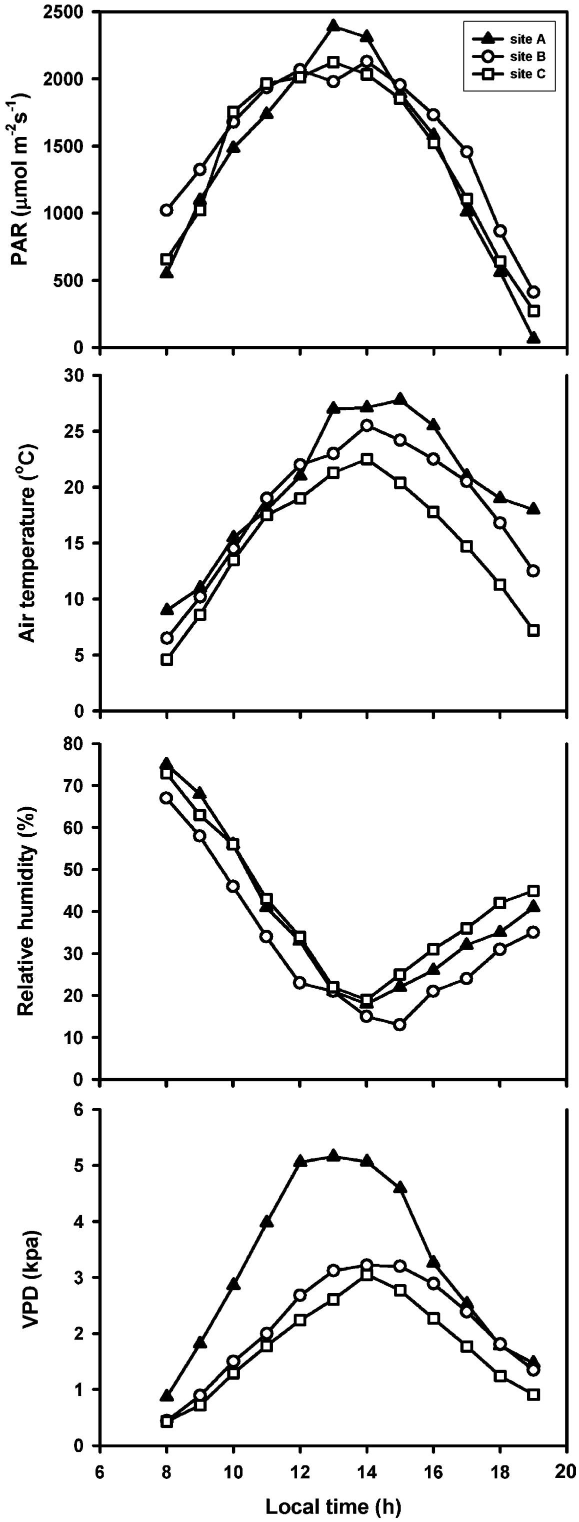

Fig. 1. Diurnal variations of PAR, air temperature, relative humidity and VPD at site A (▲–▲), B (○–○) and C (□–□) in the Hengduan Mountains.

1413.6 ± 186.6 μmol m^{-2} s^{-1} between 08:00 and 19:00 h at sites A–C, respectively. Maximum mean PAR was at site B, but there were no significant differences among three sites (LSD = 0.198, $p = 0.821$). Air temperature decreased as altitude increased (LSD = 2.208, $p = 0.04$). Relative humidity did not vary significantly among three sites (LSD = 0.786, $p = 0.464$), but VPD decreased with increasing elevation (LSD = 5.096, $p = 0.012$).

At all sites, the maximum values of stomatal conductance occurred in the early morning, decreased towards mid-day and increased in the afternoon (Fig. 2). The diurnal variation of G_s was similar to that of relative humidity, an inverse trend to VPD. The G_s of plants at lower altitudes did not significantly differ from those of plants at higher altitudes (LSD = 0.392, $p = 0.676$).

