The Effects of Mineral Nutrition on Hardening-Off of Conifer Seedlings[1]

Ivor K. Edwards[2]

Edwards, Ivor K. 1989. The Effects of Mineral Nutrition on Hardening-Off of Conifer Seedlings. In: Landis, T.D., technical coordinator. Proceedings, Intermountain Forest Nursery Association; 1989 August 14-18; Bismarck, ND. General Technical Report RM-184. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station: 98-102. Available at: http:www.fcnanet.org/proceedings/1989/edwards.pdf

Abstract.--Containerized lodgepole pine, jack pine, red pine, Scots pine, white spruce, and black spruce were hardened off under different nutrient regimes to determine the effect of nutrition on development of cold hardiness. There was only weak correlation between cold hardiness and nutrient concentration or uptake in the shoot of seedlings. In lodgepole pine, cold hardiness was associated with a low nitrogen regime and in all other species, it was associated with either high phosphorus or high potassium. The implication of a lower level of cold hardiness achieved in pines is discussed.

INTRODUCTION

Over 30 million containerized seedlings are produced annually in the prairie provinces and annual production is projected to be 50 million in three years.

Overwintering has become a very important phase in the production of containerized seedlings as double- and, in one instance, multiple cropping have become more common. It is necessary if outplanting of the first crop is not possible but it is mandatory in the case of the second or subsequent crop because the latter seedlings have attained their desired size too late in the year for outplanting to occur. In preparation for overwintering, the stock has to be hardened off. Dormancy (i.e., cessation of shoot growth and the initiation of terminal buds) is induced by reducing temperature, light intensity, and photoperiod. Following cessation of shoot growth, budset and bud development occur with a moderate amount of cold hardiness (Glerum 1985). With appropriate low temperature acclimatization, further cold hardiness is developed and the stock is ready for overwintering outdoors.

Nutrition during the conditioning phase to induce dormancy and cold hardiness is believed to be important. Unlike the rapid growth phase, when high nitrogen (N) is required for vegetative growth, low N and high phosphorus (P) and/or potassium (K) are required during hardening off (Levitt 1956). Timmis (1974) found that the level of cold hardiness developed in Douglas fir (Pseudotsuga menziesii [Mirb.] Franco) seedlings was not related consistently to bud development or individual N, P, and K regimes but was more closely related to K/N ratio in the foliage. The use of a finisher-type fertilizer containing low N and high P or K during hardening off is widespread in the seedling production industry in the prairie provinces. However, the practice resulted from advice given by fertilizer companies, based on research with other species and in other parts of Canada. Objective experiments, utilizing prairie conifers, were required. Lodgepole pine (Pinus contorta Dougl. var. latifolia Engelm.), jack pine (Pines banksiana Lamb.), red pine (Pinus resinosa Ait.), Scots pine (Pinus svlvestris L.), white spruce (Picea glauca (Moench) Voss), and black spruce (Picea mariana (Mill.) BSP.), are grown in the prairie region for reforestation and it is necessary to work with appropriate provenances of these species. The objective of this study was to determine which nutrient regimes were associated with cold hardiness in the various conifer species and to determine if specific nutrient concentrations in plant tissue was required for development of cold hardiness.

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[1] Paper presented at the Annual Meeting of the Intermountain Forest Nursery Association, Bismarck, North Dakota, August 14-18, 1989.

[2] Ivor K. Edwards is *Research Scientist,* Forestry Canada, Northern Forestry Centre, Edmonton, Alberta.

