Root cold hardiness and native distribution of subalpine conifers

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Root and needle cold hardiness were compared in seedlings of subalpine conifers to determine if differences existed among species originating from either cold continental climates or mild maritime climates. *Ahies amabilis* (Dougl.) Carr. and *Tsuga mertensiana* (Bong.) Carr. are exclusively distributed in maritime environments, while *Abies lasiocarpa* (Hook.) Nutt. and *Pinus contorta* Dougl. are more generally distributed in both continental and maritime environments. Because of the differing winter soil conditions of these two climatic types, special emphasis was placed on root cold hardiness. Cold hardiness for root samples, as measured by a decrease in the electrolyte leakage, was much greater for *A. amubilis* and *A. lusiocurpa* than for *P. contortu* and *T. mertensiana* (−1 1.4, −1 1.5, -7.5, and −7.5°C, respectively). Thus, subalpine conifer species distribution was not found to be influenced by root cold hardiness. Root cold hardiness of field-grown seedlings paralleled changes in soil temperature through February. Under constant temperature conditions (3°C) the maximum cold hardiness achieved in 6 weeks was not subsequently maintained in *A. amabilis* and *A. lusiocurpu.* Injury in unhardened roots was coincident with bulk freezing, whereas hardened roots were able to tolerate bulk freezing. Needles had more than three times the level of cold hardiness of roots when measured in December. All species except *P. contorta* reached needle cold hardiness levels below -40°C.

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La resistance au froid des racines et des aiguilles a été comparée chez des semis de conifères subalpins pour verifier s'il existe des differences entre les especes qui viennent de climats continentaux froids et celles qui viennent de climats maritimes doux. *Abies amubilis* (Dougl.) Carr. et *Tsuga mertensianu* (Bong.) Carr. se retrouvent exclusivement en milieu maritime alors que *Abies lasiocarpa* (Hook.) Nutt. et *Pinus contortu* Dougl. sont plus généralement distribués autant en milieu continental que maritime. A` cause des conditions de sol différentes en hiver sous ces deux types de climat, une emphase particulibre a été mise sur la resistance au froid des racines. La resistance au froid des tchantillons de racines, telle que mesurée par une diminution de la perte d'électrolyte, Ctait beaucoup plus élevée chez *A. amabilis* et *A. lusiocurpa* que chez *P. contortu* et *T. mertensianu* (−11,4, −11,5, −7,5 et −7,5°C, respectivement). Par consequent, la distribution des espèces de conifères subalpins n'est pas influencée par la resistance au froid des racines. La resistance au froid des racines de semis cultivts au champs suivait les changements de temperature dans le sol pendant le mois de février. Sous des conditions de temperature constante (3°C), la resistance maximum au froid atteinte après 6 semaines n'était pas subséquemment maintenue chez *A. amubilis* et *A. lusiocurpu.* Les racines non endurcies exposées à un gel global subissaient des dommages, alors que les racines endurcies pouvaient tolérer un gel global. Les aiguilles étaient plus de trois fois plus résistantes au froid que les racines lorsque la resistance au froid Ctait mesurée en décembre. Les aiguilles de toutes les especes, à l'exception de *P. contorta,* ont atteint un degré de resistance au froid inférieur à -40°C.

[Traduit par la redaction]

Introduction

The role of cold temperatures as a limiting factor in species distribution has been described for perennial plants (George et al. 1974; Sakai and Larcher 1987). The emphasis, however, has been almost exclusively on aboveground plant parts. It is also possible that cold hardiness of roots may affect the distribution of perennial species. To our knowledge, this possibility has not been examined. Over 90% of the fine roots of perennials in montane to high-elevation sites are located in the upper IO- 1.5 cm of the soil profile (Vogt *et* al. 1980). Cold injury of roots may limit plant survival in areas where soil temperatures near the surface decrease below freezing.

The two subalpine environments examined in this study present contrasting circumstances in which freezing soil temperatures may differentially affect species survival and productivity. Maritime subalpine environments are characterized by relatively mild conditions, deep, persistent snowpacks, and extensive periods when soils are between 2 and 0°C (Zabowski 1988). Under these conditions tree root growth may occur year-round (Vogt et al. 1980; Keyes 1982). In contrast, continental subalpine climates are much colder (Baker 1944), and prior to snowpack formation, in wind-swept areas or under low snowfall conditions, freezing soil tetnperatures are possible (Hadley and Smith 1987). There is little year-round root growth information available on trees in the continental-subalpine environment, yet typically tree roots do not grow when soils are below 2°C (Lyr and Hoffmann 1967; Teskey and Hinckley 1981) and are, therefore, able to cold harden. Some subalpine tree species are distributed in both continental and maritime environments, while others are only found in the maritime climate. If root cold hardiness is limiting distribution, it should be greater in species capable of survival in the more extreme environment.