The diurnal variation of E, P_n of *Q. pannosa* showed a significant mid-day depression. The maximum value of E was observed at around noon at site B, but there were no significances among three sites (LSD = 0.327, $p = 0.723$). The average P_n of five leaves at different altitudes peaked rapidly after dawn, before subsiding during the middle of the day. They reached maximum values at about 09:00–10:00 h at site C. After 15:00 h, P_n increased gradually again and reached a second-peak in the late afternoon. Both daily mean photosynthetic rate (P_{day}) and light-saturated photosynthesis (P_{max}) of plants decreased with elevation (Fig. 2). The light-saturated photosynthetic rates of *Q. pannosa* at sites A–C were 8.17 ± 0.53, 7.11 ± 0.71 and 5.47 ± 0.35 μmol m^{-2} s^{-1}, respectively. Plants at higher altitude had higher P_{max} than those at lower altitude (LSD = 7.622, $p = 0.006$).

The quotient between P_n and E was used to estimate WUE. The diurnal variations of WUE were similar to P_n. They were higher in the early morning, reaching a minimum at about mid-day and increasing after 15:00 h (Fig. 2). There were no significant differences in WUE among three sites (LSD = 0.276, $p = 0.760$).

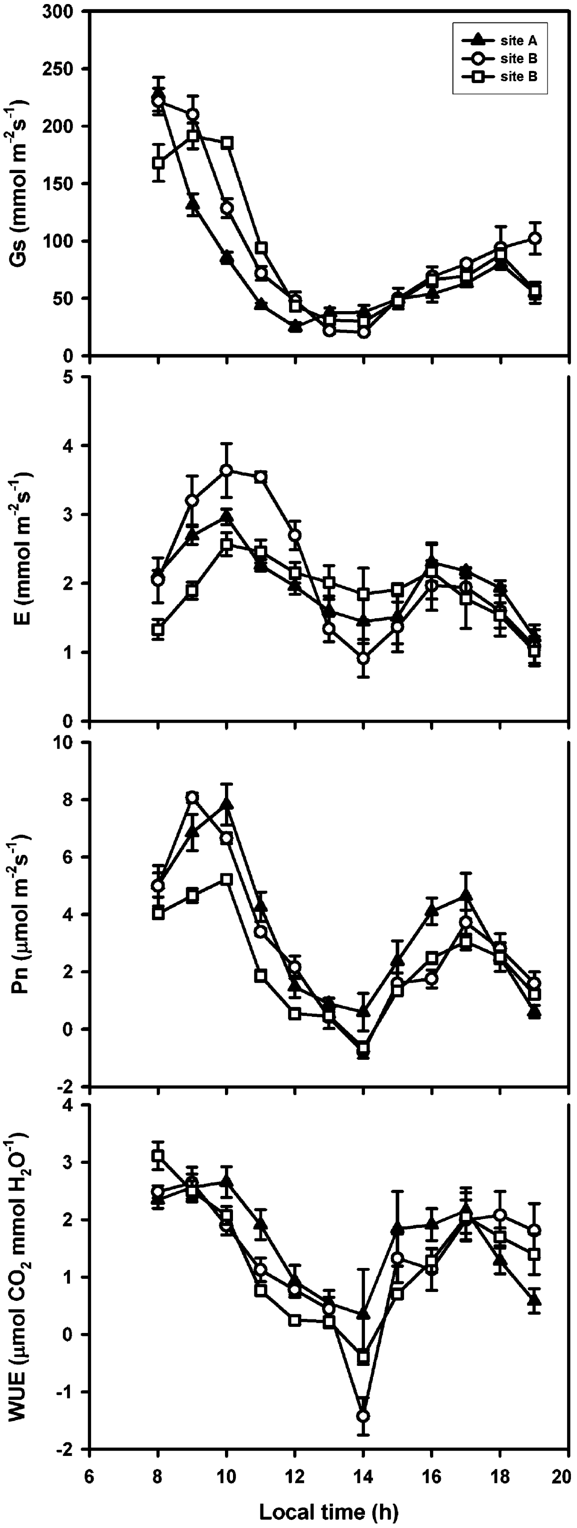

Fig. 2. Diurnal patterns of stomatal conductance (G_s), transpiration rate (E), net photosynthesis (P_n) and water use efficiency (WUE) of *Q. pannosa* grown at site A (▲–▲), B (○–○) and C(□–□). Each point is a mean of five measurements. Error bars represent ±1S.E.

3.2. The effects of environmental factors on photosynthesis

The photosynthetic optimum temperatures (T_{opt}) of *Q. pannosa* decreased as altitude increased (Fig. 3c). The T_{opt} of *Q. pannosa* at sites A–C were 19.2, 17.1 and 14.3 °C, respectively. Net photosynthesis decreased over 50% when leaf temperature was over 30 °C at all sites.

