COLD HARDINESS AND BUD DEVELOPMENT UNDER SHORT DAYS IN BLACK SPRUCE AND WHITE SPRUCE SEEDLINGS

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Abstract.--Cold hardiness of first-year seedlings of black spruce *(Picea mariana* B.S.P.) and white spruce *(P. glauca* [Moench] Voss) was increased by exposure to 8-hr photoperiod at 20°C. After five weeks of short days, a temperature of -9°C did not cause damage in either species. Shoot elongation ceased and bud development began after exposure to short days. The development of cold hardiness was strongly correlated with the decreasing rate of shoot elongation and the increasing number of needle primordia in the terminal buds. After eight weeks of short days, bud development in both species was virtually complete.

Résumé.--On a augmenté la résistance au froid de semis d'épinette noire *(Picea mariana* [Mill.] B.S.P.) et d'épinette blanche *(Picea glauca* [Moench] Voss) de moins d'un an en les soumettant à une photopériode de huit heures à 20°C. Après cinq semaines dans ces conditions, une température de -9°C n'a causé aucun dommage aux deux espèces. L'allongement des pousses a cessé et les bourgeons ont commencé à se développer après l'exposition à la courte photopériode. L'augmentation de la résistance au froid était fortement corrélée avec la réduction de l'allongement des pousses et l'augmentation du nombre de primordiums foliaires dans les bourgeons terminaux. Après huit semaines de photopériode courte, le développement des bourgeons était pratiquement complet chez les deux espèces.

INTRODUCTION

In Ontario, winter damage to container-grown spruce seedlings is currently a serious problem limiting the availability of vigorous stock for outplanting. A principal cause of winter damage is that container seedlings are hardened outside, under prevailing weather conditions. Seedlings are then susceptible to freezing damage until sufficient cold hardiness has developed in response to shortening daylengths and cool temperatures. One way to reduce these losses is to ensure adequate cold hardiness by exposure to short days before moving the trees outside.

During cold hardening in tree seedlings, a series of changes takes place in shoot elongation and bud formation. However, the interrelationships of these processes have not been thoroughly investigated for eastern boreal coniferous species such as black spruce *(Picea mariana* [Mill.] B.S.P.) and white spruce *(P. glauca* [Moench] Voss).

It was decided, therefore, to test the effects of 8-hr days at 20°C on cold hardiness, shoot growth and bud development of black spruce and white spruce seedlings.

MATERIALS AND METHODS

Black spruce and white spruce seed from single-tree collections made in the vicinity of the Petawawa National Forestry Institute (Lat. N. 46° 00', Long. W. 77° 26') was germinated and grown in a glasshouse for 12 weeks at temperatures of 17-25°C, under natural

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daylength supplemented to 16 hr with high-intensity sodium vapor lamps providing 175 $uE.m^{-2}.s^{-1}$ of photosynthetically active radiation (PAR).

Seedlings were grown in 350 cm^3 Styrofoam pots (four seedlings per pot) filled with a peat moss:vermiculite mixture (2:1 v/v). Twice a week, beginning 29 days after sowing, 70 ml of nutrient solution (Hocking 1971) were applied to each pot. After 12 weeks, pots were placed for eight weeks in growth chambers under an 8-hr short-day treatment with a PAR of 246 and a constant day-night temperature of 20 °C. Fertilization was continued on a weekly basis with half-strength Hocking's solution.

The effect of 8-hr days was assessed by measuring cold hardiness, bud development and shoot elongation at weekly intervals for the eight weeks of short-day treatment.

Cold hardiness was tested by exposing the seedlings to freezing temperatures (-9 °C). The freezing test consisted of preconditioning 16 seedlings (four pots) of each species at 5 °C for 12 hr. Eight seedlings served as controls and were left at 5 °C. The remaining eight seedlings were placed in a Styrofoam chest, with vermiculite insulation around the pots to protect the roots from freezing. The chest was sealed and placed in a freezer for 6 hr, during which time air temperature in the chest decreased approximately 10 °C in the first hour and 0.8 °C•hr^{-1} over the next 5 hr down to -9 °C. The chest was post-conditioned for 12 hr at 5 °C before opening.

The degree of cold hardiness was determined after freezing both by measuring the leakage of electrolytes from damaged shoots and by assessing the development of visible symptoms of damage.

