

Effect of Nursery Culture and Bud Status on Freeze Injury to *Pinus taeda* and *P. elliottii* Seedlings

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SYNOPSIS

The effects of nursery practices on freeze tolerance of *P. taeda* and *P. elliottii* seedlings were examined by placing the seedlings in a freezing chamber and lowering the temperature to -10 °C. Injury was evaluated 10 days after the freeze treatment by examining cambial tissue (browning test). Top-pruning *P. taeda* seedlings (three times) increased freeze tolerance while root-wrenching (six times) had no effect. Cambial injury of *P. elliottii* was negatively related to seedling diameter but injury was not affected by either top-pruning or root-wrenching. Although potassium fertiliser is often recommended for fall fertilisation in the southern United States, this study found no beneficial effects on freeze tolerance. In fact, fertilisation in October decreased freeze tolerance of *P. elliottii*. Data from the freeze test and from natural freezes in the nursery were analysed to determine if freeze tolerance was related to the presence of a well-formed terminal bud in early November. Covariance analysis from the freeze test indicated no relationship between freeze tolerance and bud presence for either species. Survey data indicate that *P. taeda* seedlings with well-formed terminal buds were not more tolerant of freezing in November than seedlings without terminal buds.

INTRODUCTION

During the fall, pine seedlings in bare-root nurseries can be damaged by sudden freezes. Exposure to low temperatures and short photoperiods usually increases the ability of pine seedlings to withstand freezing temperatures (Aronsson, 1975; Mexal *et al.*, 1979; Menzies *et al.*, 1981a; Greer *et al.*, 1989; Repo *et al.*, 1991). Unfortunately, managers of bare-root nurseries have little control over temperatures during the fall. Injury to roots of *Pinus taeda* and *Pinus elliottii* can occur during years when a sudden freeze (-16 °C) follows a warm fall (Rowan, 1985). This injury can occur even after the photoperiod has reached a minimum. Nursery managers need to know if cultural practices will affect the seedling's ability to tolerate freezing temperatures.

Except for continued overhead irrigation during freezing temperatures (used to produce latent heat during the formation of ice), there is little information on management practices which would protect pine seedlings from freeze injury. Menzies (1977)

proposed that undercutting and wrenching could improve frost-tolerance of *Pinus radiata*. However, there is a lack of information on the effects of root wrenching, top-pruning and fertilisation on frost-tolerance of southern pine seedlings.

It is generally believed that fertilisation practices affect the susceptibility of seedlings to cold injury. In addition, practices such as undercutting and top-pruning are used to "condition" (*sensu van Dorser*, 1977) bare-root seedlings in the southern United States, but little is known about the effects of these practices on freeze tolerance. Research has not been attempted to determine if these practices interact with fertilisation to cause differential response in frost tolerance. A study was established to investigate such potential interactions.

Two additional studies involving freeze tolerance were conducted in 1991. The second study involved a record breaking freeze which occurred in November 1991. Secondary needles of *P. taeda* seedlings in several bare-root nurseries in Alabama were injured by the freeze. This early natural freeze provided an

opportunity to test the hypothesis that freeze injury is related to the presence of a well-formed terminal bud. A third study was conducted to determine the effects of top-pruning on leachate conductivity of *P. taeda* seedlings.

MATERIALS AND METHODS

Study 1: Artificial freeze test

A study was established in 1990 at the Union Camp Nursery near Inverness, Alabama (3° 06' N, 85° 43' W, altitude 140 m). The climate for this area is warm and humid with a mean annual precipitation of 1 288 mm. In this study *P. taeda* seed was from a half-sib family while the *P. elliottii* seed was from an orchard mix. Prior to sowing on April 19, the area was fertilised with 24 kg/ha of P (from triple super phosphate) and 56 kg/ha of K (from potassium chloride). During June and July, seedlings received a total of 171 kg/ha of N (60 % as ammonium nitrate and 40 % as ammonium sulphate). Seedlings were top-dressed with an additional 56 kg/ha of K (from potassium chloride) in August. Routine nursery management practices were followed until the installation of the study in the first week of August. The *P. taeda* study (275 seedlings/m²) was installed adjacent to the *P. elliottii* study (234 seedlings/m²). For each species, the experimental design was a split-plot with four replications. Main plots consisted of top-pruning and wrenching treatments; fertiliser treatments were sub-plots.