With the increase of altitude, the apparent quantum yields (AQY) of *Q. pannosa* were reduced. They were 0.030, 0.023 and 0.022 mol CO_2 mol photons^{-1} at sites A–C, respectively. There was no significant difference in photosynthetic light compensation point (21–24.9 μmol m^{-2} s^{-1}), but the photosynthetic light-saturation points (PAR to achieve 99% of P_{max}) of oak at site B was higher than those at sites A and C (Fig. 3b). However, there was no evidence of photoinhibition at any site under high light intensity.

Both photosynthetic rate and carboxylation efficiency were higher at low altitude sites (LSD = 7.62, $p = 0.023$) (Table 1). The CO_2 compensation point decreased with the increasing elevation (Fig. 3a). In addition, VPD had significant effect on the photosynthesis ($R = 0.462$, $p = 0.005$) (Table 2).

3.3. The relationship between chlorophyll fluorescence and PAR or temperature

Photoinhibition of photosynthesis of *Q. pannosa* was determined by F_v/F_m. The relationships between F_v/F_m and temperature or PAR were addressed using a second-degree polynomial equation (Fig. 4). Both high temperature and high PAR inhibited the photosynthesis of *Q. pannosa*, but the effect of temperature on F_v/F_m was more significant than that of PAR (Table 2).

3.4. Leaf characteristics of Q. pannosa

The content of chlorophyll *a* and chlorophyll *a* + *b* of *Q. pannosa* were significantly different among altitudes (Table 1). High-altitude plants tended to have higher contents of chlorophyll *a* (LSD = 32.667, $p = 0.001$) and chlorophyll *a* + *b* (LSD = 16.370, $p = 0.004$). However, the chlorophyll *a*:*b* at site B were the highest among three sites.

Leaf N content per unit leaf area of *Q. pannosa* decreased with increasing altitude (LSD = 3.4,

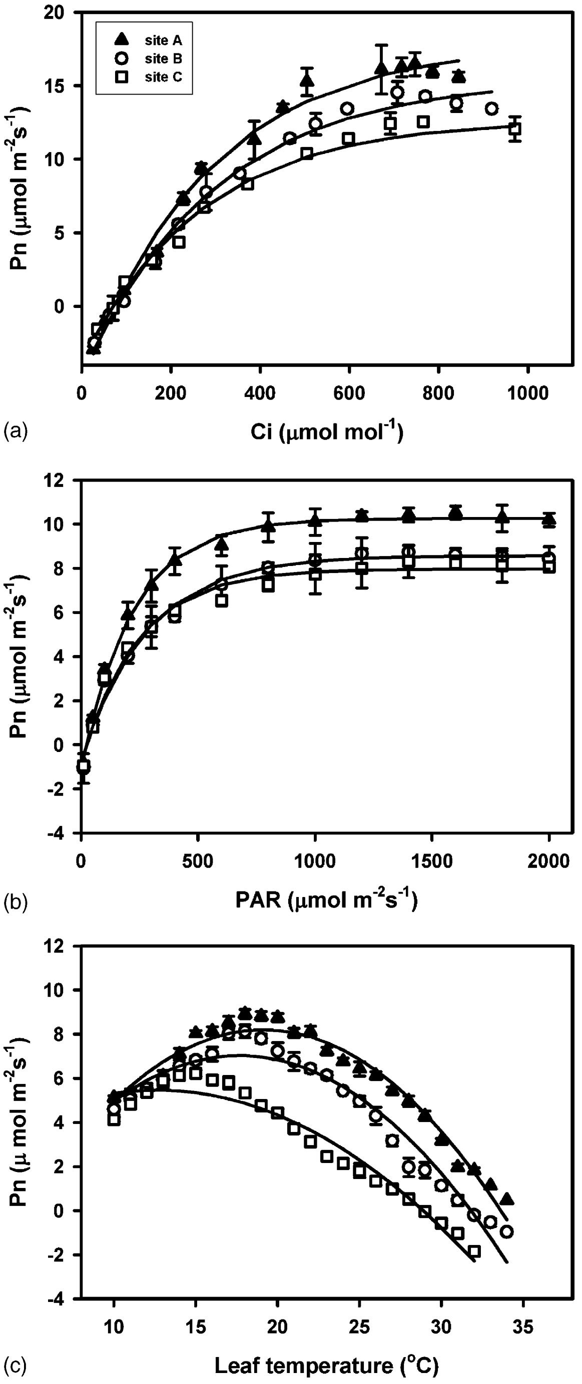

Fig. 3. Photosynthetic responses of *Q. pannosa* to CO_2 (a), PAR (b) and leaf temperature (c) at site A (▲–▲), B (○–○) and C (□–□). Vertical bars indicate standard errors of means for three measurements.

