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Difference of high-light stress sensitivity in the two firs, *Abies mariesii* and *Abies veitchii*, in early spring

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Abstract: *Abies veitchii* and *Abies mariesii* are dominant species at the tree-line in Central Japan. Recently, we observed needle death, probably due to photodamage of the photosynthetic apparatus at the tree-limit during March–April. *A. veitchii* survives winter without any needle death due to photodamage at the tree-line. However, there is no conspicuous damage between the two species because this phenomenon is only observed at the tree-limit. In this study, we examined the difference in winter down-regulation of PS II between *A. veitchii* and *A. mariesii* and observed the following results: In March, (1) Fv/Fm of both species was about 0.1, showing the photochemical efficiency being severely inhibited. (2) The de-epoxidation state, expressed as [(A+Z)/(V+A+Z)], was about 0.35 for both species. (3) Chlorophyll (Chl) content of *A. veitchii* was much less than that of *A. mariesii* and Pchlide was found only in *A. veitchii.* In April, (1) Fv/Fm increased and [(A+Z)/(V+A+Z)] decreased for both species. (2) Chl content of *A. veitchii* increased by four-fold while Pchlide nearly dissappeared. These results indicate the following: During cold periods, most of Chl of *A. veitchii* may have been converted to Pchlide which is easily re-converted to Chl in spring, an intermediate of Chl biosynthesis. Winter conversion from Chl to Pchlide in *A. veitchii* may provide effective protection from photodamage of the photosynthetic apparatus. Furthermore, this may explain the higher ability of *A. veitchii* to prevent photodamage compared to *A. mariesii.*

key words: Down-regulation of PS II, winter-induced photoinhibition, xanthophyll cycle, *Abies* species

Introduction

Evergreen conifers retain their needles for several years and so their photosynthetic apparatus must survive severe winters, allowing recovery of photosynthesis when warmer periods return in spring. Winter stress, with the interaction of light and low temperature, results in inhibition of the photochemical efficiency δ of PS II (Fv/Fm) in evergreen conifers (Martin *et al.*, 1978; Strand and Öquist, 1988; Han and Mukai, 1999). Reversible photoinhibition of PS II is recognized to be a means for long-term down-regulation of PS II to prevent further irreversible photodamage to the thylakoid membrane by excess light energy, when low temperature causes inhibition in the Calvin cycle (Strand and Öquist, 1988; Ottander and Öquist, 1991). Decrease in Fv/Fm

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during autumn–winter is caused by increase in the de-epoxidation state of the xanthophyll cycle, loss of D1-protein associated with PS II complex, loss of chlorophyll and changes in the thylakoid polypeptide compositions (Ottander *et al.*, 1995). Thus, conifers of the boreal zone have been considered to be completely protected from photodynamic destruction of the photosynthetic apparatus during winter (Ottander *et al.*, 1995). Recently, we observed winter needle death, probably due to photodamage of the photosynthetic apparatus in *Abies mariesii* at tree-line in the temperate zone (Maruta and Nakano, 1999). This indicates that long-term down-regulation of PS II could not solely prevent photodamage under the harsher conditions at the alpine tree-line compared to the boreal zone.

In Central Japan, *A. mariesii* dominates at the tree-line, protected by heavy snow cover on the Japan Sea side of Japan. *A. veitchii* dominates at the tree-line on the Pacific Ocean side with only a small amount of snow cover and survives winter without any needle death due to photodamage. Thus, *A. veitchii* and *A. mariesii* have different geographic distributions probably due to different susceptibilities to winter photodamage.

The purpose of this study is (1) to compare the mechanism of winter-induced photoinhibition between *A. veitchii* and *A. mariesii* during the critical period (March–April) for photodamage and (2) to evaluate the relationship between susceptibility to winter photodamage and geographic distribution for both species.

Materials and methods

Study site and plant materials

The study site was chosen at 2270 m a.s.l. on the gentle northern slope of Mt. Shimagare [36°03′N, 138°20′E, 2395 m above sea level (a.s.l.)], one of the mountains of the "Yatsugatake" ("8 peaks" massif) located in the central mountainous area of Japan in Nagano Prefecture. The ground is covered with snow from December to early-May with a maximum snow depth of *ca.* 1.5 m. The site was co-dominated by *A. mariesii* and *A. veitchii* and the tree heights and DBH (diameter at breast height) were about 6 m and 10–15 cm, respectively. On 27 March and 25 April 2001, shoots of both species were collected from the study site and brought to the laboratory, where they were kept below 5℃ in darkness. Some of the shoots were used for recovery experiments (see below). The rest were stored at −85℃ until biochemical assay.