Seasonal changes in root cold hardiness may vary among species. The level of hardiness developed in autumn depends upon the exposure temperatures and the inherent cold hardiness maximum of the species (Wildung *et al.* 1973; Mityga and Lanphear 197 1; Alexander and Havis 1980; Smit-Spinks *et al.* 1985). Loss of root cold hardiness in the spring may

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also vary among species. For example, plants adapted to the relatively consistent conditions underneath a deep, persistent snowpack may have a different pattern of dehardening and root growth in spring than those adapted to more variable conditions.

This study examined cold hardiness of four subalpine conifer species, two of which arc restricted to maritime climates, while the other two occur in both maritime and continental environments. To test the hypothesis that root cold hardiness limits distribution of maritime species in continental environments, special emphasis was placed on examining root tissue. Cold hardiness was measured on seedlings grown under field and laboratory preconditioning environments to evaluate relative hardiness among species.

Methods

Plant material

Bare-root seedlings were obtained from the USDA Forest Service Wind River nursery in Carson, Washington, United States, in April 1988. The species used, seed source, and age of material are presented in Table 1. The 20-30 cm tall seedlings were planted in 550 cm^3 (25 cm tall) Deepot tubes (McConkey & Co. Inc., Sumner, Wash., U.S.A.) containing peat moss – diatomaceous earth (1: 1) and grown under greenhouse conditions before moving to preconditioning environments. Light levels were approximately 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at midday with natural day length.

Field preconditioning environments

Seedlings were preconditioned in the field at two locations chosen to represent two different climates. *(i)* The first was a high-elevation (1800-m) field site, which represented a continental climate and was located near Deer Park campground in the Olympic National Park, Washington, United States. Seedlings were put in place on July 22, 1988. This site was chosen because of the lack of snow accumulation, resulting in cold air and soil temperatures similar to continental climatic conditions. *(ii)* The second was a low-elevation (275-m) field site, which represented a mild maritime environment and was located at the University of Washington's Pack Forest, Eatonville, Washington, United States. Seedlings were placed at this site on July 20, 1988. At both locations, seedling containers were placed on the surface of the ground inside of a wooden frame. The spaces inside the frame and surrounding the pots were then filled with the same potting mix in which the seedlings were growing. These precautions were taken to provide insulation around containers and avoid large fluctuations in soil temperature.

Temperatures at the high-elevation site were measured with thermistors and recorded using a two-channel data logger (Data Pod, Omnidata International Inc., Logan, Utah, U.S.A.). Soil temperature was measured in the potting mix surrounding the seedling containers (25 cm) at one location in the center of the wooden frame. Air temperature in the shade was measured 0.5 m above the soil surface. At the low-elevation site it was necessary to obtain temperature values from the Pack Forest weather station, which digitally recorded the output of copper-constantan thermocouples to a microcomputer disk. Soil temperature was measured at a depth of 5 cm, and air temperature in the shade was measured at 12 m. The thermocouples and the seedling material, located 50 m apart, were exposed to virtually the same environments.

Seedlings were collected from both locations monthly, except January, February, and March at the high-elevation site because of inaccessibility. Cavities created by seedling removal were filled with additional potting soil. Owing to limitations in tissue sample preparation, two seedlings per species were taken from each site at each collection time. Preparation of a number of subsamples was necessary to obtain replicate values for a range of test temperatures. This requirement along with our objective of testing a number of species limited the number of replicate plants that could be processed. Since the objectives were to identify large interspecies differences, the limited number of seedlings sampled per species was considered adequate. Small differences in cold hardiness or indistinct species responses would nullify the hypothesis.

Controlled preconditioning environments

Seedlings were transferred from the greenhouse to a controlled-environment chamber (Environmental Growth Chambers, Chagrin Falls, Ohio, U.S.A.) on September 12, 1988, and grown for 6 weeks at 15°C constant temperature and a photosynthetic photon flux density of 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 10-h photoperiod. Two seedlings from each species were sampled to determine root and shoot cold hardiness at the end of the 6-week period, and the remaining seedlings were transferred to a cold room at 3°C with supplemental lighting at a photosynthetic photon flux density of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a 10-h photoperiod. Hardiness of two seedlings of each species was measured after 6 weeks and four seedlings of each species after 8 months under these conditions.