Table 1
Photosynthetic variables and leaf traits of *Q. pannosa* at different altitudes in the Hengduan Mountains

	Altitude (m)			LSD, $p < 0.05$
	3240	3650	4170	
P_{max} (μmol m^{-2} s^{-1})	8.17 ± 0.53 a	7.10 ± 0.71 a	5.47 ± 0.35 b	6.16
CE (mol mol^{-1})	0.047 ± 0.002 a	0.044 ± 0.002 a	0.038 ± 0.002 b	7.62
Chlorophyll *a* (μg cm^{-2})	21.19 ± 0.70 a	19.94 ± 0.30 a	15.53 ± 0.49 b	32.67
Chlorophyll *b* (μg cm^{-2})	16.17 ± 0.50 a	13.47 ± 0.47 b	13.98 ± 0.37 b	10.27
Chlorophyll *a* + *b* (μg cm^{-2})	37.36 ± 1.13 a	33.41 ± 0.96 b	29.51 ± 0.78 c	16.37
Chlorophyll *a*/chlorophyll *b*	1.31 ± 0.03 a	1.48 ± 0.04 b	1.11 ± 0.03 c	35.74
C_i/C_a	0.75 ± 0.007 a	0.78 ± 0.009 b	0.84 ± 0.007 c	27.44
G_s (mmol m^{-2} s^{-1})	99.05 ± 7.16 a	93.17 ± 8.57 a	89.35 ± 7.61 a	0.39
Leaf N concentration (g m^{-2})	0.36 ± 0.02 a	0.32 ± 0.02 ab	0.29 ± 0.02 b	3.40
Leaf mass per unit area (g m^{-2})	177.96 ± 9.85 a	224.58 ± 8.96 b	263.34 ± 20.7 b	9.08

Data were means with 1S.E. The same letters (a–c) in a row indicate no significant differences ($p \geq 0.05$), light-saturated photosynthetic rate (P_{max}), carboxylation efficiency (CE), the ratio of intercellular to the ambient CO_2 concentration (C_i/C_a) and stomatal conductance(G_s).

$p = 0.048$). In contrast, leaf mass per unit (LMA) of *Q. pannosa* significantly increased with increasing altitude (LSD = 9.076, $p = 0.001$).

4. Discussion

4.1. Altitudinal patterns in photosynthesis and leaf traits

There were lots of reports on altitudinal trends in photosynthesis and leaf characteristics. However, no clear consensus on effects of altitude on photosynthesis has been reached. Most literatures indicated an increase in CO_2 assimilation and carboxylation efficiency with increasing altitude (Mächler and Nösberger, 1977; Woodward, 1986; Körner and Diemer, 1987). These results could be explained by an increase in CE, leaf nitrogen content and leaf conductance with altitude (Woodward, 1986; Friend et al., 1989; Körner and Diemer, 1987). In contrast to these results, our study provided a conflicting result. This discrepancy may be due to the differences in equipment, plant material and altitudinal range

Table 2
Regression analysis of factorial correlations for *Q. pannosa*

Y–*X*	Relationship	*F*	*R*	Significance
F_v/F_m–PAR	$Y = 0.800 + (4.238e - 5)x - (2.217e - 8)x^2$	7.755	0.342	0.0007[***]
F_v/F_m–temperature	$Y = 0.766 + 0.006x - (0.172e - 3)x^2$	38.724	0.631	0.0001[***]
P_{day}–VPD	$Y = 4.877 - 0.838x$	9.207	0.462	0.005[**]
P_{max}–chl *a*	$Y = -1.571 + 0.449x$	12.131	0.550	0.002[**]
P_{max}–chl *b*	$Y = -2.433 + 0.643x$	4.711	0.380	0.039[*]
P_{max}–chl *a* + *b*	$Y = -4.575 + 0.344x$	12.477	0.555	0.001[**]
P_{max}–leaf N	$Y = -5.344 + 37.919x$	12.012	0.548	0.002[**]
P_{max}–LMA	$Y = 13.870 - 0.031x$	12.208	0.551	0.002[**]
P_{max}–C_i/C_a	$Y = 25.751 - 23.780x$	7.531	0.460	0.010[*]
Chl *a*:*b*–PAR	$Y = -1.186 + 0.002x$	8.425	0.739	0.023[*]
CE–C_i/C_a	$Y = 0.119 - 0.096x$	17.775	0.847	0.004[**]

