

Photosynthetic characteristics of two alpine flowers, *Meconopsis integrifolia* and *Primula sinopurpurea*

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SUMMARY

Meconopsis integrifolia and *Primula sinopurpurea* are ornamental plants native to high elevations in the Himalayas, thus cultivating them at lower altitudes poses challenges. In order to understand the physiological adaptability of *M. integrifolia* and *P. sinopurpurea* to the different environments which are needed for the introduction and domestication of wild flowers, this study investigated their photosynthetic performance at lower altitudes in a nursery at an altitude of 3,260 m asl, and their photosynthetic responses to varying light and temperature conditions. On a clear day, the maximum photosynthetic rates in both species occurred at approx. 11:00 h, then decreased, indicating photo-inhibition at midday. Stomatal limitation played an important role in the depression of photosynthesis at midday. Although *M. integrifolia* had a lower light-saturated photosynthetic rate than *P. sinopurpurea*, the daily mean photosynthetic rates of the two species were not significantly different. Photosynthesis in both alpine flowers was considerably sensitive to high temperature. The significant decreases in photosynthetic rates in *P. sinopurpurea* and *M. integrifolia* at higher temperatures would limit their photosynthetic performance and growth at low altitudes.

Meconopsis integrifolia Fr. and *Primula sinopurpurea* Balf. F. ex Hutch. are well-known ornamental alpine flowers. Both species are native to rocky and grassy areas at altitudes from 3,000–4,600 m asl in western China. Large-scale cultivation under controlled conditions is necessary to supply the increasing demand for these species in the ornamental trade. As alpine plants often have very specific conditions for growth, flowering, and reproduction (Weng *et al.*, 2002), cultivating them at lower altitudes poses considerable challenges.

To cultivate wild species successfully requires sound information on their growth conditions (Aleric and Kirkman, 2005). Plants growing in alpine environments are exposed to low temperatures and high levels of irradiance. *Meconopsis integrifolia* and *P. sinopurpurea* can grow and flower under low temperatures (Norton and Qu, 1987), but we observed that high temperatures during the growing season limited their growth and development. This phenomenon may result from the fact that their tolerance of low temperature limits their adaptability to high temperatures (Sharkey, 2000). *Meconopsis punicea* and *M. betonicifolia* grown at lower temperatures had higher plant dry weights (DW) and flower sizes than those grown under warmer conditions (Still *et al.*, 2003). Buchner and Neuner (2003) suggested that the photosynthetic apparatus of alpine plants was extremely sensitive to heat stress. The growth and survival of plants are determined by the thermo-tolerance of their photosynthetic apparatus (Sharkey, 2000), as high temperatures can decrease the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase,

perturb electron transport, induce the production of reactive oxygen species (ROS), and thus affect carbon assimilation and growth (Sharkey, 2000; Crafts-Brandner and Salvucci, 2000). Plants often show a suite of adaptations to different light conditions in order to maximise their photosynthetic efficiency (Givnish, 1988). Insufficient irradiance may reduce carbon assimilation and growth, while high irradiance may damage the photosynthetic apparatus (Aleric and Kirkman, 2005; Zhang *et al.*, 2007). Unfortunately, little is known about the physiological adaptability of alpine *Primula* and *Meconopsis* spp. to their environment (Still *et al.*, 2003; Liu *et al.*, 2004; Zhang and Hu, 2008).

The growth and development of plants depends on the physiological suitability of their growing environments, which determine their rates of photosynthetic carbon fixation and growth (Wu and Campbell, 2006). Responses to temperature such as DW accumulation and flower size in alpine *Meconopsis* spp. have been studied (Still *et al.*, 2003). However, the physiological adaptations of alpine species such as *Primula* and *Meconopsis* to higher temperatures and lower irradiances, have not been well studied. Chlorophyll fluorescence and photosynthetic measurements are used widely to predict plant performance and physiological tolerance of environments (Aleric and Kirkman, 2005; Elsheery *et al.*, 2007). Such parameters can also be employed to study the physiological adaptations of alpine plants to changing temperatures and irradiance.

