# Effects of summer frosts and subsequent shade on foliage gas exchange in peatland tamarack and black spruce

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In this study, we examined the effect of summer frost on light-saturated net photosynthesis ($P_{net}$) and related parameters in 20-year-old black spruce (*Picea mariana* (Mill.) B.S.P.) and tamarack (*Larix laricina* (Du Roi) K. Koch) at a peatland site in central Alberta. Summer frosts were simulated in the field using a self-contained freezing chamber. Some trees were shaded from direct sunlight after freezing. Shaded trees received about 20% of full sunlight at midday. We found that the response of photosynthesis consisted of two phases: a depression phase, where photosynthesis declined progressively, and a recovery phase, where photosynthesis recovered gradually. The length of the depression phase varied with species but not with the degree of freezing. For both species, depression in $P_{net}$ was primarily related to decreased mesophyll conductance to $CO_2$, and a full recovery of $P_{net}$ took more than 11 days. Post-frost shade enhanced recovery in $P_{net}$ after a −3.5°C frost in tamarack, but had no detectable effect after a −6°C frost in tamarack or a −3°C frost in black spruce.

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Cette étude avait comme objectif d'étudier l'effet du gel estival sur la photosynthèse nette au point de saturation lumineuse ($P_{net}$) ainsi que les paramètres qui y sont associés, chez des épinettes noires (*Picea mariana* (Mill.) B.S.P.) et des mélèzes laricins (*Larix laricina* (Du Roi) K. Koch) âgés de 20 ans et croissant dans une tourbière du centre de l'Alberta. Le gel estival était simulé au champ à l'aide de chambres froides autonomes. Certains des arbres furent maintenus à l'ombre après avoir été soumis au gel. Les arbres ombragés recevaient environ 20% du plein ensoleillement à midi. La réaction de la photosynthèse comportait deux phases : une phase de suppression où elle diminuait progressivement et une phase de récupération où elle revenait graduellement à la normale. La durée de la phase de suppression variait selon l'espèce, mais non en fonction de l'intensité du gel. Chez les deux espèces, la suppression de $P_{net}$ était principalement reliée à une réduction de la conductivité du mésophylle au $CO_2$ et $P_{net}$ a pris plus de 11 jours pour récupérer complètement. La présence d'ombre suite au gel a favorisé la récupération de $P_{net}$ après un gel de −3,5°C chez le mélèze. Par contre, elle n'avait aucun effet notable après un gel de −6°C chez le mélèze ou de −3°C chez l'épinette noire.

[Traduit par la rédaction]

## Introduction

Summer frosts are common at high latitudes or high elevations (Christersson 1984; Christersson *et al.* 1987). Frosts can occur every month during the summer in boreal peatlands (Hayter and Proudfoot 1978; Rothwell and Lieffers 1988). These frosts can cause morphological injury or death of conifer seedlings (Christersson 1984; Christersson *et al.* 1987; Lundmark and Hällgren 1987; Lundmark *et al.* 1988). Injury from moderate to light frosts can be limited to a temporary depression of photosynthesis (e.g., in seedlings of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.), Lundmark and Hällgren 1987; Lundmark *et al.* 1988). A substantial decrease in photosynthesis was also observed in shoots cut from field-grown Engelmann spruce (*Picea engelmannii* Parry) and frost treated in the laboratory (DeLucia and Smith 1987). But none of these studies were conducted on peatland trees. A biphasic response of photosynthesis was reported for spinach (Hincha *et al.* 1989). Post-frost injury was also reported for cold-acclimated Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) and ponderosa pine (*Pinus ponderosa* Laws.) seedlings (Pharis *et al.* 1970). But processes of post-summer-frost injury and recovery of photosynthesis in trees are not well understood.

Strong light following frost can further injure the photosynthetic systems. Needle discolouration and decreases in chlorophyll concentration after summer frosts were more severe in seedlings of Scots pine and Norway spruce exposed to direct sunlight than those shaded (Lundmark and Hällgren 1987). Decreases in chlorophyll fluorescence after summer frosts were also greater in exposed than shaded Scots pine seedlings (Strand and Lundmark 1987). The recovery process of photosynthesis following summer frosts, however, is rarely monitored in subsequently shaded and exposed field-grown trees.

The objectives of this study were (*i*) to examine the effects of summer frosts on light-saturated net photosynthesis, mesophyll conductance, stomatal conductance, and photosynthetic water use efficiency, and post-frost recovery of these parameters, in 20-year-old peatland tamarack (*Larix laricina* (Du Roi) K. Koch) and black spruce (*Picea mariana* (Mill.) B.S.P.); (*ii*) to examine the effects of post-frost shading on injury and recovery of the above parameters.

## Materials and methods

The study site was a treed fen located 26 km west of Edmonton, Alberta (53°34′N; 113°31′W). The site was part of the Wagner wetland complex and classified as an extremely rich fen, particularly rich in nitrogen, phosphorus, calcium, and sulphur. Substrate pH varied between 7.4 and 7.8 in the growing season (Vitt 1990). The peat layer at the study site was more than 1 m deep. The site was flat (average slope < 1%), and the groundwater was within 0–20 cm from the peat surface during the experiments. Total precipitation and average minimum temperature from June to August were,

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respectively, 227.3 mm and 11.5°C for this general area (Environment Canada 1989, monthly record from Edmonton Municipal Airport). But the minimum temperature at the study site was generally several degrees lower.