Measurement of environmental conditions

Air temperature was measured with a thermistor sensor located 2 m above the ground, where it would not be buried under the snow and monitored hourly using a data-logger (model KADEC-HTV, KONA Systems). Photosynthetically active photon flux density (PPFD) was simultaneously measured with a portable photon sensor (Model IKS-27; Koito Industries LTD.) oriented horizontally 1 m above the ground, and the data were collected hourly with the data-logger (model KADEC-HTV, KONA Systems).

Recovery experiments

Detached current-year shoots were immediately measured for dark-adapted maximal photochemical efficiency (Fv/Fm) in the laboratory by a fluorometer (MINI-PAM; Walz) clipped with a Leaf-Clip holder equipped with the fluorometer at 20℃. After that, the shoots were placed on a damp sponge mat to absorb water and illuminated by weak light (15 μmol photons $m^{-2}s^{-1}$) at 15℃ for recovery. To follow the time course of the recovery, Fv/Fm was measured every 2 or 3 days. One experiment used six shoots, which were dark-adapted at 20℃ at least 30 min before measurement.

Determination of pigment composition by HPLC

To extract the pigments, current-year needles (0.15 g) were used. The leaf area was estimated by scanning the leaf shapes by an image scanner (EPSON GT-6500) and a microcomputer (NEC PC-9801 BA2). The needles were ground with a mortar and pestle in cold 85% aqueous acetone together with quartz sand and 4% (w/v) Polyclar AT. The mortar was then rinsed with a small amount of 100% acetone. The homogenate was centrifuged at 10000 × *g* for 5 min. The supernatant was placed on ice, and the pellet was re-extracted with a small volume of 85% acetone and recentrifugation. The acetone supernatant was combined (total 4 m*l*) and filtered through a 0.45-μm syringe filter (Millex®, Millipore) into vials. The vials were stored on ice in the dark prior to injection. All extraction procedures were carried out in a dim room. Ten microliters of extract was immediately injected into an HPLC system (model 600, Waters, CA, USA) using a Spherisorb ODS1 column (4.6 mm I.D., 250 mm length) and a Waters ODS1 guard column following a protocol described by García-Plazaola and Becerril (1999) with slight modifications: A linear gradient from 100% Solvent A containing 72 : 8 : 3 (v/v/v) of acetonitrile : methanol : Tris-HCl (100 mM, pH 8.0) to 100% Solvent B containing methanol : hexane (4 : 1; v/v) was used for the first 12 min, followed by isocratic elution with 100% Solvent B for the next 6 min. This was followed by a 2-min linear gradient from 100% Solvent B to 100% Solvent A and isocratic elution with 100% Solvent A for a further 10 min to allow the column to re-equilibrate with Solvent A prior to the next injection. All pigments were eluted from the column within approximately 20 min at a flow rate of 1.5 m*l*/min. The eluted pigments were monitored at 440 nm, and temperature was maintained at 20℃. The peak area was integrated automatically by a Waters 740 Data module. Pigment contents were determined with a standard curve.

Three independent experiments were replicated and data obtained as mean ± SD. Statistical analysis was performed with StatView ver. 5.

Results

Climate

Diurnal mean air temperature from 1 February to 1 May 2001 at the study site is shown in Fig. 1A. The temperature was below 0℃ until early April and above 0℃ from mid-April to later. Figure 1B shows the diurnal mean photosynthetically active photon flux density (PPFD) between 1 February and 1 May, 2001. PPFD was instantaneously over 1500 μmol photons $m^{-2}s^{-1}$ under subzero temperature.