Main plots were 40 m long and 1,2 m wide. The five main treatments were (1) multiple top-prunings and no undercutting; (2) one undercut; (3) undercut followed by two root wrenchings; (4) undercut followed by four root wrenchings; and (5) undercut followed by six root wrenchings. Top-pruning was done on August 6 (at a height of 200 mm), September 4 (230 mm), and October 1 (250 mm). On August 6, all non-top-pruned seedlings were undercut at a depth of 100 mm with a reciprocating, horizontally mounted blade; (using a Summit machine). Root wrenchings occurred on August 20, September 4 and 17, October 1, 15 and 19. Treatment 3 included the first two wrenchings and treatment 4 included the first four wrenchings. The reciprocating root wrenching blade was set at a depth of 100 mm with a 20 ° angle. Irrigation was conducted following each root wrenching treatment.

Fertiliser treatments were applied to sub-plots that were 18 m long and 1,2 m wide. Treatments consisted of (1) control, (2) 150 kg/ha of N; (3) 150 kg/ha of P; (4) N plus P; (5) 150 kg/ha of K. The sources used were ammonium-nitrate, triple super phosphate, and potassium chloride. The single element fertilisers were applied in two equal top dressings on October 3 and 10. The combination of N and P was applied as three equal top-dressings on October 3, 10 and 17. The October application was intended to increase seedling nutrient levels without appreciably affecting seedling morphology.

On November 5, seedlings were lifted, placed in plastic bags, and stored for two weeks at 2 °C. The

freezing test began on November 19. Ten seedlings from each plot were separated into three control seedlings and seven treated seedlings. The control seedlings were inserted into 64 ml cylindrical plastic cells (Ray Leach Single Cells) and placed into an aerated water bath. Cold exposure plants were packed into plastic bags with the roots protected by horticultural grade vermiculite (*P. elliottii*) or peat/styrene potting compost (*P. taeda*). They were placed in a commercial freezer chest which was initially at ambient room temperature (17°C). After two hours, the temperature had dropped to 1°C. Thereafter the temperature was decreased at a rate of 1,3°C/h to reach a final temperature of -10°C. Thirty minutes after reaching -10 °C the freezer was switched off and opened to allow the seedlings to thaw.

Treated seedlings were inserted into plastic containers and placed in an aerated water bath along with the controls. After 10 days, a cambium browning test (Glerum, 1985) was conducted on each seedling. The stem at the root collar was scraped to expose the cambial tissue. If brown tissue was observed, the seedling was given a damage rating of 1. Seedlings without brown tissue were assigned a rating of 0. Seedlings with *Botrytis cinerea* on the foliage were recorded using the same binomial ranking. The terminal was also examined to determine if the seedling had a well-formed terminal bud with brown bud scales. While evaluating injury on the *P. taeda* seedlings, it became evident that a relationship existed between seedling size and cambial injury. Therefore, for *P. elliottii* seedlings, the root collar diameter was measured just prior to examining the cambial tissue.

Freeze test data were analysed according to the experimental design in the nurserybeds (a split plot design). Control seedlings (those not subjected to the freeze test) were excluded from the analysis because there was no injury among treatments. A General Linear Model procedure (SAS Institute, 1985) was used to test for significant effects. Correlation analyses were used to examine the potential relationship among the presence of terminal buds, cambial injury, and presence of *Botrytis* on the needles. In addition, analyses of covariance (Snedecor and Cochran, 1967) were used on non-top-pruned seedlings in an attempt to throw light on the relationship between cambial injury and the presence of a terminal bud.

Study 2: Case studies of freeze injury

In November of 1991, *P. taeda* seedlings from three Alabama nurseries were sampled for needle injury resulting from an early freeze. Minimum temperatures were often above 15 °C during the two weeks prior to the freeze. However, within 72 hours, the minimum temperature dropped approximately 22 °C. On November 5, the minimum temperature reached -4 °C for the south central nursery, -6 °C for the central nursery, and -7 °C for the northern nursery. The nursery locations ranged from north (Red Bay, 34°26' N), central (Childersburg, 33° 17' N), to south central Alabama (Camden, 32° 6' N). At each