The study site was forested by tamarack and black spruce, with a stem density of 3000/ha. The trees were 20 years old and 2 m high (average), and appeared to be healthy. The ground was mainly covered by *Ledum groenlandicum* Oeder and *Tomenthypum nitens* (Hedw.) Loeske.

Four separate freezing experiments were conducted on black spruce and tamarack. Trees that were representative of those growing in the area were selected for treatment. Sample trees were randomly distributed on the site, but a control tree was always selected close to a treatment tree (paired). A representative branch was selected from each tree at about 1.4 m height for frost-treatment and gas-exchange measurements.

Selected branches of treatment trees were frozen to a specified temperature at 04:00, using a polystyrene freezing chamber and a NaCl–ice mixture as the freezing medium (Dang *et al.* 1991*a*). Freezing lasted for 2 h in all experiments. For the black spruce experiments in 1989, freezing chambers were also applied on control trees, but ice chips (0°C) were substituted for the freezing medium. The use of ice chips was intended to duplicate the possible crushing injury to treatment foliage from the ice–salt mixture, separating the effect of freezing from that of crushing. In subsequent experiments in 1990 on tamarack, an inner protective chamber was developed to protect foliage from this crushing injury. Polystyrene beads were then used on control trees to simulate the pressure on the inner chamber from the ice–salt mixture on treatment trees. Immediately after the freezing ended, half of the treatment trees and half the control trees were shaded from direct sunlight by placing the twigs beneath empty freezing chambers mounted on a wooden pole. The shaded twigs received about 20% of full sunlight at midday. The shaded and unfrozen trees were used as controls for the shaded and frozen trees. The unshaded and unfrozen trees served as controls for frost-treated and unshaded trees.

Foliage gas exchange was measured using an open system consisting of a portable infrared gas analyzer (LCA-2), a leaf cuvette (PLC), and an air supply unit (all from Analytical Development Corporation, Hoddeson, England). Ambient air was drawn from 4 m height using a tower, and passed through a desiccator before entry into the cuvette. Gas-exchange measurements were taken quickly (<1 min) to reduce heating inside the cuvette. All measurements were taken at or above the saturated light level for photosynthesis (800 and 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively for tamarack and black spruce). When natural light was insufficient, light levels were boosted to 1600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ using a Brinkman Q-beam spotlight.

To minimize time of day effect, all gas-exchange measurements were made between 10:00 and 11:00. According to Dang *et al.* (1991*b*), values of gas-exchange parameters were relatively stable during this time period. In addition, measurements were taken in pairs (i.e., treatment tree and adjacent control). This further reduced time effect. The shaded foliage was exposed to light at or above the saturation levels for photosynthesis for less than 3 min during gas-exchange measurements, then put back into shade again. The same twigs were used throughout an experiment. Experimental twigs were collected at the end of each experiment for foliage area determination (as described by Macdonald and Lieffers 1990).

*Black spruce, experiment 1*

Nine representative black spruce trees were selected; three were used as controls (0°C), three were cooled to −4°C, and three were cooled to −8°C. Freezing was conducted July 1, 1989. Gas exchange of current-year and 1-year-old needles of both treatment and control trees was measured on the day before freezing, on the freezing day, and 1, 2, 4, and 11 days after freezing.

The weather was sunny on most days following freezing, but partially cloudy on the 2nd day, and overcast on the 8th and 10th days after freezing. The ambient temperature was 3.5°C when the freezing started.

*Black spruce, experiment 2*

On July 25, 1989, freezing chambers were applied to 12 black spruce, six as control (0°C) and six cooled to −3°C. After the freezing chambers were removed, the treated branches from half of the control and half of the −3°C trees were shaded as described earlier. Gas exchange of current-year and 1-year-old needles of both control and treatment trees was measured on the day prior to freezing and 2 days after.

On the freezing day, it was sunny in the morning and partially and intermittently cloudy in the afternoon. On the following 2 days, it was mainly sunny. The ambient temperature was 4°C when freezing started.

*Experiments on tamarack*

Two separate freezing experiments were conducted. The design of these experiments was the same as in the second experiment on black spruce, but the number of replicates for each shading–freezing combination was increased from three to six trees. Experiment 1 (−3.5°C) and experiment 2 (−6°C) were conducted, respectively, on July 14 and 20 of 1990. The ambient temperatures at the time of freezing were 4 and 0.4°C, respectively, for experiments 1 and 2. The shading period was 4 days for both experiments. The weather was partially cloudy or rainy for part of the day on July 15, 16, 17, 19, 25, and 26, and sunny and warm on all other days during experiments.

Gas exchange of both treatment and control trees for experiment 1 was measured on the day before freezing, on the freezing day, and 1, 4, 6, and 8 days after freezing. Measurements for experiment 2 were made on the day prior to freezing, on the freezing day, and 1, 2, 3, 4, 6, and 11 days after freezing.