Ratio of variable to maximum fluorescence (F_v/F_m), light-saturated photosynthetic rate (P_{max}), carboxylation efficiency (CE), the ratio of intercellular to the ambient CO_2 concentration (C_i/C_a), photosynthetically active radiation (PAR), chlorophyll *a* content per unit leaf area (Chl *a*), chlorophyll *b* content per unit leaf area (Chl *b*), ratio of chlorophyll *a* content to chlorophyll *b* content (Chl *a*:*b*), total chlorophyll content per unit leaf area (Chl *a* + *b*), daily mean photosynthetic rate (P_{day}), light-saturated photosynthetic rate (P_{max}), leaf mass per unit area (LMA) and leaf nitrogen concentration per unit area (leaf N).

[*] $p \leq 0.05$.

[**] $p \leq 0.01$.

[***] $p \leq 0.001$.

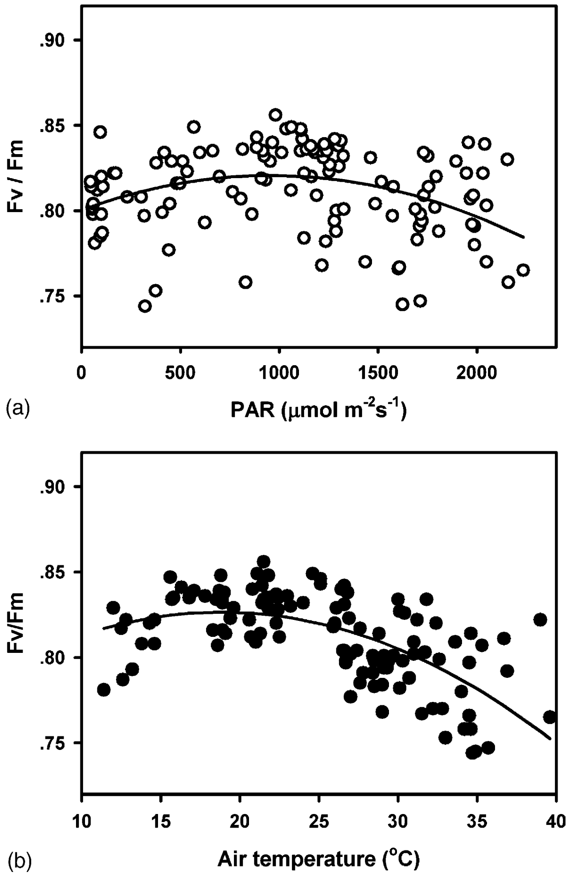

Fig. 4. The relationships between F_v/F_m of *Q. pannosa* and PAR (a) or air temperature (b) in the Hengduan Mountains.

(Körner and Diemer, 1987; Friend and Woodward, 1990; Rada et al., 1998). Gas exchange studies along low gradients (below 2500 m a.s.l.) suggested an increase photosynthetic rate with increasing altitude, but this was not true at higher altitudinal range (Rada et al., 1998; Cabrera et al., 1998).

However, the reduction in photosynthetic rate of *Q. pannosa* with increasing altitude was similar to those on other plants (Rada et al., 1998; Cabrera et al., 1998). The lower photosynthetic rates at higher altitudes have been considered to be caused by significant increase in thickness of leaf pubescence, lower stomata1 conductance and leaf nitrogen content at higher altitudes (Rada et al., 1998; Cabrera et al., 1998). In current study, several leaf characteristics should be responsible for the reduction in photosynthetic rate of *Q. pannosa* with increasing altitude.