Electrolyte leakage was measured by means of a modified version of the methods described by Aronsson and Eliasson (1970) and Green and Warrington (1978) and as developed by Dexter et al. (1932). Two seedlings from each pot in the controls and in the -9 °C treatment were decapitated 8 cm below the shoot tip and placed in test-tubes containing 25 ml of distilled water. After 24 hr at room temperature (approximately 24 °C) the test-tubes were vigorously shaken and electrical conductivity of the solution was measured with a Radiometer model CDM3 conductivity meter. The test-tubes were then placed in boiling water for 10 min and, after a further 24 hr at room temperature, the tubes were shaken and electrical conductivity was remeasured. Relative conductivity was calculated as the percentage of electrical conductivity measured before over that measured after boiling. A lower percentage of relative conductivity indicates a greater hardiness.

Visible symptoms of freezing damage (Table 1) were assessed on the four seedlings (two pots) of each species remaining from both the freezing test and its control, after they had been placed in the glasshouse for 30 days.

Table 1. Scale of visible freezing damage for white spruce and black spruce.

Rating	Symptom
0	No damage
1	Terminal alive, some needles red
2	Terminal killed, fewer than 50% of needles red
3	Terminal killed, more than 50% of needles red
4	Main stem and lateral shoots killed, just a few needles near base of epicotyl alive
5	Seedling completely dead

Shoot elongation was measured on a permanent sample of 20 trees of each species every week for the eight weeks of short-day treatment. Bud development was assessed on eight shoot apices per species at weekly intervals, from week 0 to week 8. Budscales were counted and removed to permit examination of needle primordia on the apical meristem. The total number of needle primordia was estimated by counting primordia in spirally arranged parastichies (rows) on the apical meristem and multiplying by the total number of parastichies, as described by Pollard (1973). The shoot apices from the eighth week of the short-day treatment were prepared for scanning electron micrography, according to standard procedures (Gregory 1980). Buds from production-run container stock were similarly examined for comparison.

RESULTS

The cold hardiness of both species increased with time of exposure to short days. From the first to the fourth week, relative conductivities in black spruce and white spruce exposed to the -9°C freezing treatment fell from 95.1 to 14.8% and from 39.5 to 13.1%, respectively (Fig. 1). At the same time, visible damage decreased from 4.5 to 2.0 and from 5.0 to 0.75 (Fig. 2), which also indicated a marked increase in cold hardiness.

From the fifth week on, the -9°C freezing treatment did not damage either black spruce or white spruce, as measured by the relative conductivity method, although some visible damage occurred up to the fifth week after short-day treatment began. These two assessment methods were strongly correlated (r = 0.935, P <0.01) (Fig. 3).

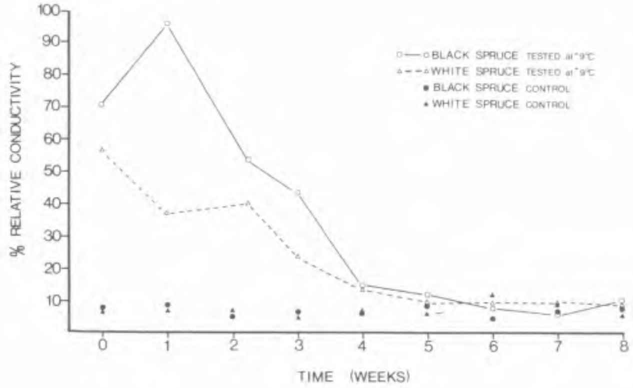

Figure 1. Development of cold hardiness as measured by relative conductivity in black spruce and white spruce under short-day (8-hr) treatment.

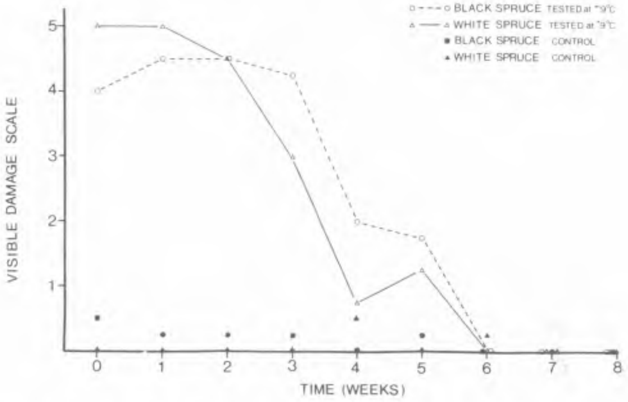

Figure 2. Development of cold hardiness as measured by visible damage in black spruce and white spruce under short-day (8-hr) treatment.