*Data analyses*

Net photosynthesis rate ($P_{net}$), leaf resistance to $H_2O$ vapour ($r$), transpiration rate ($E$), and intercellular $CO_2$ concentration ($C_i$) were determined as described by Caemmerer and Farquhar (1981). Since the high-speed fan in the cuvette and the design of the cuvette ensure a small boundary layer resistance ($r_b$) and $r_b$ is generally very small for needles, the stomatal resistance to $H_2O$ vapour ($r_s$) was assumed to be equal to $r$. The stomatal conductance to water vapour was calculated as $g_s = 1/r_s$. Stomatal conductance to $CO_2$ ($g_c$) was calculated as $g_c = g_s/1.6$ (Coombs *et al.* 1987). Mesophyll conductance to $CO_2$ ($g_m$) was calculated as $g_m = P_{net}/C_i$ (Fites and Teskey 1988). The water use efficiency of photosynthesis (WUE) was determined as WUE = $P_{net}/E$ (Larcher 1983). The parameters $P_{net}$, $g_c$, and $g_m$ were all expressed on a leaf area basis.

The data for the graphical presentations were calculated as follows. The response to frost of a parameter on a given day was determined by dividing the parameter of a treatment tree by its paired control and expressing this as a percentage. Since control and treatment trees were exposed to the same environment throughout the experiment except during the frost treatment, values of the response should be comparable between different days. A further standardization was made by adjusting the response-time line up or down, so that the response for the pre-freezing day was equal to 100%. All the above procedures were performed separately for shaded and unshaded trees. The standardized response reflected the net response of a parameter to the frost (e.g., 60% indicated the parameter was reduced by 40%). Pretreatment measurements were used in a similar way in evaluating effects of fertilizers on tree growth (Salonius *et al.* 1982; Ballard and Majid 1985).

Tests of significance of a response to treatment were performed as follows. When the mean graphical response of a parameter was greater than 70% for a post-frost day, the significance of this response was tested using analysis of covariance (ANCOVA). However, the parameters (not the response for the graphical presentation) were used in the analysis. The pretreatment measurement was used as a covariate in the analysis to control the tree to tree variation. When the graphical response was less than 70% for a post-frost day, statistical tests were generally not performed, as spot tests showed that there was clearly a significant difference with these data. As with the

TABLE 1. Analysis of covariance on the effect of a simulated summer frost of −4°C on stomatal conductance of 1-year-old needles of black spruce

| Day | Source | df | MSS | $F$ | $P>F$ |
|---|---|---|---|---|---|
| 0 | Treatment | 1 | 157.78 | 18.33 | 0.0234 |
| | Error | 3 | 8.61 | | |
| 1 | Treatment | 1 | 206.56 | 3.58 | 0.1547 |
| | Error | 3 | 57.81 | | |
| 2 | Treatment | 1 | 34.81 | 93.52 | 0.0023 |
| | Error | 3 | 0.37 | | |
| 4 | Treatment | 1 | 153.29 | 6.30 | 0.0869 |
| | Error | 3 | 24.33 | | |
| 11 | Treatment | 1 | 1.00 | 0.22 | 0.6718 |
| | Error | 3 | 4.54 | | |

NOTE: Pretreatment measurement was the covariate. Day, days following freezing; day 0 is the freezing day. MSS, adjusted mean sum of squares.

TABLE 2. Analysis of covariance on the effect of post-frost shading (i.e., frozen and shaded trees vs. frozen and unshaded trees) on photosynthesis ($P_{net}$), mesophyll conductance ($g_m$), stomatal conductance ($g_c$), and photosynthetic water use efficiency (WUE) in needles of black spruce 2 days after a simulated summer frost of −3°C

| Needle age | Parameter | Source | df | MSS | $F$ | $P>F$ |
|---|---|---|---|---|---|---|
| Current | $P_{net}$ | Treatment | 1 | 0.0084 | 0.02 | 0.8936 |
| | | Error | 3 | 0.42 | | |
| | $g_m$ | Treatment | 1 | 0.46 | 0.08 | 0.8000 |
| | | Error | 3 | 5.75 | | |
| | $g_c$ | Treatment | 1 | 58.59 | 1.68 | 0.2860 |
| | | Error | 3 | 34.87 | | |
| | WUE | Treatment | 1 | 0.055 | 0.08 | 0.7953 |
| | | Error | 3 | 0.69 | | |
| 1 year old | $P_{net}$ | Treatment | 1 | 0.16 | 2.81 | 0.1921 |
| | | Error | 3 | 0.057 | | |
| | $g_m$ | Treatment | 1 | 2.92 | 2.61 | 0.2048 |
| | | Error | 3 | 1.12 | | |
| | $g_c$ | Treatment | 1 | 17.12 | 5.27 | 0.1054 |
| | | Error | 3 | 3.25 | | |
| | WUE | Treatment | 1 | 0.51 | 6.89 | 0.0787 |
| | | Error | 3 | 0.074 | | |

NOTE: Pretreatment values were used as a covariate. MSS, adjusted mean sum of squares.

graphical presentation, all ANCOVA tests were one-way analyses, testing frozen vs. unfrozen (no shading involved) and frozen and shaded vs. frozen and unshaded (testing shading effect).