nursery, 30 or more sample plots were measured to assess the frequency of visual needle injury. The number of seedlings sampled ranged from 4 628 at the central nursery to 1 211 at the northern nursery. To avoid confounding seedling height; with bud type, seedlings were classified as either having one or two stem flushes. Surveyed seedlings were classified into two bud types. One type was well-formed terminal bud, characterised by tight, brown, bud scales. The second type lacked a well-formed bud and had the appearance of a feather-top. Feather-top seedlings have succulent shoot tips crowned with a tuft of primary needles. Seedlings with no terminal owing to mechanical injury were not included in the survey. Seedlings with signs of needle injury were ranked as (1) and uninjured seedlings as (0). Chi-square tests were conducted to determine if needle injury was related to the presence of a well-formed terminal bud.

Study 3: Leachate conductivity

The effect of top-pruning on leachate conductivity after freezing was examined using four half-sibling seed lots of *P. taeda*. Seeds were sown in April in a bare-root nursery at Bellville, Georgia (32° 9' N). The study consisted of separate randomised complete block experiments within each seed lot. Each experiment had five blocks composed of an unpruned plot and a plot that was top-pruned twice (once in August and again in September). All lots received 168 kg/ha of N (as ammonium nitrate) split into six applications from May through August. All plots received root wrenching in June, August, and September.

On November 29, seedlings from each treatment plot were lifted, placed in plastic bags, and stored for 24 h at 4 °C prior to exposure to -18 °C for 24 h. Five seedlings were randomly selected from each plot and tested using a modification of the electrical conductivity method (Dexter *et al.*, 1932). Two 25 mm stem segments were taken from each seedling, one at groundline (basal segment) and one at a height of 150 mm (aerial segment). Conductivity was measured after each segment was submerged in 5 ml of deionised water for 24 h. Subsequently, each segment was removed from the water and heated at 90 °C for 2 h. The dry segment was then submerged in 5 ml of deionised water and a second conductivity measurement was recorded after 24 h. Relative conductivity (RC) was calculated for each segment using the method outlined by Glerum (1985) [RC = (conductivity measured after freezing)/(conductivity measured after heating)]. The diameter of each segment was used as a covariate when analysing for differences in relative conductivity. The top-pruning treatment did not have an effect on the relative conductivity of segments from control seedlings that were not frozen.

RESULTS

Pinus taeda

The freeze test injured on average, 75 % of stems of undercut and wrenched seedlings (Table 1). There was no difference in injury between wrenched seedlings and seedlings that were only undercut (Table 2). However, only 17 % of the top-pruned *P. taeda* seedlings were injured. Apparently, reducing the

TABLE 1. Means of freeze injury to the cambium, percentage of seedlings with winter buds, and percentage of seedlings with Botrytis.

	<i>P. taeda</i>			<i>P. elliotii</i>		
	Injury	Bud	Botrytis	Injury	Bud	Botrytis
	-----	%	-----	-----	%	-----
Treatments						
top-prune	17	23	80	53	4	29
undercut	73	68	75	37	26	18
two wrenches	80	62	65	52	20	29
four wrenches	72	70	65	40	39	14
six wrenches	77	74	65	26	33	1
Fall fertilisation						
control	54	74	65	28	25	15
nitrogen	64	52	85	44	22	16
phosphorus	72	61	50	42	25	7
N + P	63	57	68	40	25	13
potassium	65	53	81	56	25	40

amount of foliage may have a beneficial effect on reducing freeze injury for this species. An increase in cambial injury with fall fertilisation could not be established definitively ($p = 0,107$). There was no evidence of an interaction between fall fertilisation and root or shoot pruning treatments.

Approximately 68 % of the non-top-pruned seedlings developed a terminal bud by November 5 (Table 1). By this date, there was no meaningful difference in numbers of terminal buds between the undercutting treatment and any of the wrenching treatments. However, only 23 % of the top-pruned seedlings had terminal buds. An orthogonal contrast (control vs. others) indicated that, overall, the October fertilisation delayed the formation of terminal buds (Table 2).

The presence of a terminal bud had no direct relationship to freeze injury (Table 3). Even when analysing only root cultured seedlings (Table 4), the inclusion of buds as a covariate did not account for a significant amount of variation in cambial injury.