In the present study, diurnal variations in photosynthesis and chlorophyll fluorescence in *M. integrifolia* and *P. sinopurpurea*, as well as their photosynthetic responses to light and temperature, were

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studied in a nursery at an altitude of 3,260 m asl. The main goal was to understand the photosynthetic adaptations of alpine plants to varying light and temperature conditions, and to identify the limiting factor(s) that limit their widespread cultivation at lower altitudes.

MATERIALS AND METHODS

This study was conducted at the Shangri-la Experimental Station of Alpine Flowers in southwestern China, at an altitude of 3,260 m asl. From May to September 2005, the monthly mean air temperature was 12.3°C and the total precipitation was 430 mm. The relative air humidity (RH) between May and September had an average value of 78.2% (1958 – 2000 data; Meteorological Station, Shangri-la County).

Seeds of *P. sinopurpurea* and *M. integrifolia* were collected at an altitude of 4,300 m from the Baima Snow Mountain, southwestern China in August 2003. Seeds of both species were sown in the Experimental Station nursery in March 2004. All seedlings were grown in full sunlight and fertilised each month with a liquid solution N:P:K [15:15:15 (w/w/w); Kunming Dilifert Plant Nutrition Industry Ltd., Kunming, P. R. China] during the Summer, and watered every 2 – 3 d during periods without rainfall.

Diurnal variations in gas exchange were measured during the flowering period in May 2005. Five fully-expanded leaves from different plants of each species were selected and measurements were taken every hour between 08:00 h – 19:00 h. Photosynthetic rate (P_n), transpiration rate (E), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were measured using a portable infrared gas analyser (CIRAS-1; PP Systems, Hitchin, UK). Meanwhile, photosynthetic photon flux density (PPFD) and air temperature (T_a) were recorded by a data logger (Li-1400; Li-Cor Ltd., Lincoln, NE, USA). Water use efficiency (WUE) was calculated as the ratio of P_n to E . The stomatal limitation value (L_s) was calculated using the function by Berry and Downton (1982):

$$L_s = 1 - C_i / (C_a - \Gamma)$$

where C_a was the ambient CO_2 concentration, and the CO_2 compensation point (Γ) was calculated from $P_n - C_i$ curves.

Chlorophyll fluorescence was measured in five leaves of each species every hour between 08:00 h – 19:00 h using a pulse modulated fluorometer (FMS-2; Hanstech Ltd., Norwich, UK). After the minimal fluorescence (F_o) was determined using a weak modulated light source, a 0.8 s pulse of saturating light at $6,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ was used on dark-adapted leaves (30 min) to determine the maximum fluorescence (F_m). Then, the steady-state fluorescence (F_s), minimum fluorescence (F_o') and maximum fluorescence (F_m') were measured on leaves illuminated by actinic light ($1,200 \mu\text{mol m}^{-2} \text{s}^{-1}$).

The following chlorophyll fluorescence parameters were calculated: (i) potential quantum yield of PSII: $F_v/F_m = (F_m - F_o) / F_m$; (ii) effective quantum yield of PSII: $\phi_{PSII} = (F_m' - F_s) / F_m'$; (iii) photochemical efficiency of open reaction centres: $F_v'/F_m' = (F_m' - F_o') / F_m'$; (iv) apparent rate of electron transport of PSII: ETR

$= 0.5 \phi_{PSII} Q_{abs}$, where Q_{abs} was the absorbed light energy that was calculated as PPFD \times leaf absorbance, and leaf absorbance was taken as 0.85; (v) photochemical quenching: $qP = (F_m' - F_s) / (F_m' - F_o')$; and, finally (vi) non-photochemical quenching: $NPQ = (F_m - F_m') / F_m'$.