## Results

*Black spruce, experiment 1*

The injury caused by the −8°C frost was severe, resulting in mortality and shedding of both current and 1-year-old needles. For the first 2 days following freezing, small amounts of $P_{net}$ were detectable in both current and 1-year-old needles. Current needles started turning brown at the tip on the 1st day after freezing. By the 2nd day, current needles were almost completely brown and only respiration was detected. For 1-year-old needles, a low rate of $P_{net}$ was still detected on the 2nd day, but they also started turning brown (data not shown). By the 4th day, both current and 1-year-old needles were senescent and abscising.

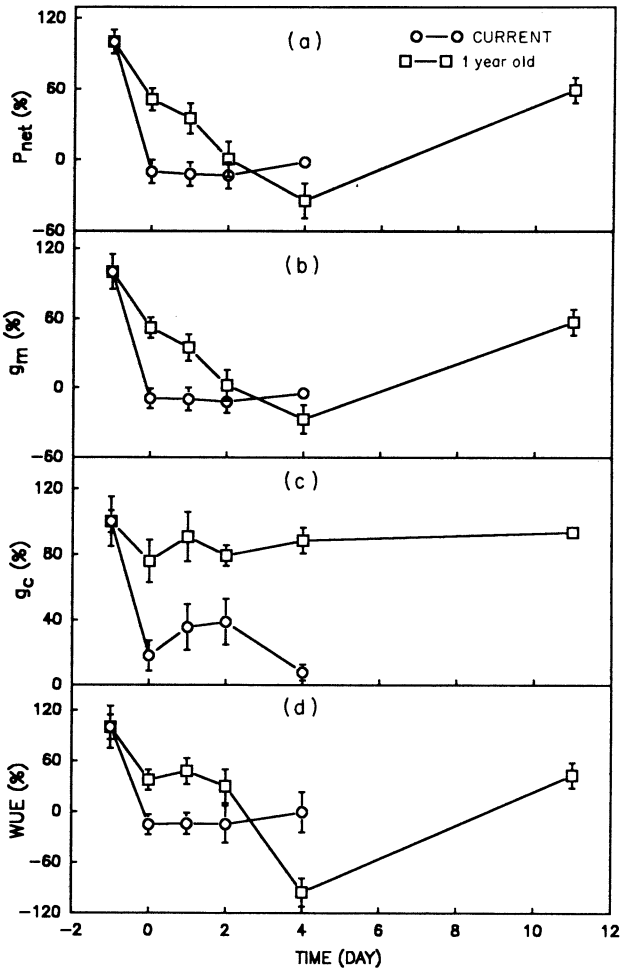


FIG. 1. Response of current and 1-year-old needles of black spruce to a simulated summer frost of −4°C. (*a*) Light-saturated net photosynthesis rate ($P_{net}$). (*b*) Mesophyll conductance ($g_m$) to $CO_2$. (*c*) Stomatal conductance to $CO_2$ ($g_c$). (*d*) Water use efficiency (WUE). On the *x*-axis, time −1 is the prefreezing day, 0 is the freezing day, and positive values are the days after freezing. Values ($\bar{x} \pm 1$ SE; $n = 3$) in the graph are expressed as a percentage of the control. Only net respiration (expressed as negative $P_{net}$) was observed in treatment trees on some post-frost days, while $P_{net}$ was measured in control trees on the same days. This resulted in negative percentage values. For the continuity of the response line, $g_m$ and WUE (negative in value) were also calculated for those days. No measurements were made on current foliage on day 11, when most needles fell off.

Current needles responded to the −4°C frost similarly as to the −8°C frost (became senescent). For 1-year-old needles, there was an immediate decrease in $P_{net}$ followed by recovery. The tips of these needles turned brown 4 days after freezing. For the first 4 days after freezing, $P_{net}$ and $g_m$ of 1-year-old needles decreased progressively (Figs. 1*a* and 1*b*). On the 4th day, no $P_{net}$ was detected. Substantial recovery of $P_{net}$ and $g_m$ occurred 11 days after freezing; however, the values were still less than 60% of those in controls (Figs. 1*a* and 1*b*). In contrast, the frost had no significant impact on $g_c$ 4 days after freezing (Fig. 1*c*; Table 1). On the freezing day and 2 days after freezing, however, $g_c$ in frozen trees was significantly lower than in the unfrozen trees ($P < 0.05$, Table 1). WUE generally responded similarly as $P_{net}$ and $g_m$, with low values

TABLE 3. Analysis of covariance on the effect of a simulated summer frost of −3.5°C (frozen and shaded trees vs. unfrozen and shaded trees) on photosynthesis ($P_{net}$), mesophyll conductance ($g_m$), stomatal conductance ($g_c$), and photosynthetic water use efficiency (WUE) in tamarack needles

