Seasonal variations in photosynthesis of *Picea morrisonicola* growing in the subalpine region of subtropical Taiwan

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Summary From January 1999 to May 2001, we investigated seasonal variations in the photosynthetic capacity of Taiwan spruce (*Picea morrisonicola* Hay.) growing in the subalpine region of subtropical Taiwan (23°29′ N, 120°53′ E, 2600 m a.s.l.). Photosynthetic capacity (near light-saturated net photosynthetic rate, *P*νsat), chlorophyll fluorescence (*F*ν/νm) and soluble protein concentration of needles all increased from mid or late spring to early winter. Even when minimum air temperature of the measuring day dropped to near 0 °C, *P*νsat remained at about 20% of the highest value observed in winter. There was a curvilinear relationship between *F*ν/νm and the minimum or mean air temperature of the measuring day. The increase in *F*ν/νm with temperature was slowed when the daily mean air temperature was above 7 °C, or the minimum air temperature was above 3 °C; however, when air temperatures dropped below these values, *F*ν/νm varied sharply. Seasonal variations in *P*νsat paralleled those in *F*ν/νm and needle soluble protein concentration. In early or mid spring when air temperature and *F*ν/νm increased, *P*νsat and soluble protein concentration remained low. Multiple regression analysis showed that seasonal variations in *P*νsat were affected by *F*ν/νm, air temperature and needle soluble protein concentration, and the multiple regression equation could be used to estimate *P*νsat in different seasons. We conclude that the decrease in photosynthetic capacity of Taiwan spruce in winter and its subsequent recovery in spring were mainly caused by photoinhibition and its reversal, and changes in needle soluble protein concentration. Another possible explanation for the delayed recovery of photosynthetic capacity in spring may be associated with the slow increase in needle soluble protein concentration.

Keywords: chlorophyll fluorescence, conifer, photosynthetic capacity, soluble protein, Taiwan spruce.

Introduction

Conifers are naturally distributed in temperate and subarctic zones of the world. However, the same coniferous species are also often present in both subalpine and alpine areas of subtropical regions such as Taiwan. Taiwan spruce (*Picea morrisonicola* Hay.), one of ~40 coniferous species found in the temperate zone of the northern hemisphere, grows on mountain slopes at altitudes from 2300 to 3000 m a.s.l. in Taiwan (21°55′ N to 25°8′ N).

Because conifers retain their needles for several years, their photosynthetic apparatus must be able to withstand periods of severe freezing and still retain the capacity for photosynthesis during intervening warm periods. Winter-induced inhibition of photosynthesis in plants is caused by the combined effects of light and temperature. Low temperature inhibits photosynthesis by reducing the activities of Calvin cycle enzymes (Öquist et al. 1987, Strand and Öquist 1988), whereas absorption of light energy in excess of that which could be used in photosynthesis at low temperatures causes photoinhibition of photosystem II (PSII) (Öquist et al. 1987, Strand and Öquist 1988, Streb et al. 1998). Photoinhibition can be estimated from the ratio of light-induced variable fluorescence and maximum fluorescence (*F*ν/νm) (Bolhar-Nordenkampf et al. 1988, Ottander et al. 1995, Maxwell and Johnson 2000).

The activity of ribulose-1,5-biphosphate carboxylase (Rubisco), the enzyme responsible for CO₂ fixation in chloroplasts of C₃ plants, varies with season, with significantly higher activity during summer than in winter (Gezelius and Smith 1985, Naidu and Swamy 1995). In many cases, a positive correlation has been found between Rubisco activity and soluble protein (SP) concentration in leaves (Friedrech and Haffaker 1980, Bravdo and Pallas 1982, Muthuchelian 1992, Naidu and Swamy 1995). In addition, chlorophyll concentration has been found to decrease in winter (Ottander et al. 1995), at low temperature (Haldimann 1998, Vogg et al. 1998) and under short-day conditions (Vogg et al. 1998). Thus, the observed reduction in photosynthetic capacity during winter could also be associated with the decrease in chlorophyll concentration (Vogg et al. 1998).

Seasonal variations in photosynthetic capacity of spruce and pine species have been observed in many field studies (e.g., Ottander and Öquist 1991, Ottander et al. 1995, Schaberg et al. 1995, 1998, Strand and Lundmark 1995, Man and Lieffers...
Materials and methods

The study was conducted at the Tatachia forest site (23°29’ N, 120°53’ E, 2600 m a.s.l.), which is characterized by acidic soil (pH = 4.1–5.1), mean monthly temperatures of ~4.6 °C (February) to 13 °C (July) and a mean monthly precipitation of ~47 (November) to 840 mm (August) (1971–2000).