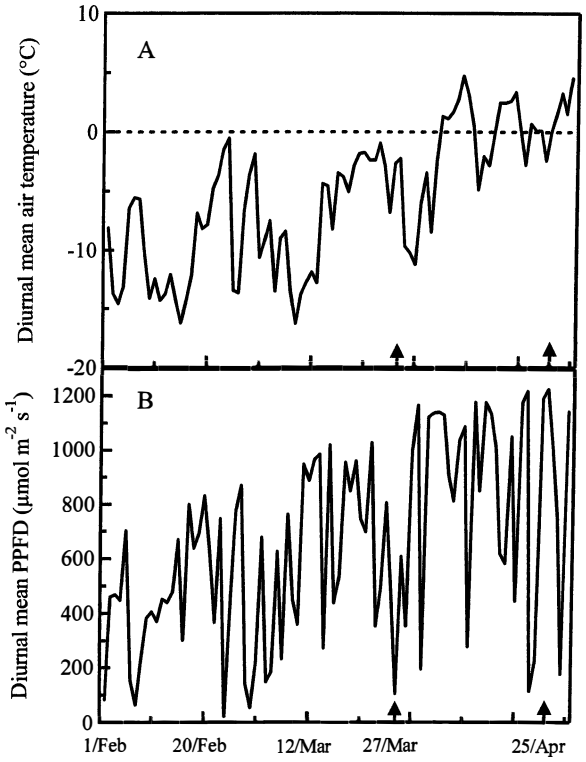

Fig. 1. Diurnal mean air temperature (A) and photosynthetically active photon flux density (B) between 1 February and 1 May, 2001, for natural *A. mariesii* and *A. veitchii* grown at 2270 m a.s.l. on the northern slope of Mt. Shimagare (36°03′N, 138°20′E, 2395 m a.s.l.). The photon sensor was located horizontally 1 m above the ground. Arrows indicate the sampling date.

Recovery experiment

Figure 2 shows the time course of recovery of the photochemical efficiency of PS II, expressed as Fv/Fm when placed at 15℃ in weak light (15 μmol photons $m^{-2}s^{-1}$) in the laboratory. In March, the Fv/Fm ratios of both species were below 0.2, and didn't significantly differ between the two species ($p > 0.05$, Student's *t*-test) at the beginning of the experiment; then the ratios showed a rapid increase and reached 0.7 within the first 10 days (Fig. 2A). After one month, the Fv/Fm ratios of both species recovered to about 0.8 of their initial values. In April, the Fv/Fm ratios of *A. mariesii* and *A. veitchii* were about 0.2 and 0.4, respectively ($p < 0.05$, Student's *t*-test), at the beginning of the recovery experiment (Fig. 2B). The Fv/Fm ratio reached 0.7 in *A. veitchii* after 10 days, while that in *A. mariesii* about 15 days. After a month, the ratio of both species recovered to about 0.8. Thus, the Fv/Fm ratio of *A. veitchii* is higher than that of *A. mariesii* in April.

HPLC analysis

Figure 3 shows the de-epoxidation state of the xanthophyll cycle expressed as (A+

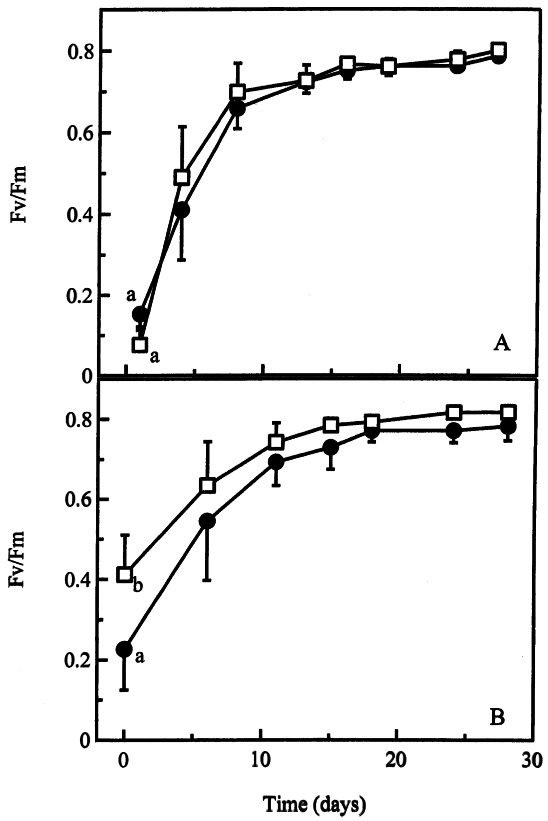

Fig. 2. The time course of recovery of the Fv/Fm ratios in *A. meriesii* (circle) and *A. veitchii* (square). After dark-adaptation, the Fv/Fm ratio was determined using the shoots collected in March (A) and in April (B). Bars indicate means±SD ($n=10$). The letter code indicates significant differences ($p<0.05$, Student's *t*-test).

Z)/(V+A+Z), where A, Z, V are the contents of antheraxanthin, zeaxanthin, and violaxanthin, respectively, in both species. The value was similar between both species ($p<0.05$) in March. One month later, the values of both species decreased. The decrease in *A. veitchii* was larger than that of *A. mariesii* ($p<0.05$, Kruskal-Wallis test).

Chlorophyll (Chl) contents of *A. mariesii* did not significantly differ between March and April ($p>0.05$, Kruskal-Wallis test), while those of *A. veitchii* were significantly lower in March than in April ($p<0.05$, Kruskal-Wallis test) (Fig. 4). The elution profiles of HPLC analysis in Fig. 5 show the clearly prominent peak at protochlorophyllide (Pchlide) in only *A. veitchii* in March. Pchlide contents in March are remarkably higher than in April (Fig. 6; $p<0.05$, Student's *t*-test).

