EFFECT OF NUTRIENT STRESS ON GROWTH, BUD SET, AND HARDINESS IN DOUGLAS-FIR SEEDLINGS 1

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Abstract.--Seedlings were grown in 2-inch[3] polystyrene containers in a greenhouse for 5 months, then cold hardened under lights at 2°C for 3 months. The effects of withdrawing various components of the weekly nutrient solution halfway through the growth period were measured. Withdrawal of P and K together produced a somewhat taller plant which otherwise did not differ significantly from controls except for a delay in achieving hardiness. Withdrawal of N produced short, chlorotic plants which, although lower in total dry weight, had 20 percent heavier root systems and a lower shoot-root ratio. Simultaneous withdrawal of all nutrients also produced small seedlings. It therefore appeared that growth and bud development were related to amount of N in the nutrient solution and in the tissue. Hardiness was associated with the balance between N and K.

INTRODUCTION

A major problem in growing container seedlings rapidly in controlled environments is that as seedlings approach the desired physical dimensions (or desired planting time), positive measures must be taken to stop growth, and encourage the development of strong terminal buds. The succulent seedlings must be adapted physiologically to the relatively harsh conditions expected at forest planting sites. We need a more detailed knowledge of the effectiveness, timing and long term effects of possible "adaptive" cultural treatments. The following results show contrasting effects of four types of nutrient stress on growth, bud development and subsequent ability to gain cold hardiness.

MATERIALS AND METHODS

Stratified seeds from a coastal Douglas fir source[3] were sown in mid-November into a 3:1 (v/v) peat-vermiculite mix in 2-cubic-inch styrofoam containers (Canadian Forest Service Styroblock 2, with seedling cavities at a density of 100 per sq. ft.), covered with a thin layer of granitic grit, and allowed to germinate and grow in a greenhouse at about 22°C.

Photoperiod was extended to 16 hours by mixed fluorescent and incandescent lighting ($\geq$ 7000 $\mu W.cm^{-2}$ photosynthetically active radiation). After germination the soil was saturated with nutrient solution twice a week and watered as necessary to keep it moist.

The standard nutrient solution (Table 1) was similar to that used by van den Driessche (1969). The five nutrient treatments are described in Table 2.

Each treatment was given to a quarter styroblock of 48 seedlings and replicated three times in a randomized block design. Height (from cotyledon to stem apex) was measured weekly on a permanent sample of five seedlings per replicate. After 21 weeks a final count was made of the number of protected terminal buds on the main stem. Sixteen plants per replicate, including the recurrently measured sample, were then harvested and measured for height, root collar diameter, and shoot and root dry weight. Four-gram samples of dried shoots from each experimental

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Table 1.--Composition of standard nutrient solution

Element	Concentration (ppm)	Source
N	50	NH_4NO_3
P	25	H_3PO_4
K	150	K_2SO_4
Ca	100	$CaCl_2$
Mg	50	$MgSO_4$
S	127	K_2SO_4+$MgSO_4$
Fe	6	Iron Chelate
Mn	0.2	$MnCl_2$
Cu	0.02	$CuSO_4$
Zn	0.02	$ZnSO_4$
Mo	0.003	$NaMoO_4$
B	0.2	H_3BO_3

unit were analyzed for N (Kjeldahl), P (vanado-molybdate) and K (atomic absorption).

An additional 10 seedlings per replicate were combined into a single sample of 30 (for each treatment) and tested for frost hardiness. Of these, a sub-sample of 10 seedlings were cooled at 5° C/hr, with roots insulated, to one of three selected freezing temperatures, maintained at this temperature (+.25°) for 2 hr, and then warmed up at 20°/hr, Injury was evaluated on a five-point scale according to the proportion of brown needle or bark tissue after 7 days in an environment favorable to growth. A 50 percent injury temperature (T_{50}) was interpolated from the injury/temperature curve obtained from the three sub-samples.