Cold hardiness evaluation

Seedlings were collected from the field sites the day before tissue preparation. Those seedlings taken from the high-elevation site were kept overnight in an insulated cooler and packed with snow. Seedlings taken from the low-elevation site were at temperatures similar to those at the low-elevation site. Seedlings preconditioned in controlled environments were prepared the day of removal. Tissue preparation was completed in 1 day, and prepared samples were kept moist at 3°C overnight before testing.

Low-temperature injury was assessed using roots <2 mm in diameter and cut to 3 cm in length for all species; whole fir and hemlock needles were used, while pine needles were cut into 3 cm lengths. All needles selected were grown in the 1988 season. Twenty-one subsamples of needles (10 each) and roots (150 ± 50 (SD) mg each) were prepared from each seedling. Three subsamples were exposed to each of seven test temperatures including an unfrozen control (2°C). Tissue subsamples were bundled using color-coded thread, dusted with synthetic fluorophlogopite (60 mesh, Mykroy Ceramics, Clifton, N.J., U.S.A.) to nucleate ice, wrapped in parafilm, and placed in a stoppered test tube (25 x 125 mm) with a thermocouple (30-gauge copper–constantan). Each tube was held at 2°C for 15 min in a low-temperature circulating bath (LT50, Neslab Instruments, Inc., Portsmouth, N.H., U.S.A.). The control samples were then removed at 2°C before the temperature of the bath was decreased at 12°C $\cdot$ hr^{-1}. After reaching the appropriate test temperature, tubes were removed and thawed at 3°C overnight. Subsamples were placed in individual test tubes (16 x 125 mm) containing 8 mL of either 0.4 mM $CaSO_4$ for roots or distilled water for needles. The tissue was infiltrated under vacuum for 1 h and shaken at room temperature overnight for 16-20 h. Electrical conductivity of the bathing solution was measured (model 212 conductivity meter, Wescan Instruments, Inc., Santa Clara, Calif., U.S.A.) to determine electrolyte leakage from the tissue (EC_{injury}). For determination of maximum electrolyte leakage (EC_{total}), conductivity was measured after samples were autoclaved at 120°C and 1.5 MPa pressure for 3 min, cooled, and shaken for 4 h. This cooling period was long enough for tissue to reach room temperature and give equilibrated conductivity values.

Index of injury was calculated according to Colombo *et* al. (1984). Briefly, electrical conductivities were first corrected for the conductivity of $CaSO_4$ and any evaporation during autoclaving by using blanks. The index of injury was calculated from relative conductivity (the ratio EC_{injury} to EC_{total}) and adjusted for the EC of nonfrozen samples. Index of injury was plotted as a function of the treatment temperature (Fig. 1). The temperature with an index of injury of 50% was determined graphically by interpolation and termed the T_{50} value. Figure 1 shows, for *Pinus contorta,* that the T_{50} of roots sampled in December occurred at a lower temperature than in September. The other species tested showed the same results (data not shown). In an initial experiment, T_{50} corresponded to visual observation of injury in needle and roots larger than 2 mm in diameter. Visual observation

TABLE 1. Source of experimental seedling material used

	U.S. national forest	Lat. and long.	Elev. (m)	Age-class
Abies amabilis (Dougl.) Carr. (Pacific silver fir)	Olympic	47°20′N 123°30′W	900	4-o
Abies lasiocarpa (Hook.) Nutt. (subalpine fir)	Gifford Pinchot	46°00′N 121°45′W	1500	4-o
Pinus contorta Dougl. (iodgepole pine)				
Western	Gifford Pinchot	46°00′N 121°45′W	1200	2-1
Eastern	Colville	48°45′N 118°30′W	1000	2-o
Tsuga mertensiana (Bong.) Carr. (mountain hemlock)	Gifford Pinchot	46°30′N 121°50′W	900	3-1

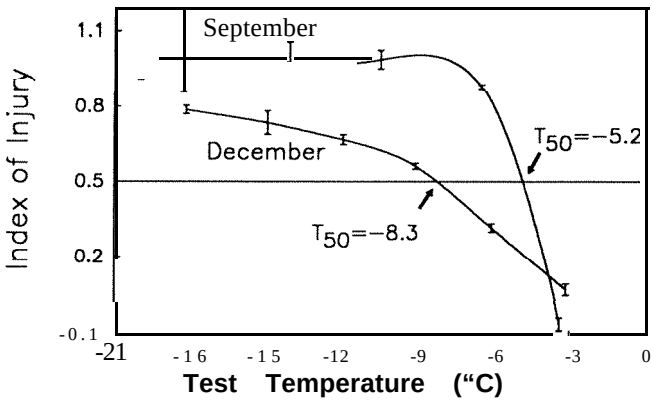

FIG. 1. Index of injury as a function of the treatment for *Pinus contortu* root tissue from seedlings grown at 275 m elevation and sampled in September and December. The 50% injury point (T_{50}) was determined graphically. Each point is the mean and standard error of three subsamples.

of injury in roots less than 2 mm diameter was not possible. Even when color change and cortical pealing associated with injury was observed in 2 mm diameter roots, no change could be observed in smaller roots.