MATERIALS AND METHODS

Lodgepole pine and black spruce, jack pine and white spruce, and red pine 'and Scots pine were grown in the Northern Forestry Centre greenhouse (latitude 53° 29' N, longitude 113° 32' W) in 1985, 1986, and 1987, respectively. Conifer seeds were sown in peat in Spencer-Lemaire "Fives" containers (cavity volume = 55 cm^3) in February and allowed to grow for 14 weeks (Table 1). Prior to filling the trays, the peat was adjusted to pH 5.2 with calcium carbonate. Environment conditions in the greenhouse were as follows: temperature 24°C day and 16°C night; relative humidity 62-65%; light intensity (high pressure sodium lamps) photosynthetically active radiation (PAR) = 260 mol. $m^{-2}.s^{-1}$

Beginning 21 days after germination, fertilizer solution, recommended for pine and spruce (Carlson 1983) and containing 125mg/l N, 60 mg/l P, and 159 mg/l K, was applied once per week. At fertilization, each cavity was completely saturated with nutrient solution. Other nutrients in the solution prescribed by Carlson (1983) were as follows: iron (Fe), 5.5 mg/l; manganese (Mn), 0.34 mg/l; boron (B), 0.30 mg/l; zinc (Zn), 0.11 mg/l; copper (Cu), 0.02 mg/l; and molybdenum (Mo), 0.01 mg/l. Between the weekly applications of fertilizer solution, the trays were watered as required.

At the completion of 14 weeks' growth, the seedlings were subjected to 12 weeks of conditioning (Table 2) which consisted of an initial two weeks during which supplementary lights were turned off, the trays were leached with water, and allowed to become dry. The drought stress was followed by an initial five-week period during which day and night temperatures were 24 and 16°C, respectively, in the greenhouse and a second five-week period in a growth chamber during which day and night temperatures were 10 and 2°C, respectively. During both five-week periods, a photoperiod of 8 hours was achieved, using a black-out cloth and one of five hardening-off solutions (Table 3) was applied. Solution III was the solution recommended to growers based on a review of the literature. Lower concentrations of N and higher concentrations of P and K were included in order to test their effect on hardening-off.

Table 1. Duration of each phase of the experiments

Phase	Duration
Growth	14 weeks
Conditioning	12 weeks
Cold Hardiness Test	24 hours
Assessment	6 weeks

Table 2. Details of conditioning phase

Time	Condition
Weeks 1-2	Supplementary lights off, leaching, drought stress
Weeks 3-7	Photoperiod 8 hours Hardening-off solution 1x/wk; Day/night temperatures 24/16°C
Weeks 8-12	Photoperiod 8 hours; Hardening-off solution 1x/wk; Day/night temperatures 10/2°C

Table 3. Nutrient regimes used during the conditioning phase (hardening-off)

Solution	N	P	K
		mg/l	
I	4.4	101	150
II	22	101	150
III	44	101	150
IV	44	202	150
V	44	101	300

Following conditioning, the seedlings were tested for cold hardiness by placing them in a -5°C or -10°C room for 24 hours. They were then returned to the 10/2°C regime in the growth chamber for 24 h before being placed in the greenhouse for a 6-week assessment. Degree of budset at completion of the conditioning phase was noted and survival and condition of shoot and roots were assessed after 6 weeks in the greenhouse. At the end of the assessment period, the seedlings were measured for height, root collar diameter, and shoot:root ratio (dry weight basis) and foliage was analyzed for N, P, and K. Assessment was conducted on a total of 192 seedlings for each treatment (hardening-off solution), comprised of 4 replicates of 48 seedlings each. Significance of differences of mean survival between treatments was assessed using Student's "t"-test (Steel and Torrie 1980). Correlation between survival and shoot concentration and uptake of N, P, and K, and the ratios N/P, N/K, and K/P was determined using the SAS procedure (SAS Institute Inc. 1985).

Nitrogen was determined by the Kjeldahl method (Bremner and Mulvaney 1982, Tecator 1985) and P and K were determined using ICAP spectrometry after nitric acid digestion (Hogan and Maynard 1984, Kalra et al. 1989).