Pinus elliottii

On average, the freeze test injured cambium in 39 % of the undercut and wrenched seedlings. There was no significant difference between undercut and wrenched seedlings. However, 53 % of the top-pruned seedlings were injured by the freeze test. The unfertilised seedlings exhibited less injury than fertilised seedlings. In particular, the fall application of

TABLE 2. Analysis of variance table, F-values, and error mean squares for freeze injury to the cambium, bud status, and presence of Botrytis

	df	<i>P. taeda</i>			<i>P. elliottii</i>		
		Injury	Bud	Botrytis	Injury	Bud	Botrytis
Replication	3	0,58	0,25	0,88	7,76 **	2,95	3,19
Treatment	4	15,04 ***	3,99 *	0,39	1,79	20,11 ***	2,17
top-P. vs. others	(1)	39,73 ***	15,19 **	0,98	2,45	56,91 ***	2,54
undercut vs. wrench	(1)	0,18	0,00	0,59	0,03	1,94	0,10
wrench-linear	(1)	0,04	0,77	0,00	4,59	9,55 **	8,02 *
wrench-quadratic	(1)	0,45	0,01	0,00	0,01	11,43 **	0,08
Error A mean square	12	0,137 8	0,213 5	0,260 0	0,134 6	0,017 4	0,104 8
Fertilisation	4	0,92	1,44	1,97	2,90 *	0,08	4,10 **
control vs. others	(1)	2,67	4,55 *	0,31	7,80 **	0,04	0,40
control vs. K	(1)	1,22	4,49 *	1,24	11,37 **	0,01	9,23 **
N vs. P	(1)	0,69	0,62	6,29 *	0,15	0,21	0,99
np vs. N and P	(1)	0,20	0,08	0,02	0,19	0,03	0,03
treatment X fert.	16	1,06	0,90	1,15	0,84	0,38	0,80
Error B mean square	60	0,092 7	0,113 7	0,194 9	0,059 9	0,036 0	0,071 3

Note: * Significant at the 5 % probability level.
 ** Significant at the 1 % probability level.
 *** Significant at the 0,1 % probability level.

TABLE 3. Matrix of simple correlation coefficients.

Species	Terminal buds	Cambial injury	Botrytis
<i>Pinus taeda</i> (n=100)			
cambial injury	0,054 9	-	-
Botrytis	-0,257 0 **	0,036 1	-
<i>Pinus elliottii</i> (n=99)			
cambial injury	-0,244 8 *	-	-
Botrytis	-0,190 4	0,660 5 ***	-
root collar diameter	0,404 7 ***	-0,491 6 ***	-0,349 7

* Significant at the 0,05 level.
 ** Significant at the 0,01 level.
 *** Significant at the 0,001 level.

potassium chloride increased the level of freeze injury to the cambium.

On November 5, 30 % of non-top-pruned *P. elliotii* seedlings had a terminal bud. By this date, there was no significant difference in terminal bud formation between the undercut-only treatment and the wrenching treatments. However, only 4 % of the top-pruned seedlings had terminal buds. The presence of a terminal bud apparently had no relationship to freeze injury since, for root cultured seedlings, there was no relationship between injury and the presence of a terminal bud (Table 4).

The presence of *Botrytis* was correlated with cambial injury (Table 3). Seedlings with brown cambial tissue tended to have *Botrytis* on the needles.

Control seedlings that were not frozen exhibited no signs of *Botrytis*.

Case studies of freeze injury

Stem cambium and terminal buds did not appear injured, but the newly-formed tissue on developing secondary needles was frequently injured, causing young secondary needles to bend at the fascicle. Regardless of bud type, primary needles and mature secondary needles did not show injury symptoms.

Needle injury was greatest at the northern nursery (Table 5). At the south central nursery, needle injury was similar regardless of bud type or number of stem flushes. However, at the other two

TABLE 4. Analysis of covariance table using only undercutting and root wrenching treatments

	df	<i>P. taeda</i>			<i>P. elliotii</i>		
		MS	F	P > F	MS	F	P > F
Replication	3	0,055 1	0,38	0,770 2	0,496 6	4,85	0,028 3
Root treatment	3	0,027 6	0,19	0,900 2	0,169 9	1,66	0,244 0
Error A mean square	9	0,145 2			0,102 3		
Covariate (bud type)	1	0,255 5	2,63	0,111 5	0,034 9	0,63	0,429 9
Fertilisation	4	0,016 9	0,17	0,693 3	0,163 9	2,97	0,028 9
Treatment X fert.	12	0,073 2	0,75	0,111 5	0,062 1	1,13	0,363 3
Error B mean square	47	0,097 2			0,059 9		

The presence of terminal bud is the covariate and cambial injury is the dependent variable (type III sums of squares).