Discussion

In March, Fv/Fm of both species was about 0.1 (Fig. 2A), showing the photochemical efficiency being most severely depressed (Ottander *et al.*, 1995). The de-

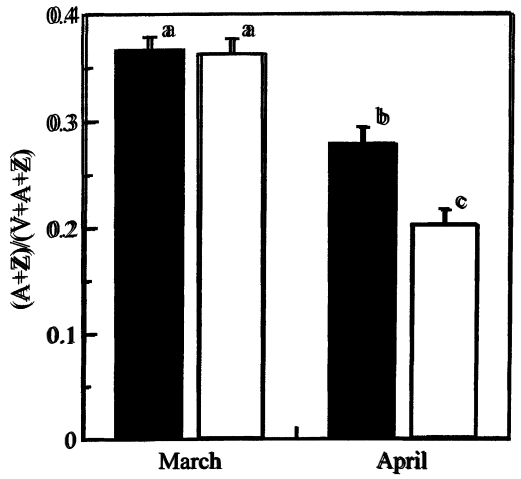

Fig. 3. The de-epoxidation state of the xanthophyll cycle expressed as [(A+Z)/(V+A+Z)] where A, Z, V are the contents of antheraxanthin, zeaxanthin, violaxanthin, respectively, in March and April. Closed bars, *A. mariesii*; open bars, *A. veitchii*. Bars indicate means±SD ($n=3$). Letter code indicates significant differences ($p<0.01$, Kruskal-Wallis test). Detailed HPLC techniques are described in the text.

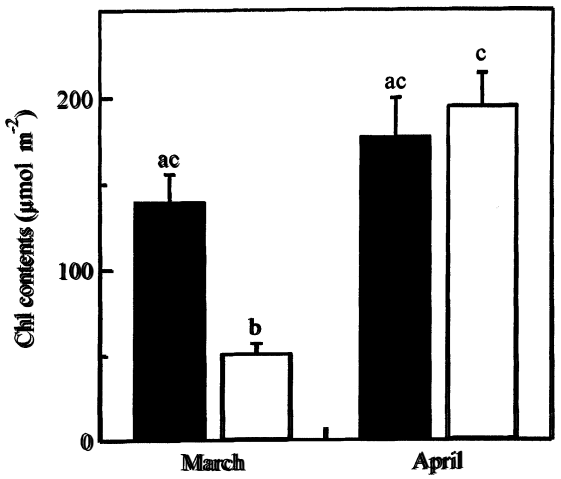

Fig. 4. Chlorophyll (Chl) contents in March and April. Closed bars, *A. mariesii*; open bars, *A. veitchii*. Bars indicate means±SD ($n=3$). Letter code indicates significant differences ($p<0.01$, Kruskal-Wallis test). Detailed HPLC techniques are described in the text.

epoxidation states of the xanthophyll cycle, expressed as [(A+Z)/(V+A+Z)], were about 0.35 for both species in March (Fig. 3). The de-epoxidation state [(A+Z)/(V+A+Z)] of other severely photoinhibited conifer species is reported to be 0.8–0.9 (Adams and Adams, 1994; Verhoeven *et al.*, 1999). Consequently, xanthophyll cycle-dependent thermal energy dissipation does not explain all depression of Fv/Fm for both