| Parameter | Day | Source | df | MSS | $F$ | $P > F$ |
|---|---|---|---|---|---|---|
| $P_{net}$ | 4 | Treatment | 1 | 0.45 | 5.10 | 0.0471 |
| | | Error | 9 | 0.088 | | |
| | 6 | Treatment | 1 | 0.90 | 6.80 | 0.0230 |
| | | Error | 9 | 0.13 | | |
| | 8 | Treatment | 1 | 0.48 | 6.71 | 0.0292 |
| | | Error | 9 | 0.072 | | |
| $g_m$ | 4 | Treatment | 1 | 15.72 | 12.37 | 0.0065 |
| | | Error | 9 | 1.27 | | |
| | 6 | Treatment | 1 | 1.64 | 9.32 | 0.0380 |
| | | Error | 9 | 0.18 | | |
| | 8 | Treatment | 1 | 10.43 | 10.31 | 0.0106 |
| | | Error | 9 | 1.01 | | |
| $g_c$ | 4 | Treatment | 1 | 3.43 | 9.69 | 0.0125 |
| | | Error | 9 | 0.35 | | |
| | 6 | Treatment | 1 | 4.50 | 12.64 | 0.0062 |
| | | Error | 9 | 0.36 | | |
| | 8 | Treatment | 1 | 11.32 | 21.97 | 0.0011 |
| | | Error | 9 | 0.52 | | |
| WUE | 4 | Treatment | 1 | 4.29 | 13.61 | 0.0050 |
| | | Error | 9 | 0.32 | | |
| | 6 | Treatment | 1 | 1.27 | 8.13 | 0.0357 |
| | | Error | 9 | 0.16 | | |
| | 8 | Treatment | 1 | 1.57 | 3.45 | 0.0962 |
| | | Error | 9 | 0.46 | | |

NOTE: Pretreatment values were used as a covariate. Day, days after freezing; day 0 is the freezing day. MSS, adjusted mean sum of squares.

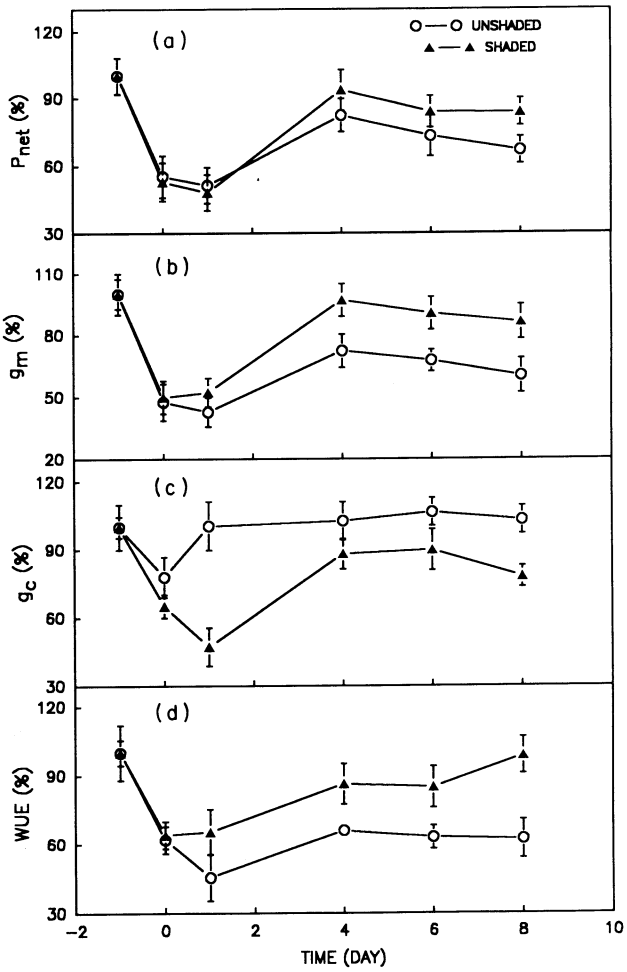


FIG. 2. Response of tamarack to a simulated summer frost of −3.5°C with and without subsequent shade. (*a*) Light-saturated net photosynthesis rate ($P_{net}$). (*b*) Mesophyll conductance to $CO_2$ ($g_m$). (*c*) Stomatal conductance to $CO_2$ ($g_c$). (*d*) Water use efficiency (WUE). On the *x*-axis, time −1 is the prefreezing day, 0 is the freezing day, and positive values are the days after freezing. Values ($\bar{x} \pm 1$ SE; $n = 6$) in the graph are expressed as a percentage of the control. Shaded trees were shaded from direct sunlight immediately after freezing.

in the first 3 days after freezing followed by recovery. WUE in frost-treated trees was 43% of that in unfrozen controls 11 days following freezing (Fig. 1*d*).

*Black spruce, experiment 2*

The −3°C frost depressed $P_{net}$, $g_m$, $g_c$, and WUE of current needles, respectively, by 69, 68, 45, and 47% 2 days after freezing. The decreases of $P_{net}$, $g_m$, $g_c$, and WUE in 1-year-old needles were, respectively, 67, 68, 18, and 62%. No colour change was observed in either current or 1-year-old needles. However, the ANCOVA results showed no significant differences in $P_{net}$, $g_m$, $g_c$, or WUE between the frozen and subsequently shaded trees and frozen and unshaded ones, in either current or 1-year-old needles (Table 2).

*Experiments on tamarack*

For −3.5°C frost, no colour change in foliage was observed for the first 3 days after freezing. On the 4th day, however, both shaded and unshaded needles had brown tips. On the day of freezing, $P_{net}$, $g_m$, and WUE were reduced by more than 40% in both shaded and unshaded trees (Figs. 2*a*, 2*b*, and 2*d*), while $g_c$ decreased by 22% for the unshaded and 35% for the shaded needles (Fig. 2*c*). Stomatal conductance in unshaded trees recovered fully 1 day after freezing ($P = 0.60$) but was still strongly depressed in the shaded trees (Fig. 2*c*). WUE of shaded trees recovered completely 8 days after freezing ($P = 0.10$, Table 3). At that time, $P_{net}$, $g_m$, and $g_c$ in frozen and shaded trees were still significantly lower than in unfrozen and shaded trees (Table 3).