TABLE 5. Needle injury in relation to the presence of a well formed terminal bud and the number of flushes of *P. taeda* at three bare-root nurseries in Alabama

Nursery location	One stem flush			Two stem flushes		
	Feather-top	bud	X ²	Feather-top	bud	X ²
	----- % injury -----			----- % injury -----		
North	67	97	153	80	100	76
Central	4	2	4,6	16	32	51
South central	18	19	0,2	19	19	0,0

Note: Chi-square values greater than 10 are significant at the 0,001 level of probability.

TABLE 6. Relative conductivity for unpruned and top-pruned *Pinus taeda* seedlings from four half-sibling seed sources at the Bellville Nursery

Seed lot	Aerial segment			Basal segment		
	Unpruned		Top-pruned	Unpruned		Top-pruned
	----- Relative conductivity -----					
A	0,618	(0,002 4)	0,295	1,461	(0,218 8)	1,320
B	0,628	(0,305 3)	0,447	1,745	(0,428 9)	1,849
C	0,913	(0,006 3)	0,402	1,581	(0,924 4)	1,623
D	0,595	(0,258 3)	0,414	2,146	(0,779 9)	2,244

Note: Probability values for a significant treatment effect are listed in brackets and were determined using analysis of covariance with segment diameter as the covariate. Differences between aerial and basal segments are significant at the 0,000 1 level of probability for all four seed lots.

nurseries, secondary needle injury was greater on seedlings with well-formed terminal buds.

Leachate conductivity

The extent of cold damage to the aerial shoot segments was reduced by top-pruning in all seed lots, although the differences for two seed lots were not statistically significant (*Table 6*). The relative conductivity was not affected by segment diameter of the aerial shoot segment for any of the four seedlots (data not shown).

The basal segment had more electrolyte leakage than did the areal segment for each seed lot. Top-pruning had no effect on the relative conductivity of basal segments. For the basal segment, only seed lot B showed a negative relationship between segment diameter and relative conductivity ($R = -0,84$ $p = 0,005$).

DISCUSSION

When compared to a single undercut, root wrenching did not improve freeze tolerance of either *P. elliotii* or *P. taeda*. Root wrenching also has little effect on freeze tolerance of *P. radiata*. Rook *et al.* (1974) reported that wrenching at the FRI Nursery reduced mortality following a -10 °C freeze by just one seedling. Apparently, nursery location (cool night-time temperature) is much more important for increasing frost tolerance than wrenching (Menzies *et al.*, 1981b). Although wrenching can improve the tolerance of *P. radiata* seedlings to storage at $1,5$ °C (van Dorsser, 1969), it apparently does not improve the ability of *P. taeda* to withstand cool storage (Hammer *et al.*, 1986). However, *P. taeda* is much more tolerant of freezing than *P. radiata* (Chaperon and Fraysse, 1986).

Nursery fertilisation is believed to affect the degree of freeze tolerance of conifers (Landis, 1985; Glerum, 1985; Skre, 1988). However, conflicting results have been achieved when fertilising with nitrogen (van den Driessche, 1991). Nitrogen fertilisation is generally thought to reduce or prevent the acquisition of freeze tolerance. Data from the artificial freeze study supports this hypothesis. However, the time of fertiliser application may be important as well as the season of the freeze (Timmis, 1974; Repo *et al.*, 1991). Nitrogen added prior to growth cessation in the fall might prolong shoot growth and thereby subject the seedlings to damage from early freezes. Nitrogen added after bud formation might increase frost resistance in the fall (Thompson, 1982), but could result in freeze damage in spring by inducing earlier shoot growth (Skre, 1988). In addition, it may be that freeze tolerance decreases at both high and low foliar nitrogen concentrations and that a moderate level may result in maximum tolerance (Aronsson, 1980).

