Photosynthesis in Relation to Reproductive Success of *Cypripedium flavum*

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Received: 6 November 2004 Returned for revision: 1 February 2005 Accepted: 28 February 2005 Published electronically: 13 April 2005

**INTRODUCTION**

The genus *Cypripedium* comprises 49 species, which are widely distributed throughout Northern America, Eastern Asia and Europe. One of the most important distribution regions of the genus is the Hengduan Mountains of south-western China, where 14 species occur, mainly found at altitudes above 2700 m (Lang, 1990). Many *Cypripedium* species have ornamental and medical value. Because large-scale cultivation of these plants under artificial conditions is not economically feasible, wild populations of *C. flavum* are the major source of material for horticulture. In recent years, ecological disturbance, unscientific and uncontrolled collection, tourism and increasing grazing pressure have resulted in considerable decline of *Cypripedium* populations in the Hengduan Mountains (Cribb and Sandison, 1998; Kull, 1999). In order to conserve and cultivate *Cypripedium* species, knowledge of the optimal growth conditions is required. Such information may help to develop effective ways to restore the natural habitat of *Cypripedium* or to introduce these species into new environments. Although there are data regarding the general ecological preferences of several species (Primack and Hall, 1990; Primack and Stacy, 1998), there is still lack of detailed physiological information for most (Kull, 1999; Weng et al., 2002). Better understanding of the physiology of *Cypripedium* species may aid their cultivation, which is still difficult.

Plants of *Cypripedium* species are long-lived (many survive more than 30 years) and generally flower in 6–10 years from seeds (Kull, 1999). In natural populations, the overall mean percentage of fruiting of *Cypripedium* species is very low (Kull, 1998; Primack and Stacy, 1998). This may depend on the environment, as flower numbers and fruit set vary greatly in different habitats. In addition, large flowering populations may improve the reproductive success, as appears to be the case for *C. acaule* (Davis, 1986). Most *Cypripedium* species grow in the forest understory; therefore they may suffer from inadequate light due to competition with tall plants (Kull, 1999). Open vegetation, with greater light penetration, enables *Cypripedium* species to intercept more light, which is beneficial for growth and reproduction (Kull, 1998).

It is commonly assumed that plant physiological adaptations to particular environments confer a reproductive advantage (Arntz, 1999). Photosynthetic activity relates to plant productivity and species success (Nagel and Griffin, 2004). By examining the way in which the photosynthetic processes of a species are adapted to diverse environments, the links between growth, production, reproduction and climate can be assessed (Luoma, 1997), and the effect of demand by developing seeds and fruits on the photosynthetic activity of adjacent leaves analysed (Lehtilä and Syrjänäinen, 1995; Obeso, 2002). Instantaneous photosynthetic rate is not always a reliable predictor of plant growth as it is very variable, responding to seasonal and diurnal changes in environmental conditions. However, plant growth can be more accurately predicted when photosynthesis is considered together with patterns of dry matter allocation (Dijkstra and Lambers, 1989), although the direct and indirect effects of photosynthesis on growth and reproduction remain to be evaluated for particular species (Arntz et al., 1998).

We hypothesize that higher photosynthetic rate is beneficial to reproductive success of *Cypripedium*. In order to
evaluate the effect of environment on photosynthesis of Cypripedium species and to examine the relationships between photosynthesis and reproductive success. C. flavum plants growing in four different habitats were studied, including photosynthetic characteristics as well as reproductive traits. The long-term aim of this study is to provide information about the mechanisms and physiological parameters that may be important for successful reproduction of C. flavum and its cultivation and management.

MATERIALS AND METHODS

Species description and habitat

Cypripedium flavum grows in rocky and grassy places in sparse woods, alpine meadows or margins of forests at altitudes of 1800–3700 m in the west of China, including Yunnan, Sichuan, Gansu, Hubei and Xizang (Chen, 1999). In the Hengduan Mountains, annual mean temperature and precipitation are 5.4 °C and 624.8 mm (30-year mean), respectively. The climate is seasonal, with 87% of annual rainfall occurring from May to October, while the dry season lasts from November to April. Cypripedium flavum grows on brown soil with abundant humic matter, and pH 6.1–6.8. It grows to 35–45 cm high, with 6–10 leaves produced from a rhizome. The growing period is about 140 d in a year. The seedling emerges above ground in mid-May and flowers appear in June. A single flower is borne on a scape arising between the basal leaves and subtended by a large, leaf-like bract. The plant sets fruit between July and September and it becomes dormant in early October. The capsule contains abundant dust-like seeds (about 6000–17 000 seeds).