The photosynthetic responses to light were measured on five fully-expanded leaves, each from a different plant, using a CIRAS-1 infrared gas analyser with a PLC-B leaf chamber (PP Systems). The light source was a tungsten lamp with a calflex filter. The CO_2 concentration was set at $370 \mu\text{mol mol}^{-1}$, and the leaf temperature was adjusted to 20°C. After an initial measurement at $0 \mu\text{mol m}^{-2} \text{s}^{-1}$, the PPFD was increased to 13 different light intensities and P_n was recorded at each PPFD. The photosynthetic light compensation point, light saturation point, and light-saturated photosynthetic rate (P_{max}) were estimated using Photosyn Assistant software (Dundee Scientific, Dundee, UK) based on the equation of Prioul and Chartier (1977).

The CO_2 responses of photosynthesis were determined using a range of CO_2 concentrations, at a PPFD of $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a leaf temperature of 20°C. CO_2 was injected into the circuit using the built-in injection system of the gas analyser (CIRAS-1; PP Systems). Measurements for the $P_n - C_i$ response curves started at $370 \mu\text{mol CO}_2 \text{mol}^{-1}$, then decreased in a step-wise manner to $0 \mu\text{mol CO}_2 \text{mol}^{-1}$, then increased to higher CO_2 concentrations. The maximum rate of carboxylation using Rubisco (V_{cmax}), light-saturated electron transport (J_{max}), and the CO_2 compensation point (Γ) were calculated using the biochemical model of von Caemmerer and Farquhar (1981).

The dependence of photosynthesis on temperature was examined on five independent leaves of each species between 08:00 h – 11:00 h using an infrared gas analyser

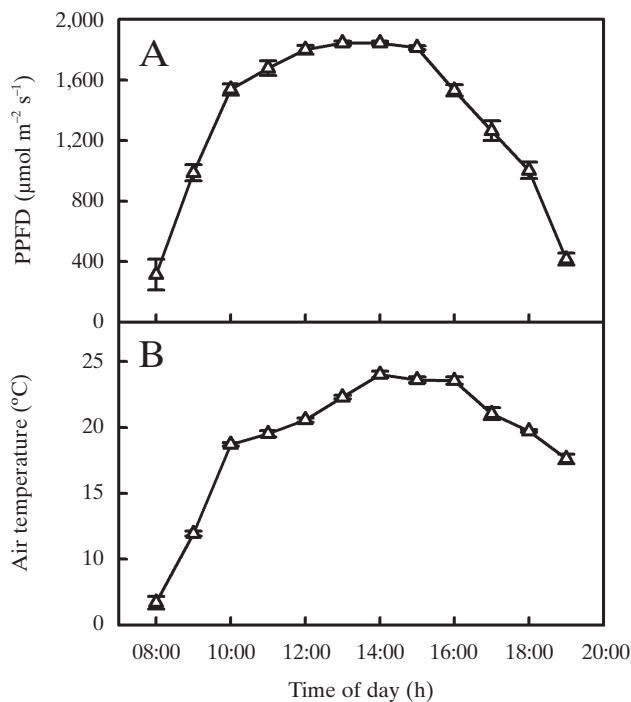


FIG. 1
Diurnal changes in photosynthetic photon flux density (PPFD; Panel A) and air temperature (Panel B) on a clear day. Values are means \pm 1 SE for three measurements on the same day.