Measurements were conducted mainly during the autumn or winter through spring period, beginning in January 1999 and ending in May 2001 (Figure 1A), with fewer measurements in summer because of road damage by thunderstorms, typhoons and heavy rains (Figure 1B). One- and 2-year-old shoots were sampled from southeastern-facing branches of 10-year-old Taiwan spruce trees, approximately 3 m high, growing in an open stand at Tatachia. Shoot samples were collected at 0630 h and immediately recut under water. The shoot was then exposed to light (600 µmol m⁻² s⁻¹ photosynthetic photon flux (PPF) from a halogen lamp), while being kept moist by spraying with water until the photosynthetic measurements had been completed.

Photosynthetic capacity was measured 800 m away from the sampling site with a portable, open-flow gas exchange system (LI-6400, Li-Cor, Lincoln, NE) connected to a conifer chamber (Li-Cor LI-6400-05). Measurements were made in saturating light (> 1000 µmol m⁻² s⁻¹ PPF) and at ambient temperature (Figure 2A), humidity and CO₂ concentration. The PPF was monitored with a quantum meter (LI-185A, Li-Cor) and when ambient PPF was less than 1000 µmol m⁻² s⁻¹, a slide projector with a tungsten halogen lamp was used to increase irradiance to the target value. Following the photosynthetic measurements, chlorophyll fluorescence was measured as the ratio between light-induced variable fluorescence and maximum fluorescence (Fv/Fm) in dark-adapted (for 20 min) needles at room temperature with a Plant Efficiency Analyzer (PEA, Hansatech, U.K.) (Blennow et al. 1998). Measurements were made between 1000 and 1500 h with four replications during each measuring period. One branch, designated as a replicate, was taken from each sampled tree and the mean of measurements for the branch was used in the statistical analysis.

When measurements of gas exchange and chlorophyll fluorescence were completed, the needle samples were taken to the laboratory for determinations of dry/wet mass ratio, and chlorophyll and soluble protein (SP) concentrations. Chlorophyll was extracted from 50-mg samples of fresh conifer needles with 80% acetone and measured spectrophotometrically (U-2000, Hitachi, Japan) at 645 and 663 nm (Arnon 1949). Soluble protein was determined by the method of Lowry et al. (1951).

Meteorological data were taken from the Alishan Meteorological Station (23°31’ N, 120°48’ E, 2408 m a.s.l.), which is about 8 km away from and 200 m lower than the experimental site. According to data collected from meteorological stations at different elevations in central Taiwan (Taichung (24°09’ N, 120°41’ E, 78 m a.s.l.), Sun Moon Lake (23°52’ N, 120°48’ E, 1014 m a.s.l.) and Alishan and Yushan (23°29’ N, 120°57’ E, 3858 m a.s.l.), the temperature decreases by about 0.5 °C per 100 m rise in elevation. Thus, to estimate the air temperature in our study, we subtracted 1 °C from the data measured at the Alishan Meteorological Station.

Data were analyzed by linear regression or multiple regression.

Results

Throughout the measurement period (January 1999–May 2001), mean daily and minimum temperatures at the experimental site were about 13–15 °C and 9–12 °C in the warmer season and 3–4 °C and –4 to 2 °C in the cooler season; monthly precipitation was 0–720 mm (Figure 1). The minimum and mean temperatures of the measuring days were 0.2–10.9 °C and 3.4–13 °C, respectively (Figure 2A).
Taiwan spruce maintained increased photosynthetic capacity (near light-saturated net photosynthetic rate, $P_{\text{nsat}}$) and chlorophyll fluorescence ($F_v/F_m$) from April–May until the following January (Figures 2B and 2C). Though the trends in temperature, $P_{\text{nsat}}$ and $F_v/F_m$ varied with seasons, there was no significant difference between the 1- and 2-year-old needles (Figures 2B and 2C). The $F_v/F_m$ values of 1- and 2-year-old needles closely followed the change in air temperature (cf. Figures 2A and 2C), with curvilinear relationships between $F_v/F_m$ and minimum as well as mean air temperature of the measuring day for all seasons (Figure 3). However, $F_v/F_m$ was more closely related to the minimum temperature ($r = 0.767, P < 0.001$) than to the mean temperature ($r = 0.637, P < 0.001$) (Figure 3). We also found that $P_{\text{nsat}}$ of Taiwan spruce closely followed air temperature most of the time (cf. Figures 2A and 2B), and $P_{\text{nsat}}$ in winter was about 20% of its maximum value, even when the minimum air temperature of the measuring day dropped to near 0 °C. However, on April 16, 1999, January 15, 2000, March 11, 2000, and March 31, 2001, $P_{\text{nsat}}$ was outside the range of variability depicted for other dates. Values for these 4 days fell well below the curvilinear relationship between $P_{\text{nsat}}$ and the minimum air temperature of the measuring day for all other dates in all seasons (Figure 4).