Study sites

Plants were studied at four sites; the number of flowers and fruit set were significantly different in these habitats, where the largest environmental difference was light in the understory due to differences in the density of the forest canopy. Sites were A (99°43'097'E, 28°11'399'N), B (99°33'463'E, 27°55'276''N), C (99°50'101'E, 27°47'760'N) and D (99°57'752'E, 27°36'568'N) at altitudes of 2910 m, 3260 m, 3450 m and 3360 m, respectively. Soil type, air temperature and water availability are very similar at the four sites.

Measurements of gas exchange in the field

Gas exchange of C. flavum was measured at the four sites in June 2003 (the flowering time). For each site, ten seedlings were selected and labelled for measurement. Diurnal photosynthetic time-courses were measured at the sites on June 3–16 in 2003. Eight fully expanded leaves at each site were randomly selected from the previously labelled seedlings; they were measured from 0800 h to 1800 h on a clear day. After steady-state of gas exchange had been achieved, photosynthetic rate (A), intercellular CO2 concentration (Ci), leaf temperature (Tl) and photosynthetically active radiation (PAR) were measured using a portable infra-red gas exchange system with a leaf chamber type PLC-B (CIRAS-1, PP Systems, UK) in an open-system configuration. In addition, a Li-188 quantum sensor, thermometer and hygrometer recorded, respectively, irradiance, air temperature and relative air humidity hourly. The defuse radiation transmission coefficient and solar beam transmission coefficient were measured using a digital plant canopy imager (CI-110, CID, USA) at midday.

Light-saturated photosynthetic rates (Amax) were measured between 0900 h and 1200 h at ambient CO2 partial pressure, air temperature 20 °C and PAR 800 μmol m-2 s-1, which was above the light-saturation point for C. flavum. Eight fully expanded leaves were measured at each site.

The photosynthetic response to light, CO2 and temperature at sites A, B, C and D were measured on 11, 16, 17 and 20 June 2003, respectively. Photosynthetic responses of mature leaves to light were measured at 14 light intensities at each study site. The CO2 concentration in the leaf chamber was 350 μmol mol-1 and temperature 20 °C. Leaves were acclimated to PAR of 800 μmol m-2 s-1 before measurements. At 0 μmol m-2 s-1, light intensity was increased in 13 steps and photosynthetic rates were recorded. The CO2 response curves of photosynthesis were determined with a range of CO2 concentrations (0–2000 μmol mol-1) at a light intensity of 500 μmol m-2 s-1 and temperature of 20 °C. After the initial measurements at 2000 μmol mol-1, CO2 concentration was reduced in steps and photosynthesis recorded after 2–3 min acclimation at each concentration. Three leaves were measured at each study site. Curve-fitting software (SigmaPlot for Windows 8.0) was used to analyse both the A–Ci and A–PAR responses using a three-component exponential function (Watling et al., 2000):

$$A = a(1 - e^{-bxi}) + C$$

where A is photosynthetic rate, x is Ci or PAR, and a, b and C are constants. Using this equation, the carboxylation efficiency (CE) was estimated as the initial gradient of the A–Ci curves (0–200 μmol mol-1), and the apparent quantum yield (AQY) was calculated as the initial slope of the A–PAR curves in the range 0–400 μmol mol-1, following Swanborough et al. (1997).

The dependence of net photosynthetic rate on temperature was measured between 0900 h and 1100 h to avoid high temperatures at midday, and provided a temperature range of 10–35 °C. During measurements, the CO2 concentration and light intensity were 350 μmol mol-1 and 500 μmol m-2 s-1, respectively. Three leaves were randomly selected and measured at each site. A second-degree polynomial equation was fitted to obtain the optimal temperature for photosynthesis.

Chlorophyll fluorescence of mature C. flavum leaves was determined using a pulse amplitude modulated fluorometer (FMS 2, Hanstech, UK). After adaptation for 25–30 min in the dark, the $F_{v}/F_{m}$ ratio ($F_{v}/F_{m} = 1 - F_{o}/F_{m}$) was used to estimate photoinhibition of PSII. A second-degree polynomial equation was used to describe the relationships between $F_{v}/F_{m}$ and temperature, and PAR.

In each population at each site, counts were made in six 5 × 5 m plots of the number of ramets and flowers during the
flowing period (June 2003), and the number of ramets and fruits during the fruiting period (September 2003). Because each ramet only had a single flower, the percentages of flowering and fruiting were calculated as the ratio of number of flowering ramets and fruiting ramets to total ramets in each plot.

Statistical analyses

Statistical analysis was conducted using SPSS for Windows version 10.0 (SPSS, USA). Differences between physiological variables were determined using analysis of variance (ANOVA) and LSD tests for multiple comparisons. The relationships between photosynthetic parameters and reproductive traits were assessed using regression analysis.