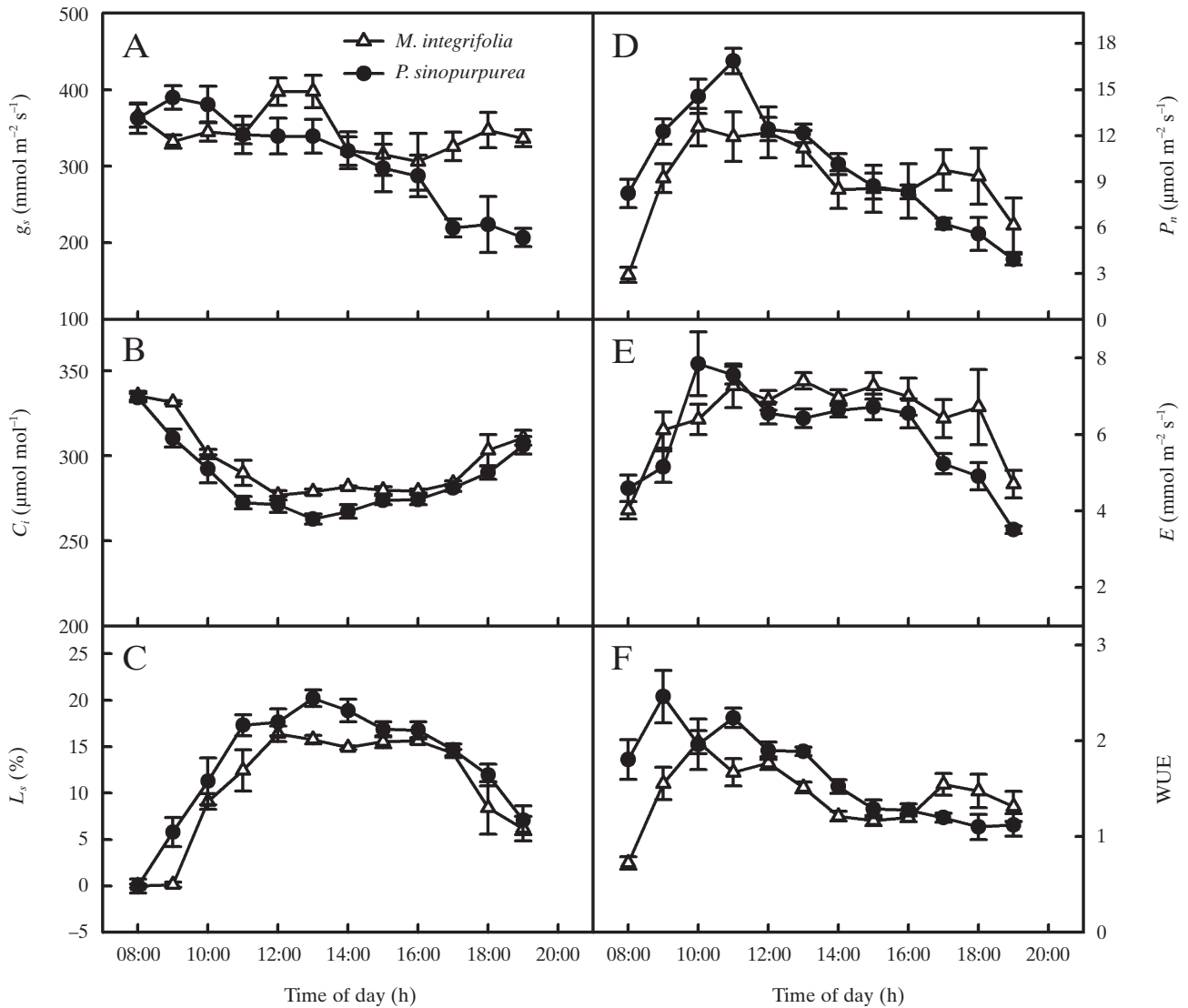


FIG. 2

Diurnal changes in stomatal conductance (g_s ; Panel A), intercellular CO_2 concentration (C_i ; Panel B), stomatal limitation (L_s ; Panel C), photosynthetic rate (P_n ; Panel D), transpiration rate (E ; Panel E), and water-use efficiency (WUE; Panel F) in *M. integrifolia* and *P. sinopurpurea* leaves on a clear day. Values are means \pm 1 SE for five leaves on separate plants of each species on the same day.