The pattern of shoot elongation in most cases resembled that of relative conductivity (r = 0.938, P <0.01) (Fig. 4 and 5). Shoot elongation decreased after week 1 and, by week 4, had ceased in both black spruce and white spruce.

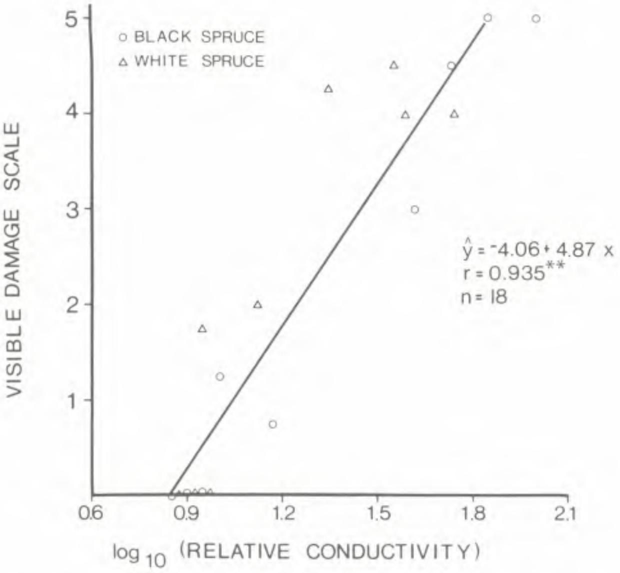

Figure 3. Relationship between relative conductivity and visual assessments of freezing damage in black spruce and white spruce.

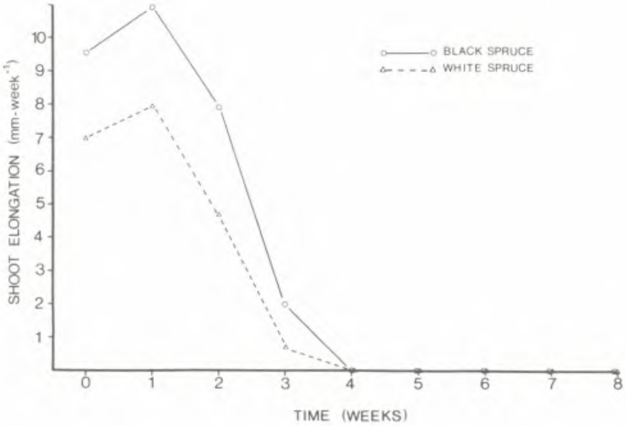

Figure 4. Shoot elongation in black spruce and white spruce under short-day (8-hr) treatment.

Bud development in both species was initiated in the first week of short-day exposure. Budscale formation was complete within two weeks, by which time 33 needle primordia (16% of the final number) had formed in black spruce, and 40 primordia (22%) had formed in white spruce (Fig. 6). Needle primordia were rapidly initiated between weeks 2 and 5. By week 5, 173 primordia (83% of the final primordia complement) had formed in black spruce and 165 primordia (90%) had formed in white spruce. After week 5, primordia were produced more slowly so that by the end of the

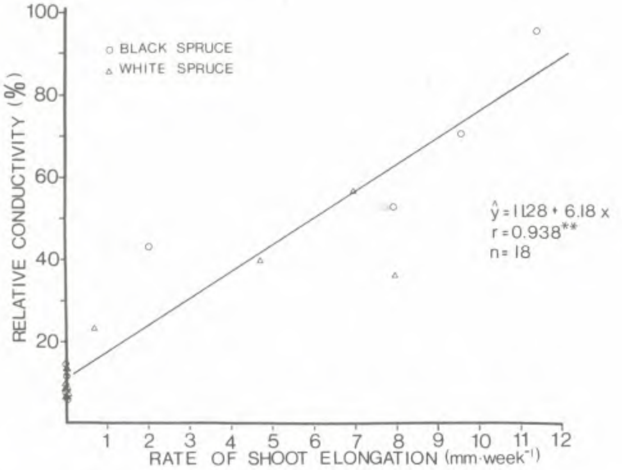

Figure 5. Correlation between relative conductivity and rate of shoot elongation in black spruce and white spruce under short-day (8-hr) treatment.