RESULTS

Environmental conditions in the understorey

The transmission coefficients for diffuse radiation show the differences between the canopies at the four sites (Fig. 1) and are related to the quantity of light reaching the herb layer. Site B had the greatest direct and diffuse radiation ($P < 0.01, n = 10$), while site A had the lowest. Canopies create complex light dynamics (sun flecks) in the understorey due to the distribution and movement of foliage. Diurnal changes of PAR in the understorey at the four sites were not uniform. The daily PAR averaged $423 \pm 71, 785 \pm 70, 497 \pm 53$ and $482 \pm 59$ µmol m$^{-2}$ s$^{-1}$ between 0800 h and 1800 h at site A, B, C and D, respectively. Minimum mean PAR was at site A, while maximal mean PAR was at site B. However, humidity and air temperature did not vary significantly between the four sites (Fig. 2).

Diurnal variation of photosynthesis

The average $A$ of leaves at all sites increased rapidly with the increasing of PAR after 0800 h (Fig. 2), reaching maximum values around noon for sites B and C, and at 1400 h

Fig. 1. The diffuse radiation transmission coefficient and solar beam transmission coefficient in the understorey at the four experimental sites. Error bars are the 95% confidence interval of the mean, $n = 10$. Different letters indicate significant differences between means ($P < 0.05$).

Fig. 2. Diurnal course of relative humidity, PAR, air temperature and photosynthesis for C. flavum at the study sites. Each point is the mean of five measurements. Error bars represent $\pm 1$ s.e.
compensation points of the four populations varied from 72.9 to 83.5 μmol mol⁻¹ and were not significantly different ($P > 0.05$). According to the slopes of $A$–$C_i$ curves in Fig. 3, carboxylation efficiencies ($CE$) of $C. flavum$ in the four populations were significantly different ($P > 0.05$), being 0.0278, 0.0237, 0.0362 and 0.0309 mol mol⁻¹ for sites A–D, respectively.

The photosynthetic responses to light at the four sites are shown in Fig. 3B. The apparent quantum yield (AQY) of $C. flavum$ was 0.0202, 0.0227, 0.0282 and 0.0257 mol CO₂ mol photon⁻¹ at sites A–D, respectively. The plants at site C had the highest AQY, and it was lowest at site A ($P < 0.05$). There were no significant differences in photosynthetic light compensation points between populations (range 25.5–32.6 μmol m⁻² s⁻¹), but the photosynthetic light saturation points (LSP; PAR to achieve 99% of $A_{\text{max}}$) were significantly different. $C. flavum$ at site B had the highest LSP (700 μmol m⁻² s⁻¹), while the lowest value (594 μmol m⁻² s⁻¹) was at site A. At values of PAR above 1200 μmol m⁻² s⁻¹ the photosynthetic rate of $C. flavum$ decreased gradually.

Photoinhibition of photosynthesis of $C. flavum$, indicated by smaller values of $F_v/F_m$, decreased linearly with increasing PAR ($R^2 = 0.58$, $P < 0.01$, $n = 120$) and $F_v/F_m$ was curvilinearly related to temperature ($R^2 = 0.61$, $P < 0.01$, $n = 120$), as shown in Fig. 4. High temperature and high PAR inhibited the photosynthesis of $C. flavum$. When the leaf temperature was above 25 °C, the photosynthetic rate decreased sharply. There were no significant differences in optimum temperature for photosynthesis ($T_{\text{opt}}$) between plants from the four sites (Fig. 3C).

Reproductive traits of different populations

The number of ramets, flowers and fruit per m² at the four sites are given in Fig. 5. The flowering percentage at sites A–D was 36.9 ± 8.7, 47.0 ± 7.5, 53.3 ± 6.7 and 42.6 ± 3.0%, and the ratio of fruit-set was 2.9 ± 0.6, 4.7 ± 1.1, 9.2 ± 1.1 and 4.2 ± 0.8%, respectively. The flowering percentage was not significantly different, but the population at site C had the highest percentage of fruiting among the sites ($P < 0.01$, $n = 6$).

The relationships between photosynthetic parameters and reproductive traits were described by second-order polynomial equations (Fig. 6). There is linearly relationship between daily mean $A$ and the number of ramet per m² ($R^2 = 0.68$, $P < 0.01$, $n = 24$) and percentage of fruiting ($R^2 = 0.56$, $P < 0.01$, $n = 24$). The ratio of daily mean photosynthetic rate to light-saturated photosynthesis ($A_{\text{daily}}/A_{\text{max}}$) was 64.0, 67.9, 72.1 and 65.9 % at sites A–D, respectively. The ratio was closely correlated with the ramet number per m² ($R^2 = 0.81$, $P < 0.01$, $n = 24$) and percentage of fruiting ($R^2 = 0.60$, $P < 0.01$, $n = 24$; Fig. 6).