RESULTS

For lodgepole pine, jack pine, white spruce, and black spruce, survival after coldhardiness testing at -5°C was high irrespective of nutrient solution applied during hardening off (Tables 4 and 5). At -10°C, survival was associated with certain treatments or hardening-off solutions and were higher in spruce than in pine. Solutions I and III favoured survival of lodgepole pine whereas Solutions IV and V were associated with high survival in black spruce (Table 4). Survival of jack pine and white spruce at -5°C was equally high and was equally low at -10°C (Table 5). Solution IV was beat for jack pine and Solution V was associated with highest survival in white spruce. The survival of red pine and Scots pine at either -5° or -10°C was lower than that of other pines in the study and there was a marked decrease in survival of Scots pine, especially, at -10°C (Table 6), indicating that it had developed very little cold hardiness. A summary of the effect of nutrient regime on hardening-off of the conifers tested is shown in Table 7.

Lower survival of pine compared to spruce in the study was correlated with the lower degree of budset observed in pine, following the 12-week conditioning period. White spruce set bud generally within 21 days after drought stress and the 8-h photoperiod were initiated. For black spruce, budset was achieved about five weeks after conditioning had begun. All pines were slower in their response to conditioning. Lodgepole pine and jack pine achieved only 50 and 30 percent budset, respectively, at the end of the 12-week conditioning period. Red pine and Scots pine were slowest to respond to the conditioning treatment and achieved only 10 and 1 percent budset, respectively, prior to the cold hardiness test.

Table 4. Survival (8) of lodgepole pine (LP and black spruce (BS) after cold treatment at -5°C and -10°C

Nutrient Solution	Survival LP	-5°C BS	Survival LP	-10°C BS
I	[1]100a	100a	82a	66c
II	97a	100a	68b	96a
III	100a	100a	78a	85b
IV	99a	97a	39d	100a
V	96a	97a	49c	99a

[1]Within a column, values followed by the same letter are not significantly different at P = 0.05 by Student's "t" test.

Nutrient concentration in foliar tissue was similar in seedlings tested at -5 and 10°C and, except for red pine and Scots Pine, results for -10°C will be discussed.

Table 5. Survival (%) of jack pine (JP) and white spruce (WS) after cold treatment at -5°C and -10°C

Nutrient Solution	Survival JP	-5°C WS	Survival JP	-10°C WS
I	[1]100a	96a	24c	40b
II	96a	88b	20c	21c
III	96a	96a	24c	8d
IV	100a	100a	56a	24c
V	100a	100a	32b	76a

[1]Within a column, values followed by the same letter are not significantly different at P - 0.05 by Student's "t" test.

Table 6. Survival (%) of red pine (RP) and Scots pine (SP) after cold treatment at -5°C and -10°C

Nutrient Solution	Survival RP	-5° C SP	Survival RP	-10° C SP
I	[1]85a	50c	46a	0
II	80a	61b	36b	0
III	71b	54bc	40b	0
IV	80a	56bc	26c	6
V	86a	75a	18c	0

[1]Within a column, values followed by the same letter are not significantly different at P = 0.05 by Student's "t" test.

Table 7. Summary of the effect of nutrient regime on hardening-off of conifer seedlings.

species	Nutrient regime 1	2	3	4	5
Lodgepole pine	X				
Jack pine				X	
Red pine					X
Scots pine					X
White spruce					X
Black spruce					X

Concentration of N, P and K in shoot was not significantly correlated with cold hardiness as expressed by survival at different nutrient regimes (Tables 4, Sand 6). The ratios N/P, N/K and K/P in shoot were calculated and they were only weakly correlated with survival after cold treatment. Only in the case of Scots pine was there a high and significant correlation (r = 0.89) between survival at 5° C and the N/P ratio in shoot. Nutrient uptake in the shoot (determined by multiplying ovendry weight of the tissue by nutrient concentration) was also found to be poorly correlated with cold hardiness.

DISCUSSION

The earlier initiation of budset in spruce, compared to pine, was a reflection of the sharper response (growth cessation) of spruce to a reduction of the photoperiod. The cessation of shoot growth, and formation of a terminal bud signal the onset of dormancy and are prerequisites for the development of cold hardiness (Glerum 1985). Under the conditioning procedure used, the degree of budset in both white spruce and black spruce was higher than in the pines and explains the greater degree of cold hardiness in spruce. Onset of dormancy in pine was obviously not achieved completely prior to the cold hardiness test and, consequently, plant tissue was prone to damage from freezing.