(CIRAS-1; PP Systems), and leaf temperatures were adjusted using the internal heating/cooling system. During all measurements, the CO_2 concentration was kept at $370 \mu\text{mol mol}^{-1}$ and the PPFD at $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$. Measurements were made between 10°C and 35°C . A second-order polynomial equation was used to fit the P_n – temperature response curves, and photosynthetic optimum temperatures (T_{opt}) were determined.

Statistical analysis was performed using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences in environmental and leaf physiological variables between species were analysed using the independent sample *t*-test.

RESULTS

The maximum PPFD occurred between 12:00 h – 15:00 h (Figure 1A), while the maximum T_a was between 14:00 – 16:00 h (Figure 1B). The g_s of *P. sinopurpurea* reached its maximum value in the early morning, then decreased. In *M. integrifolia*, the maximum g_s was found at midday, and the minimum g_s occurred between 14:00 h

– 16:00 h (Figure 2A). The diurnal variations in C_i and L_s in *M. integrifolia* were similar to those in *P. sinopurpurea* (Figure 2B,C), but L_s showed an inverse pattern to C_i in both species. The maximum values of C_i occurred at 08:00 h, decreased towards 12:00 h, and increased again at 15:00 h.

Diurnal variations in E in both species showed a dome-shaped pattern (Figure 2E), but the maximum values of E in *P. sinopurpurea* occurred at 10:00 h, then decreased more rapidly. P_n in *M. integrifolia* peaked at 10:00 h, then subsided at midday and recovered again at 17:00 h (Figure 2D). The diurnal variation in P_n in *P. sinopurpurea* exhibited a single peak with a maximum rate ($16.8 \mu\text{mol m}^{-2} \text{s}^{-1}$) reached at 11:00 h (Figure 2D). Although the maximum value of P_n in *P. sinopurpurea* in the morning was higher than that in *M. integrifolia*, there was no statistical difference in daily mean P_n ($t = 1.210$; $P > 0.05$) between the two species. The water-use efficiencies (WUE) of both species changed diurnally (Figure 2F), in parallel with P_n . *Primula sinopurpurea* had a similar daily mean WUE to *M. integrifolia* ($t = 1.210$; $P > 0.05$).