$P_{net}$, $g_m$, and WUE in frozen and shaded trees were consistently higher than in frozen and unshaded trees 4 days after freezing (Fig. 2). The difference was statistically significant on days 4, 6, and 8 for $g_m$ and WUE, but only on day 8 for $P_{net}$ (Table 4). In contrast, $g_c$ of the frozen and shaded trees was significantly lower than that of the frozen and unshaded trees on the 1st, 6th, and 8th days following freezing ($P < 0.05$, Table 4; Fig. 2*c*).

Shaded and unshaded trees generally responded similarly to the −6°C frost. All parameters measured decreased immediately after freezing (Figs. 3*a*–3*d*). A substantial recovery in all parameters occurred 2 days after freezing. But 11 days after freezing, $P_{net}$, $g_m$, and WUE in the frozen and unshaded trees were still significantly less than in the unfrozen and unshaded controls (Table 5). The effect of frost was the largest on $g_m$ and the least on $g_c$. Mesophyll conductance was 67% of control trees 11 days after freezing, while $g_c$ recovered completely 4 days in shaded ($P = 0.68$) and 2 days in unshaded trees (Table 5) after freezing.

TABLE 4. Analysis of covariance on the effect of post-frost shading (frozen and shaded trees vs. frozen and unshaded trees) on photosynthesis ($P_{net}$), mesophyll conductance ($g_m$), stomatal conductance ($g_c$), and photosynthetic water use efficiency (WUE) in tamarack exposed to a simulated summer frost of −3.5°C

| Parameter | Day | Source | df | MSS | $F$ | $P > F$ |
|---|---|---|---|---|---|---|
| $P_{net}$ | 4 | Treatment | 1 | 0.24 | 2.88 | 0.1239 |
| | | Error | 9 | 0.083 | | |
| | 6 | Treatment | 1 | 0.28 | 3.91 | 0.0794 |
| | | Error | 9 | 0.072 | | |
| | 8 | Treatment | 1 | 0.49 | 10.71 | 0.0307 |
| | | Error | 9 | 0.046 | | |
| $g_m$ | 4 | Treatment | 1 | 7.08 | 6.61 | 0.0301 |
| | | Error | 9 | 1.07 | | |
| | 6 | Treatment | 1 | 6.49 | 12.19 | 0.0251 |
| | | Error | 9 | 0.53 | | |
| | 8 | Treatment | 1 | 5.77 | 5.50 | 0.0440 |
| | | Error | 9 | 1.05 | | |
| $g_c$ | 4 | Treatment | 1 | 117.67 | 12.64 | 0.0062 |
| | | Error | 9 | 9.31 | | |
| | 6 | Treatment | 1 | 22.08 | 4.73 | 0.0577 |
| | | Error | 9 | 4.67 | | |
| | 8 | Treatment | 1 | 23.06 | 9.69 | 0.0125 |
| | | Error | 9 | 2.38 | | |
| WUE | 1 | Treatment | 1 | 2.03 | 2.93 | 0.1212 |
| | | Error | 9 | 0.69 | | |
| | 4 | Treatment | 1 | 1.15 | 7.00 | 0.0266 |
| | | Error | 9 | 0.16 | | |
| | 6 | Treatment | 1 | 2.49 | 9.32 | 0.0379 |
| | | Error | 9 | 0.27 | | |
| | 8 | Treatment | 1 | 1.28 | 12.23 | 0.0249 |
| | | Error | 9 | 0.10 | | |

NOTE: Pretreatment values were used as a covariate. Day, days after freezing; day 0 is the freezing day. MSS, adjusted mean sum of squares.