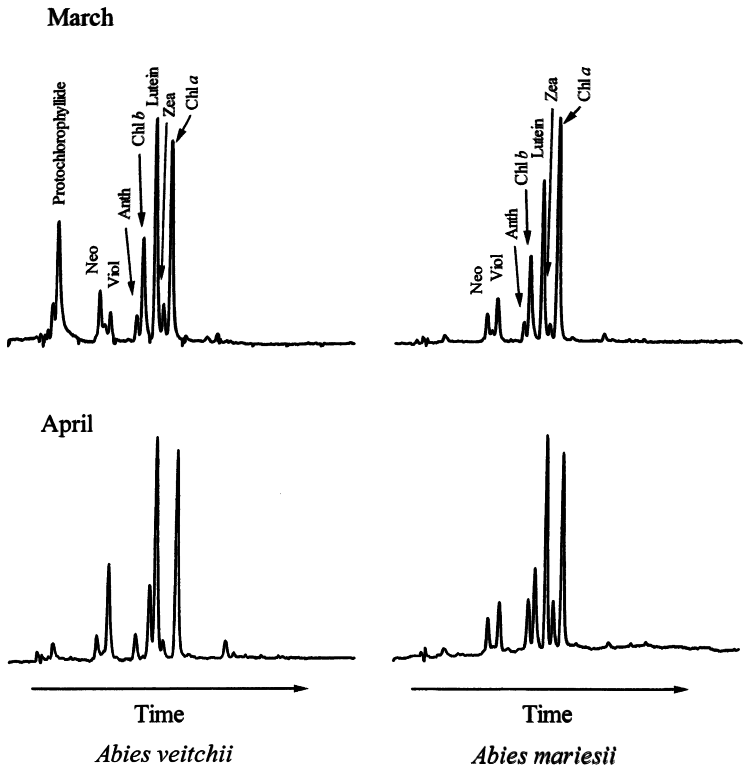

Fig. 5. HPLC chromatograms of *A. mariesii* and *A. veitchii.* Chl *a,* chlorophyll *a*; Chl *b,* chlorophyll *b*; Neo, neoxanthin; Viol, violaxanthin; Anth, antheraxanthin; Zea, zeaxanthin.

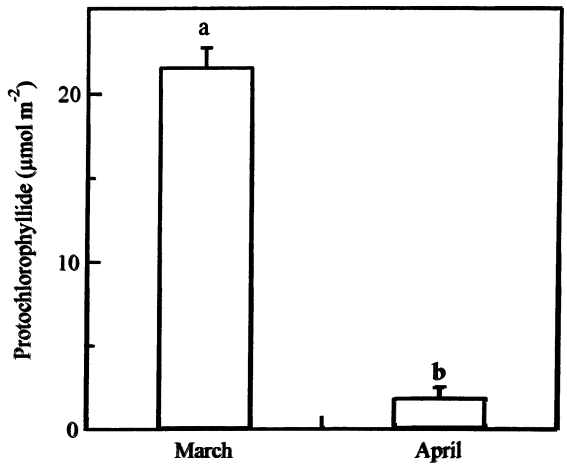

Fig. 6. Contents of protochlorophyllide (Pchlide) in *A. veitchii.* Bars indicate means±SD ($n=3$). Letter code indicates significant differences ($p<0.01$, Student's *t*-test). Detailed HPLC techniques are described in the text.

species (Ottander *et al.*, 1995). Indoor recovery of Fv/Fm in March was slow for both species and Fv/Fm increased from 0.1 to 0.7 within ten days (Fig. 2A). If depression in Fv/Fm is solely due to the xanthophyll cycle, recovery should be completed within several hours (Adams and Adams, 1994). These results indicate that loss of D1-protein in PS II and/or light-harvesting complex (LHC II) also caused depression in Fv/Fm for *A. veitchii* and *A. mariesii* in March.

In March, Chl content of *A. veitchii* was much less than that of *A. mariesii* (Fig. 4) and Pchlide was found only in *A. veitchii* (Fig. 5), whereas Fv/Fm and (A+Z)/(V+A+Z) were the same between *A. veitchii* and *A. mariesii.* During cold periods, most of the Chl of *A. veitchii* may have been converted to Pchlide which is easily re-converted to Chl in spring, an intermediate of Chl biosynthesis. Winter conversion from Chl to Pchlide in *A. veitchii* may provide effective protection from photodamage of the photosynthetic apparatus. Furthermore, this may explain the higher ability of *A. veitchii* to prevent photodamage compared to *A. mariesii.* Pchlide was found in very young beech (*Fagus sylvatica*) leaves immediately after flushing (García-Plazaola and Becerril, 2001). We present the first evidence for winter conversion from Chl to Pchlide in an evergreen conifer species.

In April, Fv/Fm increased (Fig. 2B) and [(A+Z)/(V+A+Z)] decreased (Fig. 3) for both species. These changes indicate that recovery from severe winter photoinhibition started under 0–5℃ air temperature in April (Fig. 1A). The remarkable changes included a four-fold increase in Chl of *A. veitchii* (Fig. 4) with a large decrease in Pchlide (Fig. 6). These imply that Pchlide of *A. veitchii* contributed to prevent severe photodamage during critical subzero periods and rapid recovery of photochemical efficiency when warmer temperatures return in spring. Thus, *A. veitchii* can survive severe winters at tree-line without photodamage.

In *A. mariesii* under harsh winter conditions at tree-line, long-term down regulation may not be sufficient to quench excess energy and thus prevent photodamage. Snow coverage may possibly protect needles of *A. mariesii* from photodamage Therefore, *A. mariesii* dominates at tree-line on the Japan Sea side with a large amount of snowfall, whereas *A. veitchii* dominates at tree-line on the Pacific Ocean side with only a small amount of snow cover.

Acknowledgments

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