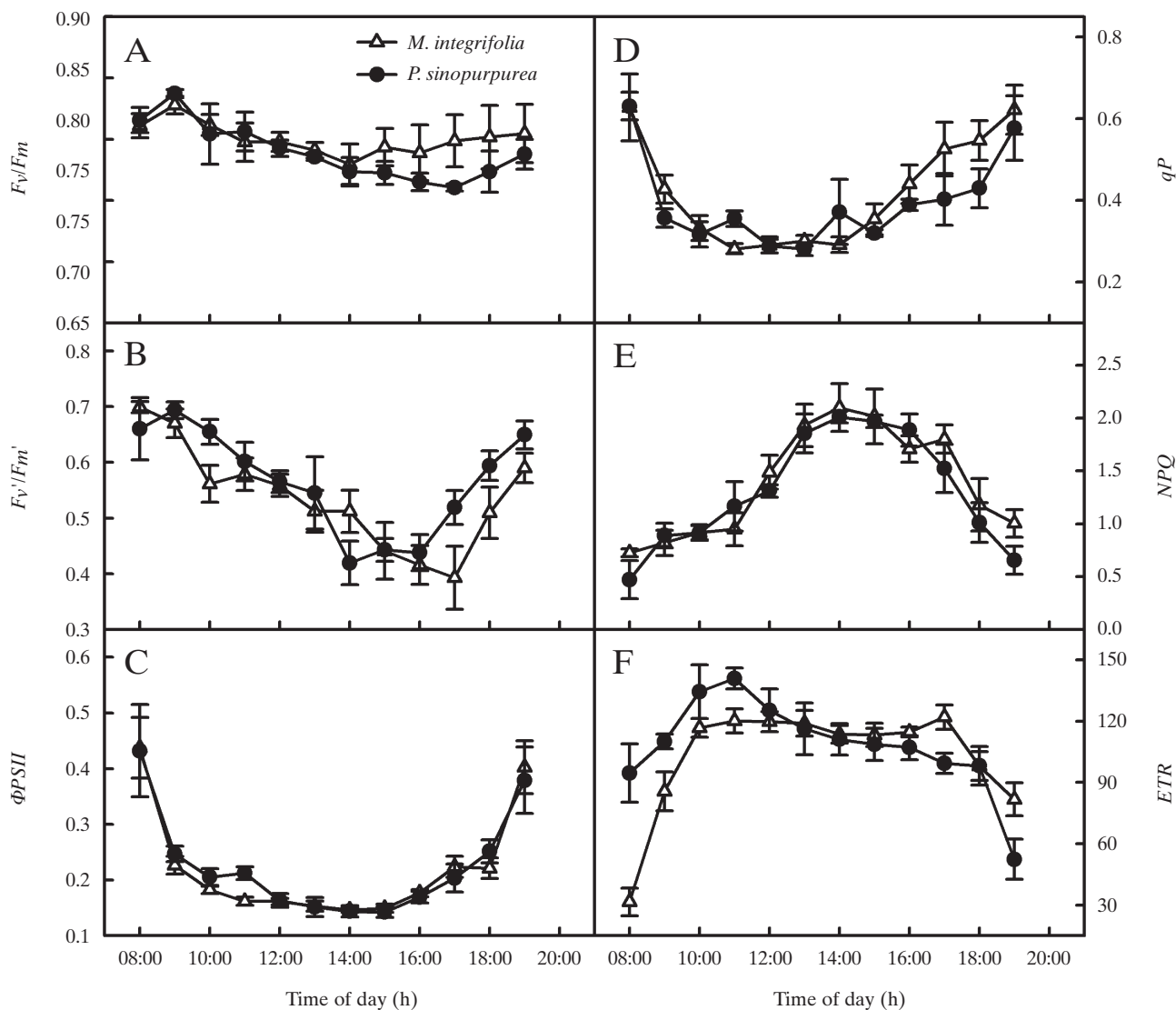


FIG. 3

Diurnal changes in the potential quantum yield of PSII (F_v/F_m ; Panel A), the efficiency of excitation energy capture by open PSII reaction centres (F_v'/F_m' ; Panel B), the effective quantum yield of PSII (ϕ_{PSII} ; Panel C), photochemical quenching (qP ; Panel D), non-photochemical quenching (NPQ ; Panel E), and the apparent rate of photosynthetic electron transport of PSII (ETR ; Panel F) in *M. integrifolia* and *P. sinopurpurea* on a clear day. Each point represents the mean \pm 1 SE of five leaves on separate plants of each species on the same day.

F_v/F_m values in *P. sinopurpurea* decreased significantly at midday (Figure 3A), while the F_v/F_m ratio in *M. integrifolia* did not decrease significantly. F_v'/F_m' and qP values in both species reached a maximum in the early morning, then decreased to their minima between 14:00 h – 17:00 h (Figure 3B,D). ϕ_{PSII} values dropped rapidly after sunrise in response to the rapid increase in PPFD (Figure 3C). The minimum ϕ_{PSII} value in both species was observed during the period of high irradiance, and ϕ_{PSII} increased rapidly in response to the decrease in PPFD in the late afternoon. The diurnal course of the ETR in *M. integrifolia* decreased slightly between 14:00 h – 16:00 h, then recovered slightly after 16:00 h; while the ETR in *P. sinopurpurea* peaked at 11:00 h, then decreased (Figure 3F). NPQ values in both species changed diurnally, in parallel with T_a , and reached their highest values at 14:00 h (Figure 3E).