The pines, especially red pine and Scots pine, require further work in determining their requirements of light, temperature, water, and nutrients for hardening-off. Their relatively slow response under the conditioning regime used in this study supports the hypothesis[1], based on fluorescence and photosynthetic studies, that the triggering mechanism for onset of dormancy in pine is completely different from that in spruce, with regard to light and temperature requirements. Pine is able to maintain photosynthesis at lower photoperiod and temperature levels. Also, cold hardiness in roots develops as the growth medium freezes (Glerum 1985). Temperature of the root environment in this study was not monitored but it would not have been less than 2°C, prior to cold treatments. The role of nutrients in the development of cold hardiness is not well understood. In general, nitrogen promotes shoot growth and succulence, thus delaying the development of cold hardiness. Phosphorus and potassium, on the other hand, promote cold hardiness (Levitt 1956). However, research shows no consistent results. Timmis (1974) found no consistent relationship between development of cold hardiness and either bud development or level of N, P, and K in Douglas fir. He concluded that the K/N ratio of the shoot was inversely related to cold hardiness and suggested that a ratio of 0.6 was critical. The weak correlation between cold hardiness and nutrient concentration and uptake in shoot in this study may also indicate that, anatomically, the area of interest is inappropriate. Roots are more sensitive than shoot to damage by low temperature (Steponkus _et_ _al_. 1976) and therefore nutrient concentration and uptake in this tissue should be examined also.

Results from the present study indicate that a nutrient regime with low N was associated with cold hardiness in lodgepole pine, and regimes with high levels of P and K were associated with cold hardiness in jack pine and the other species, respectively (Table 7). Still, inconsistencies remain.

Although Benzian (1965) found a positive effect of K on cold hardiness of Western hemlock (_Tsuga heterophylla_) and sitka spruce (_Picea sitchensis_) in bareroot seedbeds, Christersson (1973) found no effect of K on cold hardiness of potted Scots pine. It is recognized (Glerum 1985) that, biochemically, solute concentration in cells increases as cold hardiness develops and N, P, and K contribute to the accumulation of biochemical constituents, depending on their amounts, relative proportion, and time of application.

Results of this study indicate that the nutrient regime presently recommended for hardening-off of prairie conifer species needs to be revised. The recommendation had been based on a review of the literature (Tinus 1974, Van Eerden 1974) but no work on prairie provenances and cultural practices had been conducted prior to this study.

CONCLUSIONS

Both white spruce and black spruce achieved budset more easily than the pines during conditioning and developed a greater degree of cold hardiness as shown by survival following cold treatment. Cold hardiness in spruce appeared to be related to the degree of budset, generally. The pines, especially Scots pine and red pine, respond more slowly to the stimuli of light and temperature used during conditioning and leads to the conclusion that the mechanism for induction of dormancy in this species is not well understood and is worthy of further research.

Except for Scots pine, the data indicated no significant relationship between degree of cold hardiness and nutrient concentration or nutrient uptake in the shoot of seedlings. Investigation of nutrient uptake in roots also merits further study in view of their lower

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[1] Vidaver, W. 1989. Personal conversation. Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.

resistance to freezing. However, the nutrient regime presently recommended during conditioning (hardening-off) should be amended. Low N enhanced cold hardiness in lodgepole pine, whereas high P and high K promoted cold hardiness in jack pine, and in the other tested species (red pine, Scots pine, white spruce and black spruce), respectively.

ACKNOWLEDGEMENTS

The technical assistance of Mr. Joe van Dyk, Soil Research Technician, and Ms. Wendy Mills, Greenhouse Assistant, is gratefully acknowledged. During the summer months, they were ably assisted by students Robert Koski, Roman Wasarab, and Caroline Slupsky in 1985, 1986, and 1987, respectively.

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