**DISCUSSION**

**Light availability under different forest understoreys**

Light intensity at the ground surface is affected by the forest canopy cover, which creates complex light dynamics for
C. flavum due to the distribution and movement of foliage. Effective acquisition and use of light are critical, especially for plants inhabiting in the forest understorey, as shading may not be favourable to growth and reproduction (Hughes et al., 1988). Seedlings of C. flavum under the sparsest forest (site B) received most direct and diffuse irradiation, followed by D, C and A.

Leaf photosynthetic traits in understorey environments

Light conditions affect the morphological and physiological characteristics of understorey plants. Plants of C. flavum at site C had the highest values for $A_{\text{max}}$, apparent quantum yield and carboxylation efficiency. Those at site A had the least. The photosynthetic light compensation points of C. flavum at the four sites were very low (ranging from 25.5 \( \mu\)mol m\(^{-2}\) s\(^{-1}\) at site A to 32.6 \( \mu\)mol m\(^{-2}\) s\(^{-1}\) at site B); this is an important feature of the carbon balance as low LCP improves carbon gain in shade environments (Muraoza et al., 2003).

Light is a limiting factor for the establishment of seedlings in populations of C. calceolus in Europe (Kull, 1998). Sites suitable for seedling establishment have more extensive moss cover, more moisture, but less vascular plant cover and more intense light (Kull, 1998). The proportion of reproductive ramets is larger in more intense light, and in shady localities small clones dominate (Kull, 1999). A fraction of 1/14 – 1/20 of full light is optimal for C. calceolus in the taiga zone of Europe (Kull, 1995). The poor growth for C. calceolus in shade conditions may be due to this limited resource. In our study, the photosynthetic light-saturated points (LSP) of C. flavum were from 593 to 700 \( \mu\)mol m\(^{-2}\) s\(^{-1}\), which are equal to one-quarter to one-third of full sunlight. The seedlings at site B had the highest LSP due to the higher light intensity. Cypripedium flavum at site C had the highest photosynthetic potential due to suitable light. Because C. flavum is adapted to lower light conditions in the understorey, exposure to strong light (above 1200 \( \mu\)mol m\(^{-2}\) s\(^{-1}\) PAR) resulted in photoinhibition of photosynthesis. The value of $A$ for the populations is depressed by the excess sunlight at site B and by shade condition at site A.

**Relationship between photosynthetic capacity and reproduction**

The growing season for C. flavum is short (about 140 d) in the Hengduan Mountains. In order to reproduce, C. flavum must complete the process from emergence, through flowering to fruiting in a short period, allowing sufficient time to store adequate energy for the next year. Shading may reduce the photosynthetic capacity and the ability of perennial species to store resources for subsequent growth and reproduction (Vallius, 2001). So the light availability under different forest canopy coverage would explain the differences in reproductive success of C. flavum at the different sites.

It is commonly assumed that instantaneous photosynthetic rates of leaves are the consequence of environmental factors, but they may also be affected by plant demand for assimilates from growth and seed production (Burton and Bazzaz, 1995; Tissue et al., 1995; McAllister et al., 1998). When growth is limited by light, water or nutrient availability, the morphological and physiological traits involved in the acquisition and allocation of limiting
resources are expected to influence growth and production (Arntz, 1999), and thus affect populations and their reproduction. A higher photosynthetic rate should increase carbon gain and in turn increase accumulation of biomass, leading to an increase in fitness (Arntz et al., 2000). Within a season, fruit production may be limited by pollinators, but the availability of resources ultimately limits fruit production and reproductive success (Snow and Whigham, 1989; Zimmerman and Aide, 1989; Sugiura et al., 2001). In our study, the ratio of daily mean photosynthetic rate to light-saturated photosynthesis ($A_{\text{daily}}/A_{\text{max}}$) for C. flavum at sites A–D was 64/66, 67/69, 72/71 and 65/69 %, respectively. The daily mean photosynthetic rate ($A_{\text{daily}}$) and $A_{\text{daily}}/A_{\text{max}}$ are closely correlated with the number of ramets per m² and percentage of fruiting (Fig. 6).

It is concluded that photosynthesis and thus reproduction of C. flavum are affected by light availability in the understory, supporting the hypothesis that higher photosynthetic rate increases reproductive success and fitness of C. flavum. The ratio $A_{\text{daily}}/A_{\text{max}}$ expresses the effect of the currently prevailing environment on photosynthesis, and because of the relationship with fruiting it can also be regarded as a proxy of reproductive success. 

**ACKNOWLEDGEMENTS**

We thank Professor Cunxin Li and Mr Hua Wang for their assistance with the experiments, and we also thank Dr Hazel Wilkinson, Dr David Melick and Dr Shaotian Chen for improving the English. In addition, the comments and suggestions of anonymous two referees significantly improved the manuscript. We are grateful to the National Natural Science Foundation of China (grants 30270151, 30170077), the National Key Basic Research Program (grant 2003CB415102) and Natural Science Foundation of Yunnan Province (grant 2002C0062M).

**LITERATURE CITED**