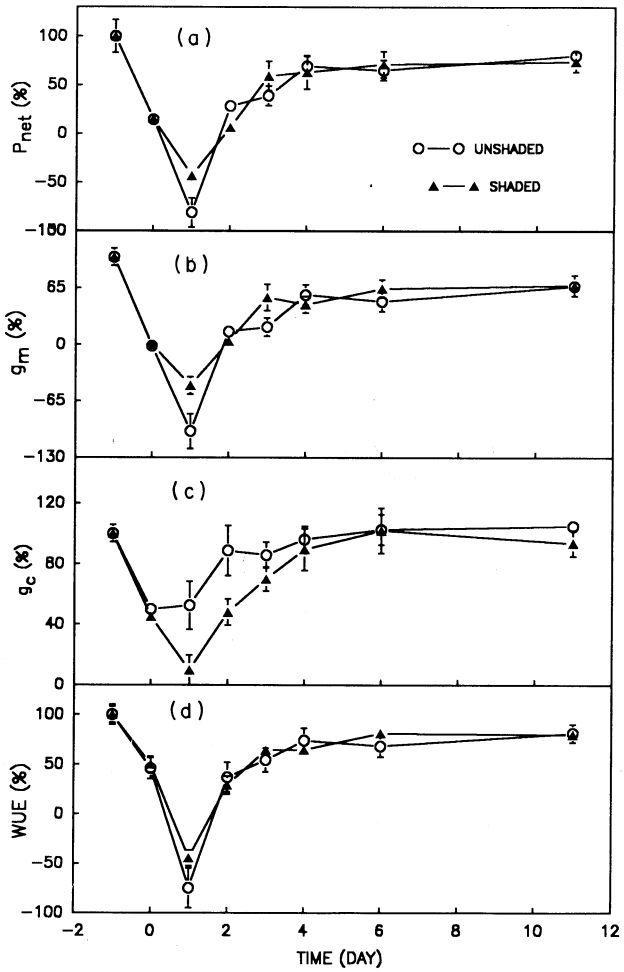


FIG. 3. Response of tamarack to a simulated summer frost of −6°C with and without subsequent shade. (*a*) Light-saturated net photosynthesis rate ($P_{net}$). (*b*) Mesophyll conductance to $CO_2$ ($g_m$). (*c*) Stomatal conductance to $CO_2$ ($g_c$). (*d*) Water use efficiency (WUE). On the *x*-axis, time −1 is the prefreezing day, 0 is the freezing day, and positive values are the days after freezing. Values ($\bar{x} \pm 1$ SE; $n = 6$) in the graph are expressed as a percentage of the control. Shaded trees were shaded from direct sunlight immediately after freezing. See Fig. 1 for explanations of negative percentage values.

Following the −6°C frost, $P_{net}$, $g_m$, and WUE in the frozen and shaded trees were similar to those in the frozen and unshaded trees for all post-freezing days except on the 1st day after freezing, when $P_{net}$ and $g_m$ of the frozen and shaded trees were significantly higher than those of the frozen and unshaded trees ($P < 0.05$, Table 6; Fig. 3). In contrast, $g_c$ in the frozen and shaded trees was significantly lower than in the frozen and unshaded trees on the 1st and 2nd days after freezing (Table 6; Fig. 3*c*).

## Discussion

The response of $P_{net}$ to summer frosts consisted of two phases, i.e., a depression phase and a recovery phase. In the depression phase, $P_{net}$ declined progressively. The length of this phase seemed to vary with species but not with the degree of freezing. For black spruce, the depression phase lasted for at least 4 days (there were no measurements between 5th and 10th days after freezing). In contrast, the depression phase in tamarack lasted for only 1 day at both the −3.5 and −6°C frosts. Though the depression phase may have lasted up to 3 days in Fig. 2, a substantial recovery in the variable chlorophyll fluorescence was measured on the 2nd day (unpublished data). Thus it may be reasonable to infer that the depression phase was also 1 day after the −3.5°C frost. In the recovery phase, $P_{net}$ increased gradually. The recovery was generally fast for the first few days, then slowed down. Apparently, a full recovery of $P_{net}$ required more than 11 days. Pharis *et al.* (1970) found that full recovery of photosynthesis in cold-acclimated ponderosa pine and Douglas-fir required several weeks.

The depression phase of $P_{net}$ was longer in black spruce than in tamarack. This may have been due to inherent differences in cytoplasmic dehydration, membrane lipid phase separation and consequent impairment of membrane-bound enzymes (Burke and Stushnoff 1979; Levitt 1980), ion leakage and consequent environment changes around the chloroplast (e.g., altered ion concentration and electrical potential gradients) (Steffen and Palta 1987), and (or) metabolic imbalances in the cell (Burke and Stushnoff 1979). Differences in developmental stages (e.g., shoot elongation) can also have a profound effect on frost hardiness. But this did not appear to be a significant factor in this study, because shoots appeared to be nearly fully elongated and foliage fully expanded in both species when the experiments started.

TABLE 5. Analysis of covariance on the effect of a simulated summer frost of –6°C (frozen and unshaded trees vs. unfrozen and unshaded trees) on photosynthesis ($P_{net}$), mesophyll conductance ($g_m$), stomatal conductance ($g_c$), and photosynthetic water use efficiency (WUE) in tamarack needles

| Parameter | Day | Source | df | MSS | $F$ | $P>F$ |
|---|---|---|---|---|---|---|
| $P_{net}$ | 4 | Treatment | 1 | 0.67 | 7.40 | 0.0230 |
| | | Error | 9 | 0.091 | | |
| | 6 | Treatment | 1 | 0.83 | 8.00 | 0.0370 |
| | | Error | 9 | 0.10 | | |
| | 11 | Treatment | 1 | 0.67 | 5.40 | 0.0460 |
| | | Error | 9 | 0.12 | | |
| $g_m$ | 4 | Treatment | 1 | 13.39 | 10.20 | 0.0109 |
| | | Error | 9 | 1.31 | | |
| | 6 | Treatment | 1 | 16.81 | 8.13 | 0.0350 |
| | | Error | 9 | 2.07 | | |
| | 11 | Treatment | 1 | 8.90 | 9.30 | 0.0381 |
| | | Error | 9 | 0.96 | | |
| $g_c$ | 2 | Treatment | 1 | 12.20 | 0.68 | 0.4312 |
| | | Error | 9 | 17.94 | | |
| | 3 | Treatment | 1 | 18.25 | 1.60 | 0.2376 |
| | | Error | 9 | 11.41 | | |
| | 4 | Treatment | 1 | 4.03 | 0.30 | 0.5994 |
| | | Error | 9 | 13.43 | | |
| | 11 | Treatment | 1 | 0.50 | 0.080 | 0.7803 |
| | | Error | 9 | 6.25 | | |
| WUE | 4 | Treatment | 1 | 0.66 | 8.50 | 0.0340 |
| | | Error | 9 | 0.078 | | |
| | 6 | Treatment | 1 | 2.05 | 4.95 | 0.0495 |
| | | Error | 9 | 0.41 | | |
| | 11 | Treatment | 1 | 0.41 | 5.50 | 0.0435 |
| | | Error | 9 | 0.075 | | |