The 22 remaining plants per replicate were then hardened in a cold room at 2° C, on an 8-hour photoperiod of abou 1700 uW. cm-2 mixed fluorescent/imcandescent light. Samples of each treatment were removed after 5.5 weeks and 11.5 weeks for hardiness evaluations as described above. The timing of treatment and measurement in relation to growth and hardening periods is summarized in Figure 1.

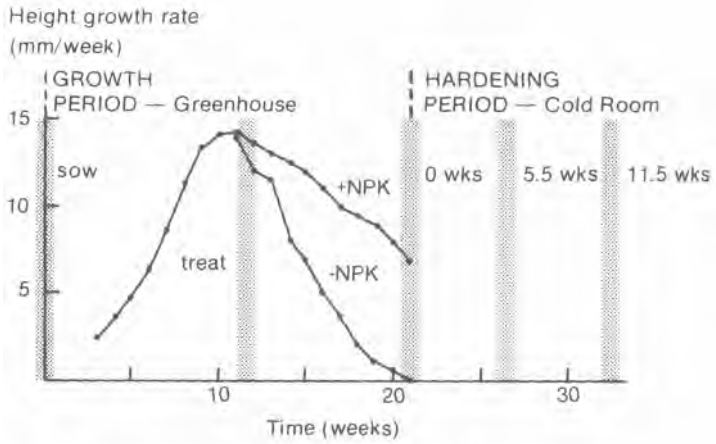

Figure 1.--Timing of treatment and measurement. Shaded bars denote the point in time at which a treatment or measurement operation was carried out. Curves represent height growth of controls (+NPK) and seedlings deprived of nutrient after the 11th week (-NPK).

Table 2.--Nutrient Solutions and Times of Application

Treatment		
Solution Composition	Time of Application	Symbol
1. standard	twice weekly for 21 weeks	NPK
2. half of K_2SO_4 omitted from standard	twice weekly for 21 weeks	$NPK_{1/2}$
3. H_3PO_4 and K_2SO_4 omitted from standard (made up to pH 4.0 with HCL)	replacing standard solution after 11th week	N
4. NH_4NO_3 omitted from standard	replacing standard solution after 11th week	PK
5. tap water	replacing standard solution after 11th week	-NPK

Growth and morphology data were logarithmically transformed (because variance was proportional to the mean) and percentage counts of buds were adjusted by arcsin transformation, prior to analysis of variance and by Duncan's multiple range test. Significance of differences between injury/temperature curves, and hence between T_{50} values, was tested by the relatively conservative method of a Duncan's test conducted on arcsin-transformed injury scores at a single temperature.

RESULTS

Growth and Morphology

The withdrawal of nutrients in N, PK and 0 treatments coincided with the time of maximum height growth (fig. 1), when demand for them was probably at its greatest. Withdrawal of N (PK and -NPK treatments) reduced height growth in the remaining 11 weeks in the greenhouse to about one half of that in controls, and produced chlorotic plants somewhat smaller in diameter (fig. 2). Withdrawal of PK resulted in significantly (p=0.05) taller seedlings.

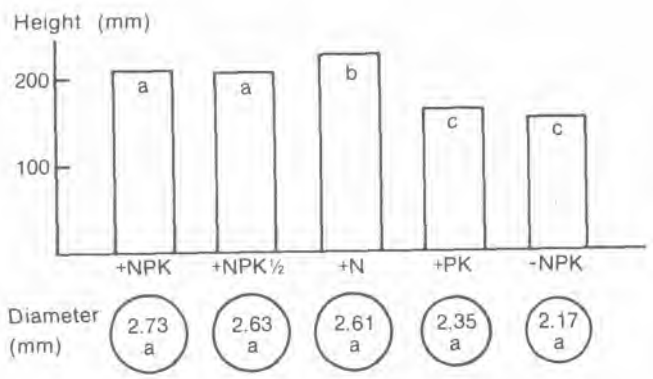

Figure 2.--Shoot heights (bars) and root collar diameters (circles) of Douglas-fir seedlings at week 21 under five nutrient regimes. Each value is an average of 4-8 observations. Bars or circles bearing the same letter are not significantly different (p=0.05) by Duncan's test.