Seasonal variation in $P_{\text{nsat}}$ of 1- and 2-year-old needles ($Y(P_{\text{nsat}})$) consistently paralleled changes in their SP concentration, i.e., a lower concentration in needles in the cool season and a higher concentration in the warm season (Figure 2). But on January 15, 2000, both $P_{\text{nsat}}$ and needle SP concentration dropped much more than the decreases in air temperature and $F_v/F_m$. On April 16, 1999, March 11, 2000, and March 31, 2001, air temperature and $F_v/F_m$ increased, whereas both $P_{\text{nsat}}$ and needle SP concentration either remained at a low value or decreased temporarily (Figures 2A–2D). From multiple regression analysis, it is evident that seasonal variations in $P_{\text{nsat}}$ of Taiwan spruce were influenced by both $F_v/F_m$ and needle SP concentration ($Y(P_{\text{nsat}}) = -321.54 + 583.01 F_v/F_m + 10.09 SP, R = 0.892, P < 0.001$), with partial correlation coefficients of $F_v/F_m$ and SP concentration to $P_{\text{nsat}}$ of 0.584 (52%) and 0.533 (48%), respectively. Thus, the influence of SP concentration on $P_{\text{nsat}}$ was similar to that of $F_v/F_m$.

Taking into consideration the relationships of $P_{\text{nsat}}$ to $F_v/F_m$ and SP, and of $F_v/F_m$ to air temperature (Figure 3), significant multiple regressions for $P_{\text{nsat}}$ of current-year and 2-year-old needles to their SP concentration and minimum or mean air temperatures of the measuring day ($T_{\text{min}}$ or $T_{\text{mean}}$) were found ($Y(P_{\text{nsat}}) = 106.07 + 11.86SP - 30.46/T_{\text{min}}, R = 0.874, P < 0.001$; and $Y(P_{\text{nsat}}) = 151.00 + 10.56SP - 386.11/T_{\text{mean}}, R = 0.770, P < 0.01$). The $P_{\text{nsat}}$ estimated from the multiple regression equation for $P_{\text{nsat}}$ versus $F_v/F_m$, SP concentration and minimum air temperature were close to measured $P_{\text{nsat}}$ values. Also, $P_{\text{nsat}}$ measured on April 16, 1999, January 15, 2000, March 11, 2000, and March 31, 2001, fell within the range of
Discussion

Seasonal variation in photosynthetic capacity of spruce species in temperate and subarctic areas has been reported by several researchers (Ottander and Öquist 1991, Ottander et al. 1995, Schaberg et al. 1995, 1998, Strand and Lundmark 1995, Man and Liefers 1997, Lundmark et al. 1998, Vogg et al. 1998). These results indicate that, under field conditions, changes in photosynthesis generally parallel seasonal changes in ambient air temperature. The critical daily mean temperature at which photosynthesis of both red spruce (Picea rubens Sarg.) and white spruce (Picea glauca (Moench.) Voss) decreased to nil in autumn and then commenced again in spring was about 0 °C (Man and Liefers 1997, Schaberg et al. 1998). Lundmark et al. (1998) also reported that, during spring, photochemical efficiency of PSII (Fv/Fm) in Norway spruce (P. abies (L.) Karst.) recovered when the daily mean air temperature increased above 0 °C up to a maximum of about 12 °C in late spring, and Fv/Fm followed to a slight extent (> 0.7) when daily mean air temperature was above 10 °C.

We demonstrated that variations in Fv/Fm closely followed the changing patterns of air temperature (Figures 2A, 2B and 3). Close examination revealed that, when the mean daily air temperature was above 7 °C, or the minimum air temperature increased to 3 °C and higher, Fv/Fm tracked temperature quite closely (Fv/Fm > 0.7). This result indicates that the temperature threshold for the recovery of Fv/Fm for Taiwan spruce is about 3 °C lower than for spruce species growing in high-latitude regions. Colom et al. (2003) point out that Pinus leucodermis Ant. originating from high altitudes was probably naturally selected for resistance to photoinhibition induced by low temperature or excess light, or both. Despite a higher winter temperature in the subalpine region of subtropical Taiwan compared with lowland high-altitude regions, Taiwan spruce was better able to limit photoinhibition at low temperature than Pinus leucodermis, probably because tropical alpine environments are characterized by high-amplitude diurnal, rather than seasonal, temperature fluctuations.