The altitudinal difference in photosynthesis could be caused by differences in photosynthetic pigments (Friend and Woodward, 1990). The content of chlorophyll *a* and chlorophyll *a* + *b* of *Q. pannosa* were significantly different among altitudes (Table 1). High-altitude plants tended to have lower contents of chlorophyll *a* and chlorophyll *a* + *b* (Todaria and Thapliyal, 1980). Chlorophyll *a* and chlorophyll *a* + *b* content per unit area were positively correlated with P_{max} (Table 2). Lower chlorophyll contents of *Q. pannosa* at higher altitude appear to be linked to lower P_{max}.

Although most studies show that leaf N content on an area basis increased with elevation (Friend and Woodward, 1990; Cordell et al., 1999), leaf N content of *Q. pannosa* decreased with increasing altitude (Table 1). Similar result was observed at *Espeletia Schultzii* (Rada et al., 1998). Photosynthetic capacity (P_{max}) generally increased with leaf nitrogen content per unit area because photosynthetic enzymes such as RuBP carboxylase contained large quantities of N (Evans, 1989). The leaf N concentration per unit area was lower and allocation of leaf N to the photosynthetic apparatus was smaller in high-altitude plants compared with low altitude plants (Hikosaka et al., 2002). Lower leaf N content would be an important determinant of lower P_{max} of *Q. pannosa* at higher altitude (Rada et al., 1998).

Changes in CE and δ^{13}C value should be correlated with the ratio of intercellular to the ambient CO_2 partial concentration (C_i/C_a) (Farquhar et al., 1982). Ratio of C_i/C_a could be affected by stomatal conductance and RuBP carboxylase. If C_i/C_a increased with altitude, the CE and δ^{13}C value of leaf would be expected to decrease (Körner and Diemer, 1987). Most studies indicated that δ^{13}C values and carboxylation efficiencies increased with increasing elevation (Körner and Diemer, 1987; Friend and Woodward, 1990). However, altitudinal pattern in δ^{13}C value was related to annual climate (Friend et al., 1989). In Hengduan Mountains, although stomatal conductance of *Q. pannosa* increased slightly, there were no significant differences among altitudes (LSD = 0.392, $p = 0.676$). Carboxylation efficiency of *Q. pannosa* decreased with altitude, but C_i/C_a increased with altitude. The higher carboxylation efficiency at lower altitude was linked to lower C_i/C_a ratio.

Likewise, the trend in leaf morphology has also been correlated with photosynthetic variables that varied with altitude. The observed trends in leaf morphology of *Quercus* with increasing altitude included: progressively higher LMA, thicker leaf pubescence, larger stomatal size and lower stomatal density (He et al., 1994) and relatively constant stomatal conductance. Increase in stomatal density with increasing altitude might compensate for the reduction in CO_2 partial pressure, but stomatal conductance might not necessarily be related to stomatal size and stomatal density (Kao and Chang, 2001). The thicker leaf pubescence and higher LMA were caused by lower temperature at higher altitude (Meinzer et al., 1985; Friend and Woodward, 1990; Kao and Chang, 2001). Thickness of leaf pubescence and, therefore leaf boundary layer resistance, also increasing with altitude resulted in both potentially higher leaf temperature relative to air temperature and higher leaf to air vapor pressure gradient (Meinzer et al., 1985). High LMA was often associated with higher leaf $\delta^{13}C$ value (Hultine and Marshall, 2000), higher leaf nitrogen content and photosynthetic rate per unit leaf area at higher altitude (Friend and Woodward, 1990; Körner, 2003), but this is not true in *Q. pannosa*. Similar results were also observed by other researchers (Mediavilla et al., 2001; Kao and Chang, 2001). Longer diffusive path found in thicker leaf would increase resistance, and thus reduce CO_2 concentrations at the site of carboxylation (Kao and Chang, 2001). In addition, in species with higher LMA, photosynthesis was reduced by non-stomatal limitations, possibly because of a lower allocation of N to the photosynthetic machinery than in species with lower LMA (Mediavilla et al., 2001).