Shoot dry weight revealed essentially the same pattern of dependence on N as did height growth, with reduction of K or withdrawal of PK having no significant effect (fig. 3). However, dry weight of roots was significantly higher (by 20 percent) for seedlings deprived of N than for controls, and significantly lower than controls in PK-deprived and reduced-K treatments.

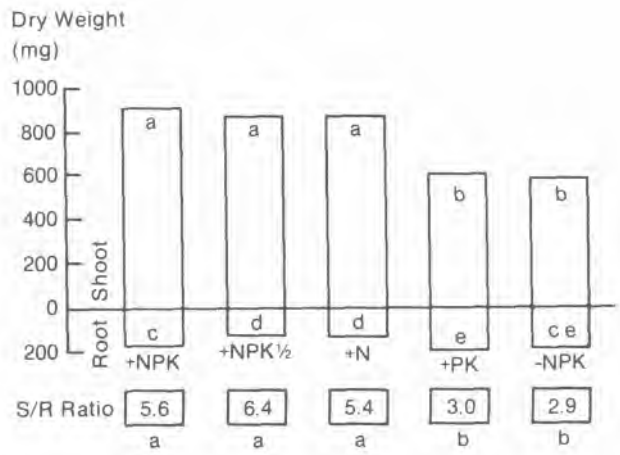

Figure 3.--Shoot and root dry weights (bars) and shoot/root ratios (lower numerals) under 5 nutrient regimes. Each value is an average of 48 seedlings. Values bearing the same letter are not significantly different (p=0.05) by Duncan's test.

Budset

Terminal buds had formed on 50 percent of the seedlings within 45 days of withdrawing N. By the end of the growth period 85-95 percent of all N-deprived plants (PK and -NPK) had buds compared with less than 5 percent in other treatments (fig. 4). A concurrent series of treatments, in which NPK was withdrawn at different times, showed a significant age effect in the bud set response. Response time for 50 percent bud set decreased

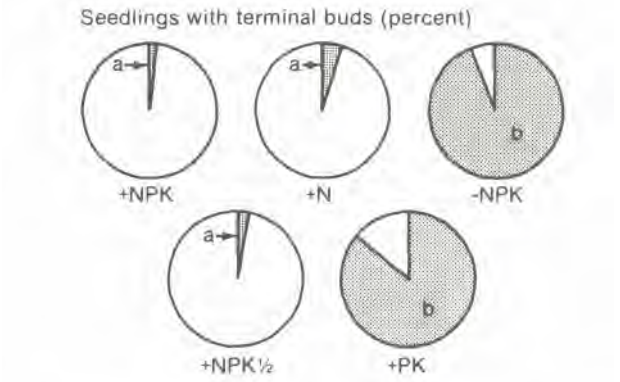

Figure 4.--Percent seedlings with protected terminal buds (shaded segments of circles) at week 21 under five nutrient regimes. Each value is based on 144 seedlings. Segments bearing the same letter are not significantly different (p=0.05) by Duncan's test.

from 51 to 45 to 29 days corresponding with NPK withdrawal at the 10th, 12th and 14th week respectively.

Hardiness

The development of hardiness (fig. 5) was not consistently related either to the chlorosis, bud development or the level of any one of the three manipulated nutrients.

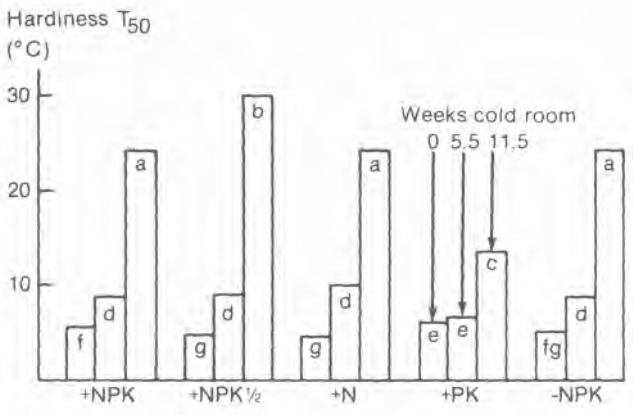

Figure 5.--Cold hardiness of seedlings grown under five nutrient regimes, measured (as temperature causing 50 percent foliar injury) before, during, and after 11.5 weeks of cold room treatment. Bars bearing the same letter are not significantly different (p=0.05) by Duncan's test.