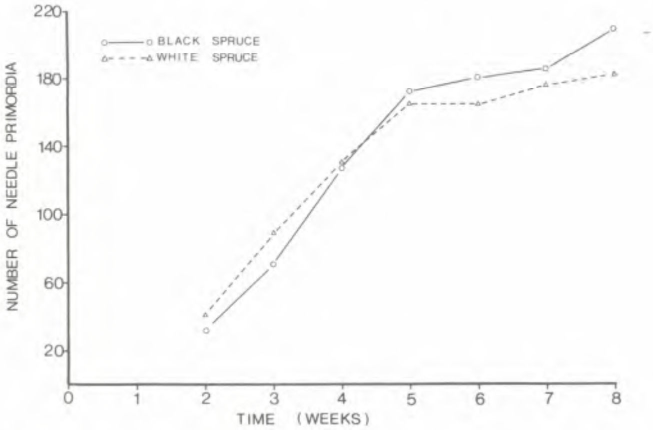

Figure 6. Needle primordia initiation in black spruce and white spruce under short-day (8-hr) treatment.

eighth week the complement of needle primordia was 209 in black spruce and 182 in white spruce. Development of needle primordia in both species was negatively correlated ($r = -0.937$, $P < 0.01$) with relative conductivity (Fig. 7).

Seedlings of both species exposed to eight weeks of short days in growth cabinets produced large apical meristems with many needle primordia (Fig. 8a). In comparison, the apical meristems of production-run black spruce container seedlings were small and, on average, produced only 95 needle primordia (Fig. 8b) (Colombo and Glerum 1982).

DISCUSSION

Photoperiod and temperature are two major environmental factors influencing cold hardiness development in conifers (Aronsson 1975, Glerum 1976, Christersson 1978). In the present experiment, cold hardiness increased in both species after two weeks of exposure to 8-hr days. After six weeks all seedlings were hardy to at least -9 C, the limit of the present test. Christersson (1978) found that, in Norway spruce *(Picea abies* [L.] Karst.), six weeks of short days and warm temperatures (20 C) induced cold hardiness to -16 C without visible damage. Since damage in the present test was not observed in either black spruce or white spruce exposed to -9 C after six weeks of short days, the seedlings may well have been hardy to a temperature similar to that found by Christersson.

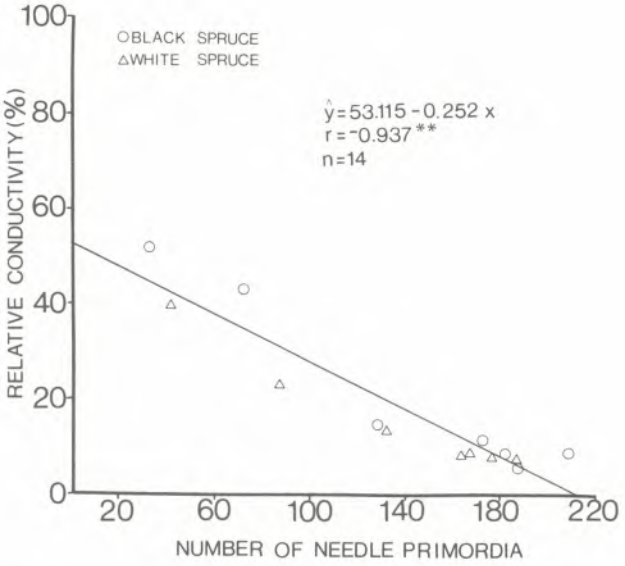

Figure 7. Correlation between relative conductivity and number of needle primordia in black spruce and white spruce under short-day (8-hr) treatment.

The electrical conductivity method could be a useful tool of the container nurseryman for making operational decisions concerning the cold hardiness of his stock, since the state of cold hardiness was obtained as reliably ($r = 0.934$) using conductivity measurements as with visible damage assessments. Similar correlations between the electrical conductivity and visible damage assessment methods have been shown for Scots pine *(Pines sylvestris* L.) (Aronsson and Eliasson 1970) and radiata pine *(Pinus radiata* D. Don) (Green and Warrington 1978). Conductivity has also been used as a measure of cold hardiness in Norway spruce (Aronsson 1975) and Douglas-fir *(Pseudotsuga menziesii* [Mirb.] Franco) (van den Driessche 1969, 1976).