Differential thermal analysis

Differential thermal analysis was performed on tissue from the same seedlings examined for cold hardiness. Two subsamples (300 ± 10 mg) of needles and roots were prepared from each seedling. Subsamples were dusted with synthetic fluorophlogopite and wrapped in parafilm with a thermocouple (30-gauge copper-constantan). The wrapped tissue was placed in the circulating water bath inside a stoppered test tube (100 x 16 mm). Dry references (one per four tissue samples) were prepared by wrapping the thermocouple in dry tissue paper and parafilm before inserting into the test tube. The bath temperature was lowered at a rate of 14°C·h^{-1}. Temperatures were recorded by computer (NEC PC-8300, Nippon Electric Co. Ltd., Tokyo, Japan) every minute. The total number of samples prepared on a sampling date (32) were run simultaneously. The temperature of the dry reference was subtracted from the sample temperature. The resulting difference was plotted against the dry reference temperature, showing a positive peak when the exothermic reaction of freezing occurred.

Statistical analysis

A two-way factorial analysis of variance was used to compare among the four species and three preconditioning environments (3°C controlled environment and both high- and low-elevation field sites). All data used in the analysis were collected in December. There were two replicate plants for each species-environment combination. Species means were separated using Student-Newman-Keuis test.

Results and discussion

Differences among species

Distinct differences in root cold hardiness were detected among the species tested (Fig. 2A) in December. The data presented in Fig. 2 are only from the December measurement for two reasons: *(i)* differences among species and levels of cold hardiness were greatest in December and *(ii)* material grown under controlled-environment conditions were measured in December and could, therefore, be compared with measurements taken from material grown at high- and low-elevation sites. Thus, the statistical analysis allowed comparison, not only of species differences, but also environmental preconditioning differences.

The most important finding regarding the control of distribution by root cold hardiness concerned relative hardiness levels among species. *Abies lasiocarpa* and *Abies amabilis* achieved the greatest levels of root cold hardiness; *Tsuga mertensiana* and *P. contorta* roots were least cold hardy. The distribution into or exclusion of species from harsh continental climatic conditions was not correlated with root cold hardiness found in this test. For example, *A. amabilis* is found exclusively in maritime regions and was not expected to have very great root cold hardiness, yet it was among the hardiest of those tested when grown under our conditions. In contrast, *P. contorta* was expected to have greater root cold hardiness because of its continental distribution, yet roots of this species were the least cold hardy. These data suggest that root cold hardiness is not a primary determinant in distribution patterns. It must be acknowledged that the particular seed source of the species chosen may have affected the results. Nevertheless, the hypothesis questioned the species' ability to withstand cold soil temperatures, regardless of the adaptation of the family chosen.

TABLE 2. Temperature summaries (°C) for 10 days prior to sampling in December from the low- (275 m) and high- (1800 m) elevation sites

	Site elevation	
	275 m	1800 m
Air temperature		
Average	4.3	1.9
SD	3.6	3.5
Minimum	-2.7	-8.0
Maximum	14.4	9.5
Soil temperature		
Average	4.9	0.6
SD	2.7	0.2
Minimum	1.9	0.5
Maximum	9.5	1.5

NOTE: The minimum, maximum, and standard deviations (SD) are for average daily temperatures.