Seedlings deprived of N, but receiving P and K, were unable to harden off to an extent that would have allowed them to survive a normal winter in their native site (T50 = -13° C). However, when all nutrients were withdrawn (treatment -NPK) hardening proceeded normally. This was true whether NPK was withdrawn at week 10, 11.5 or 14. The hardiness attained by seedlings in N and NPK treatments, which were actively growing at the start of cold treatment, was the same (-24° C) as that in NPK-deprived seedlings, which had ceased height growth and developed terminal buds. The hardiest seedlings (T50= -30° C) were those receiving the continuous diet of reduced K (treatment NPK 1/2). As with controls, these were actively growing at the start of cold treatment, but their hardiness level was the same as that attained by normally nourished seedlings in which height growth cessation and bud set had first been induced by short day treatment in the greenhouse (data not shown).

Hardiness levels measured immediately after the greenhouse period (fig. 5) were not predictive of the later levels noted above. Before cold room treatment, the PK seedlings (subsequently least hardy) were significantly hardier than controls while NPK 1/2 seedlings (subsequently hardiest) were significantly less hardy than controls. Seedlings receiving treatment N were least hardy of all at first. Bud development was again not related to hardiness. Although the differences in T50 in these cases are relatively small (1-2° C), they represent a difference of 50 percent or more in the number of plants surviving a given frost.

The detrimental effect on cold hardening of withdrawing N, but not N, P and K together, suggests that it may be the balance between these elements that is important. The ratio of K to N in the shoot (from Table 3) shows a trend that is inversely related to cold-induced hardiness (fig. 6). In contrast, hardiness before cold treatment tends to have a direct relationship to the K/N ratio.

Table 3.--Concentrations of N, P and K in shoots at week 21.

Treatment	percent of dry weight[1] N	P	K
NPK	1.53_a	0.43_a	1.15_a
NPK 1/2	1.64_a	0.41_a	1.03_b
N	1.13_b	0.18_b	0.71_c
PK	0.77_c	0.36_a	1.04_b
-NPK	0.83_c	0.25_c	0.80_c

[1]Percentages of a given element with the same subscript letter do not differ significantly (p=0.05) by Duncan's test.

DISCUSSION

The present data, although based on a rather small number of extreme treatments, show clearly that neither the size, growth status, bud development nor foliage color of a seedling provides a reliable indication of its present level of hardiness or its subsequent ability to harden off more deeply under cold conditions. The presence or absence of N appeared to determine the main growth, bud set and morphology responses, including a stimulation of root growth in response to sudden nutrient deprivation.

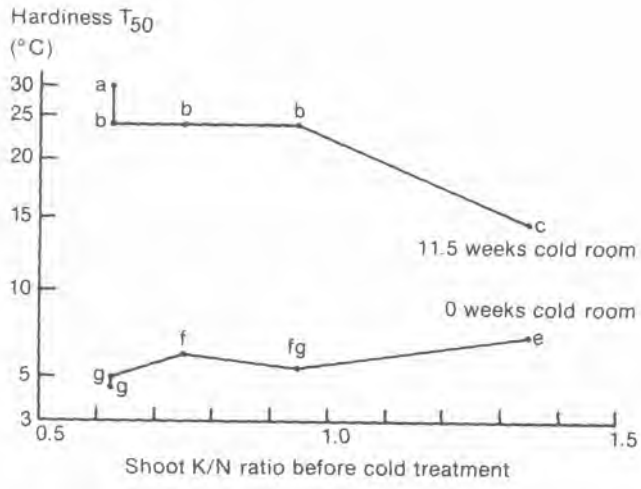

Figure 6.--Relationship between cold hardiness (50 percent foliar injury temperature) and prior shoot K/N ratio, before and after 11.5 weeks of cold room treatment. Hardiness scale is logarithmic to emphasize differences between points on the lower curve, where, in fact, a given decrement of frost temperature causes a much greater increase in injury. Points bearing a common letter do not differ significantly in hardiness (p=0.05) by Duncan's test.