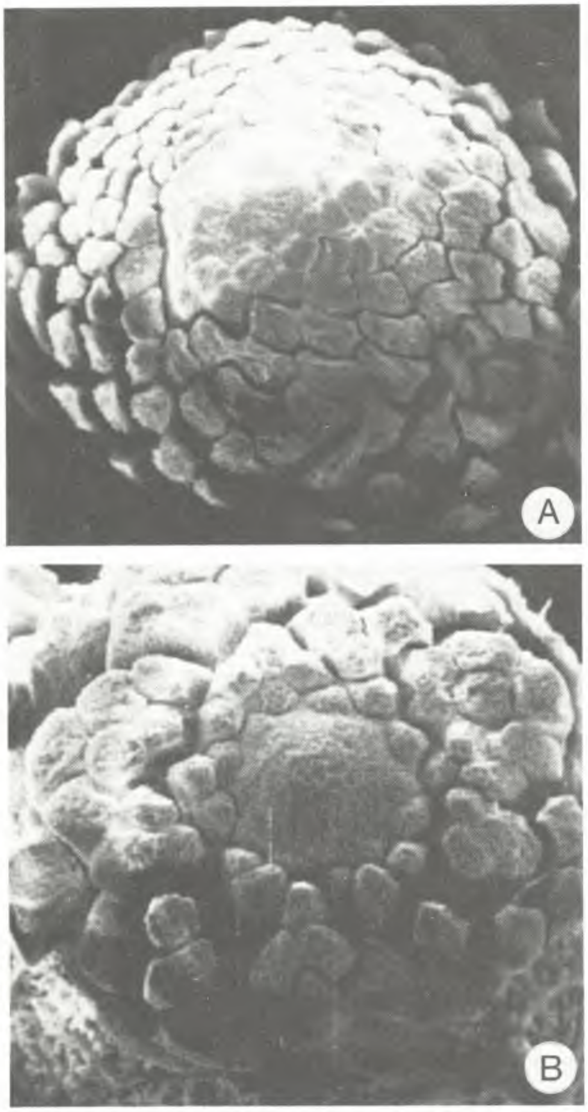

Figure 8. Representative bud development in black spruce seedlings: (A) after eight weeks of short day (8 hr) treatment (72x) and (B) from a production-run overwintering bed (98x).

Shoot elongation and bud development were equally good indicators of the state of cold hardiness (r = 0,938 and 0.937), and may be useful for predicting the initial development of cold hardiness in black spruce and white spruce seedlings in the container nursery. Shoot elongation measurements are simple to perform, but provide information for only the first few weeks of cold hardiness development. Bud development gives an indication of cold hardiness beyond the time when shoot elongation ceases, but is less easily determined. The relationship of cold hardiness to shoot elongation and bud development is now being investigated under operational conditions, to determine whether under different environmental and nutritional regimes these correlations remain strong.

Although not examined in this experiment, short-day pretreatment followed by periods of low temperature and frost is one method of achieving maximal cold hardiness (Timmis and Worrall 1975). While low temperature exposure preceding or during short-day treatment can likewise result in maximal cold hardiness (Timmis and Worrall 1975, Christersson 1978), this treatment will inhibit bud development (Heide 1974, Pollard and Logan 1977, 1979). In Ontario, container seedlings are placed outside for overwintering without the benefit of short-day treatment. In this instance, not only are seedlings susceptible to damage by freezing temperatures which commonly occur in late summer in northern Ontario, but bud development may be reduced by temperatures below the optimum. In the experiment reported in this paper, production-run black spruce seedlings formed less than half as many needle primordia as were formed in controlled environmental conditions, apparently because bud development was reduced by low outdoor temperatures.

It is recommended that seedlings grown in greenhouses be exposed to short, warm days before being put outside in the fall. This would allow buds to develop large numbers of needle primordia, while cold hardiness could increase without the risk of freezing damage.

Short, warm days can be achieved artificially, when natural daylengths would otherwise allow continued shoot elongation, by shading seedlings in the greenhouse for a period of four to six weeks, after which the seedlings can be moved outside. Alternatively, cold hardiness and bud development can be promoted without shading, by leaving seedlings in a heated greenhouse later in the fall (Sandvik 1980), under naturally declining daylengths. In northern Ontario bud development in extended greenhouse culture is largely completed between late September and early October (Colombo, unpublished data), at which time temperatures in the greenhouse can be gradually lowered to promote greater levels of cold hardiness, before seedlings are put outside.

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