It is often believed that fall fertilisation with potassium can assist in growth cessation and bud

development (Landis, 1985). However, for *P. taeda*, applying potassium chloride in October apparently delayed bud formation. For *P. elliotii*, only about 25 % of the seedlings had well-formed terminal buds in November, and potassium fertilisation in October did not increase this percentage.

Potassium fertilisation in the fall is recommended to hasten the "hardening-off" process of southern pines (Davey, 1984). This recommendation may be based on findings by Kopitke (1941) who measured the freezing point, osmotic pressure and simple sugar content of expressed sap, but not the freeze tolerance of intact seedlings. More recently, Christersson (1973, 1975, 1976) and Aronsson (1980) found that potassium levels in shoots of *P. silvestris* seedlings were not related to frost hardiness. In contrast, Menzies *et al.* (1981b) found high levels of potassium ($>0,77$ % dry weight) associated with greater frost damage to *P. radiata* in the winter. It may be that improved performance attributed to potassium fertilisation in older studies was due to improved drought resistance when soil is frozen instead of improved cold hardiness (van den Driessche, 1991).

In the southern United States, there is no data to show that fall applications of potassium chloride improved frost tolerance of *P. taeda* (Rowan, 1987). In fact, Dierauf (1982) reported that survival decreased when seedlings were fertilised in September with 186 kg/ha of potassium and subsequently stored for two weeks. In addition, fall fertilisation with just potassium does not benefit new root growth of *P. taeda* (Byran, 1954) or *P. radiata* (Donald, 1988). These findings cast doubt on the practice of applying potassium chloride in the fall.

It is generally believed that nursery practices that promote bud development will simultaneously increase freeze tolerance. However, in this study, there was no positive relationship between presence of a terminal bud and freeze injury in either the natural or artificial freeze studies. For non-top-pruned *P. taeda*, 75 % and 77 %, respectively, of the seedlings with or without well-formed terminal buds were injured. With *P. elliotii*, non-top-pruned seedlings with well-formed terminal buds had 35 % injury while those without well-formed buds had 40 % injury. When exposed to an early fall freeze, seedlings with well-formed terminal buds were not less likely to have injured secondary needles (*Table 5*). In fact, at two nurseries, seedlings with two flushes and terminal buds were more likely to have injured secondary needles. This can be explained because primary needles associated with feather-top seedlings were not injured, while developing secondary needles (associated with terminal buds) were injured. With this freeze, injury was related to the degree of secondary needle elongation rather than the presence or absence of a terminal bud *per se*. Timmis (1974) also reported no relationship between bud set and ability to withstand freezing temperatures for *Pseudotsuga menziesii*. Skrøppa (1991) found that bud-set could not be used to predict frost hardiness;

for full-sib families of *Picea abies*. Although Colombo (1990) found an empirical relationship between bud dormancy and frost hardness for *Picea mariana*, seedlings three weeks after bud initiation were not more frost hardy than seedlings one week prior to bud initiation.

Although top-pruning reduced the percentage of seedlings with well-formed terminal buds, it also reduced cambial injury and relative conductivity of aerial segments of *P. taeda* seedlings. Rowan (1985) also reported reduced cambial injury for *P. taeda* in Georgia. Seedlings top-pruned in early October to a height of 150 mm had only 10 % cambial injury while unpruned seedlings had 50 % injury. Dierauf (1991) reported that multiple top-pruning increased survival by 19 % when seedlings were planted just before a hard, natural, freeze (-16 °C) in December of 1983. These data suggest that the absence of a well-formed terminal bud does not necessarily mean that the seedling has not developed cold tolerance.

However, shoot length is an important seedling attribute with regard to freeze injury of conifers in bare-root nurseries. At the central and northern nurseries, the taller seedlings with two stem flushes were more exposed and more likely to be injured than shorter seedlings with only one stem flush (Table 5). Skrøppa (1991) reported a positive relationship between first-year height growth and freeze injury to *Picea abies* seedlings. A 20 or 30 mm difference in height growth made a large difference in the amount of needle injury. Similar results were reported by Blake *et al.* (1979) for *Pseudotsuga menziesii*. Dierauf (1977) reported a significant linear regression ($R = 0,62$) between freeze damage and seedling height of unpruned *P. taeda* seedlings. Approximately 34 % of his seedlings that were 250 mm tall had freeze damage while only 10 % of the 150 mm seedlings were injured. This may help to explain why top-pruning of *P. taeda* in Virginia is successful since the seedlings are more often exposed to hard freezes than in other regions of the South.