The T_{opt} (i.e., the temperature range attaining $> 99\%$ P_{max}) in *M. integrifolia* and *P. sinopurpurea* were 16° – 21°C and 12° – 18°C, respectively (Figure 4A). The two species attained higher photosynthetic rates (P_n) at a PPFD of

approx. 1,200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and showed similar photosynthetic adaptation to PPFD (Figure 4B). Leaves of *P. sinopurpurea* had higher V_{cmax} ($t = 3.002$; $P < 0.05$) and J_{max} ($t = 4.406$; $P < 0.05$) values than those of *M. integrifolia* (Figure 4C). The P_{max} of *P. sinopurpurea* ($19.27 \pm 0.66 \mu\text{mol m}^{-2} \text{s}^{-1}$) was also higher than that of *M. integrifolia* ($13.93 \pm 1.01 \mu\text{mol m}^{-2} \text{s}^{-1}$; $t = 4.411$; $P < 0.05$).

DISCUSSION

Our results revealed the physiological limitations in high temperature adaptation of two alpine plants in terms of carbon assimilation and photo-inhibition, and may help to explain the reduced growth and flower size seen in some alpine plants grown under warmer temperatures compared to lower temperatures (Still *et al.*, 2003). The decline in photosynthesis of *P. sinopurpurea* and *M. integrifolia* in the afternoon was linked to high PPFD and temperature, and resulted mainly from lowered stomatal conductance. On a clear day, both *P. sinopurpurea* and *M. integrifolia* showed a

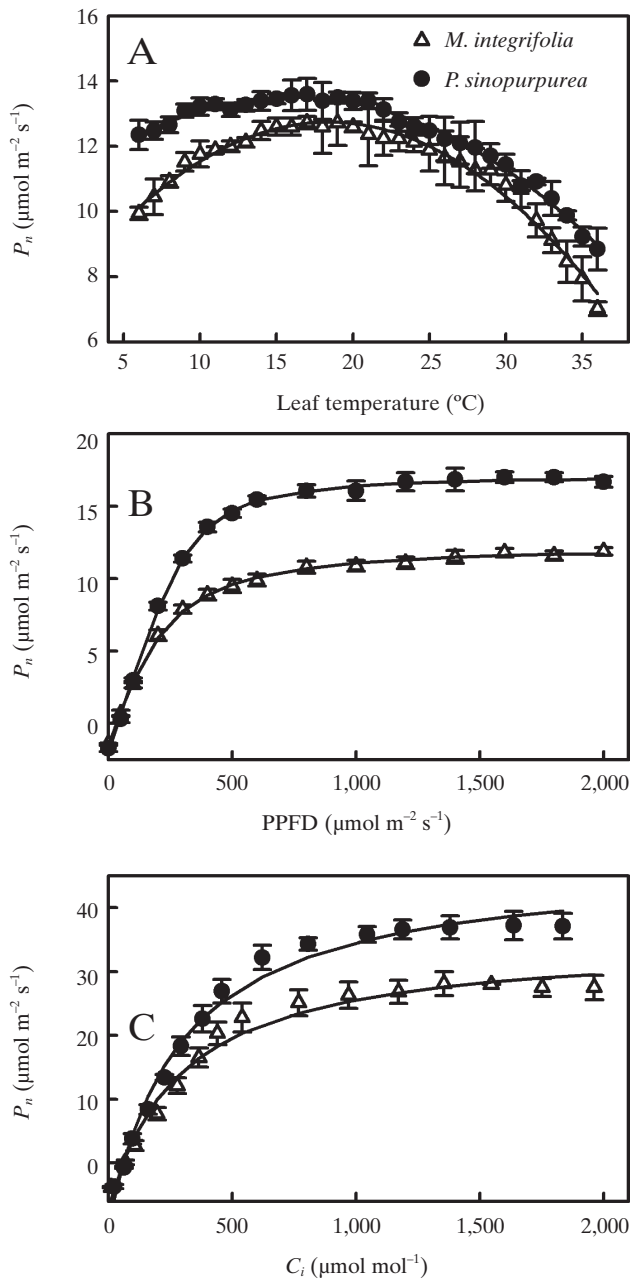


FIG. 4

Photosynthetic responses of *M. integrifolia* (open triangles) and *P. sinopurpurea* (solid circles) to leaf temperature at a PPFD of $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a CO_2 concentration of $370 \mu\text{mol mol}^{-1}$ (Panel A), to photosynthetic photon flux density (PPFD) at a temperature of 20°C and a CO_2 concentration of $370 \mu\text{mol mol}^{-1}$ (Panel B), and to intercellular CO_2 concentration (C_i) at a PPFD of $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a temperature of 20°C (Panel C). Each point represents the mean ± 1 SE for five leaves on separate plants of the same species on the same day.