NOTE: Pretreatment values were used as a covariate. Day, days after freezing; day 0 is the freezing day. MSS, adjusted mean sum of squares.

TABLE 6. Analysis of covariance on the effect of post-frost shading (frozen and shaded trees vs. frozen and unshaded trees) on photosynthesis ($P_{net}$), mesophyll conductance ($g_m$), stomatal conductance ($g_c$), and photosynthetic water use efficiency (WUE) in tamarack exposed to a simulated summer frost of –6°C

| Parameter | Day | Source | df | MSS | $F$ | $P>F$ |
|---|---|---|---|---|---|---|
| $P_{net}$ | 1 | Treatment | 1 | 0.40 | 8.10 | 0.0365 |
| | | Error | 9 | 0.049 | | |
| $g_m$ | 1 | Treatment | 1 | 10.50 | 5.00 | 0.0480 |
| | | Error | 9 | 2.10 | | |
| $g_c$ | 1 | Treatment | 1 | 6.51 | 16.21 | 0.0024 |
| | | Error | 9 | 0.40 | | |
| | 2 | Treatment | 1 | 6.88 | 8.65 | 0.0171 |
| | | Error | 9 | 0.80 | | |
| | 3 | Treatment | 1 | 3.50 | 3.05 | 0.1200 |
| | | Error | 9 | 1.15 | | |
| WUE | 1 | Treatment | 1 | 0.91 | 0.31 | 0.5984 |
| | | Error | 9 | 2.94 | | |

NOTE: Pretreatment values were used as a covariate. Day, days after freezing; day 0 is the freezing day. MSS, adjusted mean sum of squares.

Nevertheless, experiments were conducted in 2 different years (1989 for black spruce; 1990 for tamarack) and this may also have had an impact.

The most likely reason for the difference in the length of depression phase between the two species, however, might be related to differences in rates of frost injury repair in the two species. Recovery from freezing injury is an active process, requiring energy (Steffen and Palta 1987). In this study, the repairing energy could have come from three sources: (*i*) photosynthetic products of the injured foliage, (*ii*) breakdown of cell reserves, and (*iii*) carbohydrates translocated from uninjured foliage. Contribution from the first source was probably small, particularly for the first few days. It could be possible that tamarack needles had more internal reserves or faster translocation of sugars from uninjured tissues to the injured foliage than black spruce; this resulted in a shorter depression phase.

In this study, $P_{net}$ decreased and recovered in parallel with $g_m$ in both species (Figs. 1–3). This is in agreement with earlier findings that depressed $g_m$ was primarily responsible for the low rate of photosynthesis after summer frosts (DeLucia and Smith 1987; Lundmark *et al.* 1988). The depression in $g_m$ may have resulted from frost injury to photosystem II (Klosson and Krause 1981; Grafflage and Krause 1986; Strand and Lundmark 1987), changes in the environment around the chloroplasts (Steffen and Palta 1987), membrane lipid phase transition (Levitt 1980), and (or) subsequent impairment of membrane-bound enzymes (Burke and Stushnoff 1979).

The response of $g_c$ to summer frosts in this study was in contrast with studies by DeLucia and Smith (1987) and Lundmark *et al.* (1988), where $g_c$ generally decreased in parallel with $P_{net}$ following frosts. In our study, however, changes in $P_{net}$ following frosts generally followed a different pattern from that of $g_c$ (Figs. 1, 2, and 3). A particularly interesting result is that $g_c$ in trees exposed to direct sunlight following frosts recovered much faster than in the shaded trees. This appeared to indicate that stomatal opening after frosts required a substantial amount of incident light, but the mechanism is not clear.

The effect of post-frost shading on ecophysiological parameters of tamarack varied with the degree of freezing. The shading significantly enhanced the recovery of $P_{net}$ and related parameters after –3.5°C frost, but generally had no detectable effects after the –6°C frost. This is despite the fact that there were greater numbers of clear days following the –6°C frost. The same phenomenon was observed in cold-acclimated Scots pine (Strand and Öquist 1985). This difference might be related to the sites of frost injury to photosynthetic apparatus. The effect of post-frost shading on photosynthesis was related to photoinhibition (Strand and Lundmark 1987). Photoinhibition is caused by excess trapped light energy when the biochemical reaction of photosynthesis are inhibited. It is possible that the –6°C frost caused more severe injury to the photochemical apparatus of photosynthesis compared with the –3.5°C frost. In other words, there might have been less excess trapped light energy after the –6°C frost.

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