Genetics might explain why top-pruning would increase freeze tolerance of *P. taeda* but not *P. elliotii*. It is well known that freeze tolerance of the southern pines is related to the geographic seed source (Minkler, 1951). The natural range of *P. taeda* is more temperate than *P. elliotii* and therefore *P. taeda* is more tolerant of natural freezes (Rowan, 1985). It has been suggested that the average minimum temperature may be the most critical factor limiting the distribution of *P. elliotii* (Burns and Honkala, 1990). Therefore, *P. taeda* may have genetically different mechanisms for cold acclimation.

Although top-pruning *P. taeda* did not reduce freeze injury to needles (as evidenced by the presence of *Botrytis*), injury to stem cambium was reduced. It is conceivable that foliage removal allowed more cool air to reach the stems during the month of October.

While evaluating cambial injury for *P. taeda*, it became apparent that there was a negative relationship between root-collar diameter and cambial in-

jury (unfortunately diameters were not recorded prior to destroying the cambium). However, root-collar diameters were measured for each *P. elliotii* seedling and it was determined that mean root-collar diameter could account for 24 % of the variation in cambial injury (Table 3). This finding is similar to that reported for other conifers. Menzies and Chavasse (1982) found that on a hard frost site, survival and growth of *P. radiata* was better for "first-grade" stock (selected on the basis of size and form) than for "second-grade" stock. Seedbed density can affect both seedling size and freeze tolerance of conifers. For *Pseudotsuga menziesii*, shorter seedlings with larger root collar diameters were produced at lower densities and these seedlings were more tolerant of freezes (Timmis and Tanaka, 1976). Lower seedbed densities can also increase the freeze tolerance of *P. taeda* (Dierauf, 1977) and *P. radiata* (Menzies *et al.*, 1981a).

For *P. taeda* and *P. elliotii*, the percentage of seedlings with a well-formed bud is related to seedling size (Wakeley, 1954; Williams *et al.*, 1988). During the winter, seedlings greater than 5 mm have a higher probability of having a well-formed bud than seedlings that are less than 3 mm in diameter (Wakeley, 1954). Since seedling size can be related to freeze tolerance, it will be very important to not confound differences in heights or diameters with differences in bud type. If seedling size is confounded with bud type (e.g. Skrøppa, 1991), correlations between bud class and freezing injury may be statistically significant.

Botrytis frequently infects seedlings which have experienced freeze injury (Davis *et al.*, 1942; Pawsey, 1964; Hepting, 1971; Russell, 1990). This was apparent in the artificial freeze study because only frozen seedlings were subsequently infected by *Botrytis*. In general, cambial injury and *Botrytis* infection were greater for *P. taeda* than for *P. elliotii*. With *P. elliotii*, there was a positive correlation between cambial injury and presence of *Botrytis* (Table 3). With this species, fertilisation with potassium chloride increased *Botrytis* on the needles and caused more cambial injury. Since *Botrytis* is more a symptom of needle injury than of cambial injury, some seedlings with no apparent cambial injury had *Botrytis* on the needles. For example, with *P. taeda*, 80 % of the top-pruned seedlings had *Botrytis* while only 17 % exhibited cambial injury. Therefore, presence of *Botrytis* is symptomatic of freeze injury to needles and not necessarily symptomatic of injury to stem tissue.

CONCLUSION

Although the prime factor in acclimating southern pine seedlings to freezes is exposure to low temperature, managers of bare-root nurseries have little control over temperatures. Some nursery practices such as growing seedlings at low seedbed densities can improve freeze tolerance of conifer seedlings. However, results from this and other studies in

Georgia indicate that a fall application of potassium chloride does not improve freeze tolerance. Multiple root wrenchings also does not appear to improve freeze tolerance more than a single undercutting in August. Multiple-top pruning appears to increase freeze tolerance of the stem but not the needles of *P. taeda*. Contrary to popular belief, the presence of a well-formed terminal bud in November is not a reliable indicator of freeze tolerance. Other morphological traits (such as diameter or height) are more related to freeze injury of pine seedlings than the stage of bud development.

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