But cold hardiness was more closely related to the balance between nutrients--N and K in this case--than to the level of any single element. Furthermore, a tissue nutrient balance which might favor survival of early fall frosts could severely limit on-site adaptation to mid-winter freezes.

It has often been found that application of nitrogen causes a decrease in hardiness, which has been attributed to its effect in prolonging growth and delaying the onset of dormancy (Levitt, 1956). Under this hypothesis the lack of any consistant effect of nitrogen on initial hardiness in the present study is due to the fact that hardiness and growth status were not related. This independence was noted in earlier studies of Douglas-fir (Timmis, 1973) and other woody species (Irvine and Lanphear. 1967).

The increase in root dry weight following N deprivation was not observed in a concurrent study on western hemlock (Cheung, 1973). It could be viewed as the removal of conditions that were in some way inhibitory to root growth, since in the control treatment the proportion of dry matter in the root system was abnormally low. Or it is possible that root systems accustomed to an abundance of nutrient and then deprived may respond differently from those grown (e.g. by van den Driessche, 1969) at continuously low nutrient levels.

The importance of tissue K/N balance to the level of hardiness attained after cold treatment presumably reflects the participation of both these elements in the biochemical processes of the second stage of hardening (Weiser, 1970). Nitrogen is necessary for protein synthesis leading to augmentation or modification of protoplasm and membranes (reviewed by Levitt, 1972); potassium participates in many enzymatic reactions. The involvement of the balance between two elements, and the occurrence of both short term and long term effects on hardiness must have contributed to the inconsistent effects of N and K on hardiness in conifers reported by different investigators (table 4). The only concensus is that K usually increases the initial hardiness as did the higher K/N ratio in the present study (fig. 6), possibly through an increase in cell sap osmotic pressure (Sato and Muto, 1951; Kopitke, 1941). However, in growing seedlings for high elevation forests it is more important to inculcate a high responsiveness to the hardening temperatures encountered on site. The present results suggest that we should aim for a K/N ratio around 0.6 and circumvent any initial root growth, bud set and hardiness disadvantages by, for example, short day treatment in the greenhouse. Further work is needed on these possibilities.

ACKNOWLEDGEMENT

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Table 4.--Summary of literature on the relation of K and N to cold hardiness in conifers.

Author	Species	Effect[1] of increasing: K measured at hardening stage[2]:			N measured at hardening stage[2]:			Conditions
		1	2	?	1	2	?	
Alden (1971)	Douglas-fir	+	0			+		Normal and K-deficient plantations
Benzian (1966)	w. hemlock		+			+		Nutrients applied during dormancy in nursery beds
	sitka spruce		+			+		
Christersson (1973)	scots pine	0						Potted plants
Coultas (1966)	w. red cedar			+			-	Potted plants-effects not seen in 2nd season
	juniper			0			0	
Kopitke (1941)	white spruce	+						Nursery beds. Relative hardiness based on osmotic pressure of expressed cell sap only.
	white pine	+						
	red pine	+						
Pümpel (1973)	Norway spruce	0				±		Pots. Results very variable depending on soil type and exact NPK proportions
	stone pine	0				-		
Pellet & White (1969)	juniper						0	
Sato et al (1952)	Japanese species of spruce, true fir	+						Pots. Different effects according to time of K application during growth.
		+						
	cypress & pine	+						
Timmis (1972)	Douglas-fir				-			Small containers
Tranquillini (1963)	stone pine						-	Plantation survival vs. foliage color in nursery
Tukey & Meyer (1968)	yew		0					Pots

[1]Hardiness: +increased, -decreased, 0 unchanged.

[2]Stage 1 is before, and stage 2 after a substantial period of cold treatment (after Weiser, 1970).

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