However, we found that the relationship between Pnsat of Taiwan spruce and air temperature did not hold, and an underestimation of Pnsat value was observed on 4 days (Figure 4). On 3 of these 4 days, i.e., April 16, 1999, March 11, 2000, and March 31, 2001, Pnsat activity either remained low or dropped temporarily, despite increases in air temperature and Fv/Fm (Figures 2A–C).

It has been reported that winter-induced inhibition of photosynthesis of Scots pine (Pinus sylvestris L.) needles is fully overcome within days or even a few hours in the laboratory under favorable conditions (Lundmark et al. 1988, Ottander et al. 1995). However, under natural conditions, it might take several months before photosynthesis is fully restored (Lundmark et al. 1988, Ottander and Öquist 1991, Wieser 1997, Schaberg et al. 1998). Schaberg et al. (1998) pointed out that a temperature-induced increase in photosynthetic capacity of winter-inhibited red spruce occurred within 3 h in the laboratory, whereas a plateau at only 37% of the mean photosynthetic rate was observed during the growing season in the field. Thus, even under near-optimal environmental conditions, a short-term, significant limitation to winter photosynthesis remains (Schaberg et al. 1998). Ottander and Öquist (1991) also reported that, in Scots pine, photochemical efficiency of PSII...


Water deficit, another factor that limits photosynthesis, was probably not a factor in our study because the Taiwan spruce shoots were sampled at predawn and recut under water immediately. Besides, we found that $F_v/F_m$ values of Taiwan spruce on February 14 and March 30, 2003, were 0.544 ± 0.035 and 0.746 ± 0.008, respectively, and the ratios of intercellular to ambient CO$_2$ concentration were 0.601 ± 0.099 and 0.606 ± 0.025, respectively, indicating that, at low temperatures, both stomatal factor and non-stomatal factors were equally affected.

Seasonal variation in $P_{\text{nsat}}$ of Taiwan spruce was closely related to needle SP concentration and $F_v/F_m$ as well as to air temperature (Figures 2 and 4). Soluble proteins in leaves com-

**Figure 3.** Relationship between chlorophyll fluorescence ($F_v/F_m$) of Taiwan spruce needles (1 and 2 years old) and temperatures of measuring day in all measured seasons. Asterisks indicate statistical significance ($P < 0.001$).


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prise mainly the enzymes of photosynthesis (Friedrich and Haffaker 1980). There is a positive correlation between Rubisco and foliar SP concentration (Bravdo and Pallas 1982, Muthuchelian 1992, Naidu and Swamy 1995), and the photosynthetic capacity of a single leaf shows a positive linear relationship to its SP concentration in many cases (Patterson et al. 1977, Bravdo and Pallas 1982, Weng and Chen 1987, Muthuchelian 1992). Ottander and Öquist (1991) concluded that, in Scots pine, the recovery of the photoinhibition component in PSII was faster than that of the frost inhibition component at the enzymatic level. Figure 2 shows that both $P_{\text{nsat}}$ and needle SP concentration of Taiwan spruce recovered from winter stress much later than $F_{v}/F_{m}$, suggesting that one reason for the delay in recovery of photosynthetic capacity could be the slow increase in needle SP concentration.

Gezelius and Hallen (1980) stated that the needle SP concentration in Scots pine began to decrease in April and then to increase in July, and that Rubisco activity was higher in summer, suggesting that the new shoots used proteins (including Rubisco) as the nitrogen source. Figures 1 and 2 show that 2001 had a warm winter (January to February) and the SP concentration of the needles was unaffected by air temperature. On March 31, 2001, however, needle SP concentration and $P_{\text{nsat}}$ dropped temporarily, despite the rising air temperature. The same tendency has been found in two other conifers, *Pinus taiwannensis* Hayata and *Tsuga chinensis* Franchet (Pixsel) ex. Diels (authors’ unpublished data). These results provide strong circumstantial evidence that the needle soluble proteins act as the nitrogen source to support growth of new shoots.

Many studies have shown that the chlorophyll concentration in needles decreases in response to low temperature or short-day conditions (Ottander et al. 1995, Vogg et al. 1998). Although we found that the chlorophyll concentration in needles of Taiwan spruce was higher in summer and lower in winter (Figure 2), the relationship between $P_{\text{nsat}}$ and chlorophyll concentration was less marked than the relationships between $P_{\text{nsat}}$ and $F_{v}/F_{m}$ or between $P_{\text{nsat}}$ and leaf SP concentration.

In conclusion, we found that, in the subalpine areas of subtropical Taiwan, Taiwan spruce retained about 20% of its photosynthetic quantum yield, chlorophyll organization, and energy distribution between the two photosystems in Scots pine. Can. J. Bot. 64:748–755.


References