significant depression in photosynthesis in the afternoon, indicating that they experienced photo-inhibition. In the afternoon, the T_a and PPFD were higher than the T_{opt} and photosynthetic light saturation point of both species. High PPFD and high temperature would result in a decrease in RH and an increase in leaf-air vapour pressure deficit (VPD; Xu, 2002; Mohotti and Lawlor, 2002). Previous studies showed that a high VPD was the primary environmental factor depressing photosynthesis under low RH conditions, even though the plants were irrigated adequately (Xu, 2002; Zhang *et al.*, 2009). This indicated that high PPFD and high temperature were the

environmental factors causing midday depression of photosynthesis in *P. sinopurpurea* and *M. integrifolia*.

The diurnal pattern in photosynthesis can be linked to stomatal limitation and to non-stomatal limitation (e.g., carboxylation efficiency; Pons and Welschen, 2003). In our study, the decreases in P_n in *P. sinopurpurea* and *M. integrifolia* at midday were accompanied by a decrease in C_i and an increase in L_s (Figure 2). If the decrease in P_n was accompanied by decreasing C_i and increasing L_s , the midday decline in photosynthesis would mainly be the result of stomatal limitation (Farquhar and Sharkey, 1982; Xu, 2002).

During a diurnal cycle, with increases in T_a and PPFD, the efficiency of PSII declined, indicating that PSII centres could not use excessive light energy (Zhang and Gao, 1999). Most of the PSII centres in both species are closed under high irradiance and high temperature in the afternoon, which meant that high temperature and irradiance could lead to the inactivation of PSII and block electron transfer from PSII to PSI. The decrease in PSII efficiency can result from the reduced photochemical quenching and photochemical efficiency of open PSII centres (Valentini *et al.*, 1995), and can act as a down-regulation that maintains a balance between light-driven linear electron flow and the requirement for reducing power for both carboxylation and oxygenation of RuBP (Demmig-Adams and Adams, 1996). Most of the decline in PSII efficiency was related to NPQ, due to energy dissipation (Valentini *et al.*, 1995). Light energy allocated to NPQ is dissipated as heat, mainly through de-epoxidation of the xanthophyll cycle, in order to protect the photosynthetic apparatus (Niyogi, 1999; Ögren, 1991). High irradiance and high temperature during the day led to photo-inhibition in both species, while NPQ increased, indicating that much of the light energy was dissipated *via* NPQ.

The temperature range maintaining 99% P_{max} in *P. sinopurpurea* was narrower than that in *M. integrifolia*. Thus, *P. sinopurpurea* could attain a greater P_n than *M. integrifolia* under favourable conditions, but the daily average P_n in the two species was similar. Our study confirmed that the photosynthetic apparatus of alpine plants is extremely sensitive to heat stress (Buchner and Neuner, 2003). High midday temperatures can depress Rubisco activity and the efficiency of PSII, and enhance photorespiration, which can affect net carbon assimilation (Valentini *et al.*, 1995; Crafts-Brandner and Salvucci, 2000). The decline in P_n at midday by high temperature, as well as the low T_{opt} of alpine plants, may explain the higher DWs and flower sizes of some alpine plants grown at lower temperatures than under warmer temperatures.

In conclusion, the intolerance of photosynthesis to high temperature in two alpine flowers would limit their carbon assimilation and consequently limit their growth and development at lower altitudes.

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