4.2. Photosynthetic adaptability of Q. pannosa

Resource availability and environmental conditions would influence the distribution and functional characteristics of species. Low temperature, strong irradiance and low CO_2 pressure have been considered to mold many features of alpine plant. In the Hengduan Mountains, *Q. pannosa* experienced a pronounced mid-day depression in P_n, WUE and G_s, this was similar to the responses found for Mediterranean oaks *Q. suber* and *Q. ilex* (Tenhunen et al., 1981, 1984; Pathre et al., 1998). The mid-day depression in photosynthesis of *Q. pannosa* was accompanied by the reducing stomatal conductance and relative humidity, the increasing irradiance and temperature and VPD. The sclerophyllous oaks could withstand much higher PAR (up to 2000 μmol m^{-2} s^{-1}) than their saturation limit without decreasing the photosynthesis substantially, provided temperature was favorable (Fig. 3). The T_{opt} of *Q. pannosa* was relatively low. When leaf temperature was over 25 °C, the photosynthetic rate decreased sharply at all sites. In fact, the leaf temperature was generally above 25 °C from 11:00 to 15:00 h at three sites. High temperature resulted in the decrease in humidity and increase VPD, therefore reduced G_s (Filella et al., 1998). Our experiments provided evidence for the dominant role of high temperature and low humidity on mid-day depression of *Q. pannosa*.

Comparing with Mediterranean oaks, *Q. pannosa* had lower T_{opt} due to the lower growth temperature. Previous studies showed that the favorable leaf temperature for Mediterranean oaks allowing 90–100% of P_{max} was 14–28 °C, decreasing over 50% when leaf temperature were, respectively, below 6 °C and over 37 °C (Gratani et al., 2000). When leaf temperatures were above 35 °C, the photosynthetic rates of *Q. pannosa* at three sites reduced to zero. Apparently, *Q. pannosa* exhibited more sensitive to high temperature than Mediterranean oaks. The water use efficiencies of Mediterranean oaks were significantly higher than those of *Q. pannosa* (2.5–3.8 μmol versus 1.3–1.6 μmol CO_2 mol^{-1} H_2O) (Gratani et al., 2000; Ogaya and Peñuelas, 2003). *Q. pannosa* showed greater water availability response dependence than Mediterranean oaks due to more humid condition in the Hengduan Mountains. Likewise, the photosynthetic light responses of Mediterranean oaks and *Q. pannosa* were similar. Both of them could withstand very high PAR without decreasing the photosynthesis (Ogaya and Peñuelas, 2003). The LMA of *Q. pannosa* was higher than those of Mediterranean oaks (178–263 g m^{-2} versus 86–242 g m^{-2}) (Damesin et al., 1997; Mediavilla et al., 2001). Lower temperature in the Hengduan Mountains could reduce leaf extension of *Q. pannosa* and result in thicker leaves (Friend and Woodward, 1990).

Temperature would determine altitudinal tree distribution in different ways: affecting survival through freezing or by a negative carbon balance by lower photosynthetic rate (Cavieres et al., 2000). Similar sclerophyllous oaks (*Q. engleriana*, *Q. ilex*, etc.) were distributed in the xerothermic regions, but *Q. pannosa* occurred in the relatively cold and moist habitats in the Hengduan Mountains. Its drought-tolerance was usually covered by cold-tolerance trait. The T_{opt} of this oak was significantly lower than that of Mediterranean sclerophyllous oak (25–30 °C) (Tenhunen et al., 1984; Gratani et al., 2000). Sakai (1981) found that *Q. semecarpifolia,* occurring through Himalaya to southwest China, could resist the low temperature of -15 °C. The physiological and morphological evidences suggested that *Q. pannosa* had a strong tolerance for low temperature. The strong adaptability to low temperature was one of the most important factors affecting its distribution. At other hand, the reduction in photosynthetic capacity of *Q. pannosa* would be caused by low temperature stress because the trees would suffer from freezing at the night of growing season in the Hengduan Mountains.

In conclusion, the reduction in photosynthetic capacity of *Q. pannosa* with increasing altitude could be linked to the increasing LMA and C_i/C_a, the decreasing leaf N concentration and photosynthetic pigments, also low temperature stress. In addition, *Q. pannosa* exhibited a strong tolerance to lower temperature but high temperature. This cold-resistance played an important role in the distribution patterns of *Q. pannosa* in the Hengduan Mountains. Our study provided support for the hypothesis that sclerophyllous oaks could adapt well to cold climate (Zhou, 1993; Zhou et al., 2003).

Acknowledgements

The authors thank Prof. Cunxin Li and Mr. Hua Wang for experiment assistance and Dr. David Melick and Dr. Shaotian Chen for English improving, and also for the comments and suggestions of two referees significantly improved the manuscript. We are grateful to the National Key Basic Research Program (973) (2003CB415102), National Natural Science Foundation of China (30170077, 30270151) and Natural Science Foundation of Yunnan Province (2002C0062M).

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