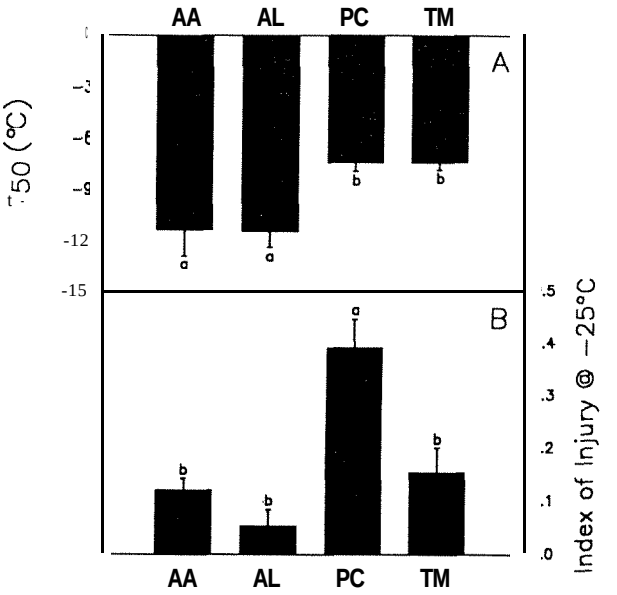

FIG. 2. Cold hardiness values for each of four species examined: *Abies amabilis* (AA), *Abies lasiocarpa* (AL), *Pinus contorta* (PC), and *Tsuga mertensiana* (TM). (A) Root tissue T_{50}. Differences among species (i.e., means with different letters) were significant at the 0.012 probability level. (B) Minimum needle tissue index of injury for the -25°C test temperature (P = 0.001). Measurements were taken in December, and each bar represents the mean of the three growing environments (controlled-environment chamber and high- and low-elevation field sites) for which cold hardiness was not significantly different (see text). Each bar represents the mean and standard error of six seedlings.

Foliage tolerated much lower temperatures than roots. This finding is consistent with other reports showing that roots, especially those smaller than 2 mm in diameter, are much less cold hardy than shoots (Wildung et *al.* 1973; Wiest and Steponkus 1976; Alexander and Havis 1980; Smit-Spinks et *al.* 1985). When data from the four study species grown at the low-elevation site were averaged, the needle T_{50} value in December was below −33°C, while the mean root T_{50} value was −9.6°C. The average increase in hardiness for all species from August 31 to December 21 was 2.4 times for foliage, but only 1.4 times for roots.

Distinct species differences were also found for needle cold hardiness (Fig. 2B) in December. Cold hardiness of needles exceeded the minimum test temperature used (−40°C), especially for *A. lasiocarpa* (see Fig. 4). The temperature of 50% index of injury, T_{50}, was not reached and, therefore, could not be used to compare cold hardiness of species. Alternately, index of injury values at a given test temperature (−25°C) were used for the response variable in the analysis of variance for needles. Since differences in index of injury among species were large at -25°C and this test temperature was included in each of the seasonal measurements, the -25°C test temperature was chosen for comparison of index of injury values.

Pinus contorta developed the least cold hardy needles (Fig. 2B). Individuals from this species have previously been shown to differ widely in cold hardiness depending on elevational and geographical origin (Rehfeldt 1980). Jonsson (1985) exposed the shoots of intact seedlings from several sources of *P. contorta* to -10°C and found the families from Montana (945 m elevation) to be intolerant, while survival was 100% in families from northern British Columbia. Becwar et *al.* (1981) observed no stem or needle injury at -60°C in a high-elevation Colorado source. Becwar *et al.* (198 1) also examined *A. lasiocarpa* for cold hardiness and found stems of plants from a Colorado source to be tolerant to -40°C. Needle cold hardiness of *Tsuga mertensiana* could not be distinguished from that of the two *Abies* species in our experiments (Fig. 2B). Needle cold hardiness exceeded the minimum test temperature used (−40°C), especially when grown at the high-elevation site (data not shown). Gordon-Kamn (1980) also found that *T. mertensiana* and *A. amabilis* had similar levels of cold hardiness of about -50 and −60°C, respectively.

The cold hardiness of both roots and needles was unaffected by preconditioning treatment as measured in December. From the analysis of variance, the effect of preconditioning on root T_{50} had a probability level of 0.949, and the effect of preconditioning on needle index of injury at -25°C had a probability level of 0.440. Mean air and soil temperatures for the high-elevation field site were less than those for the low-elevation site during the 10 days prior to December samplings, but there was some overlap in temperature and both were near the 3°C temperature of the controlled environment (Table 2). Although preconditioning temperature differences of 10–15°C can elicit an increase in needle and root cold hardiness (Fuchigami *et al.* 1982; Smit-Spinks *et al.* 1985), the variance in temperature among our preconditioning environments had no apparent effect on root or needle cold hardiness in any of the species tested here.

Seasonal pattern of cold hardiness

Annually, shoot growth and maximum cold hardiness occur during separate seasons (Fuchigami *et al.* 1982). Cold hardiness begins to increase in late summer after shoot growth has ceased (Glerum 1973); the process being initiated by a shortening photoperiod (Weiser 1970; van den Driessche 1969). As minimum temperatures drop below freezing, the rate of cold hardiness development increases rapidly to a peak in midwinter (Weiser 1970; Glerum 1973). By the time of bud

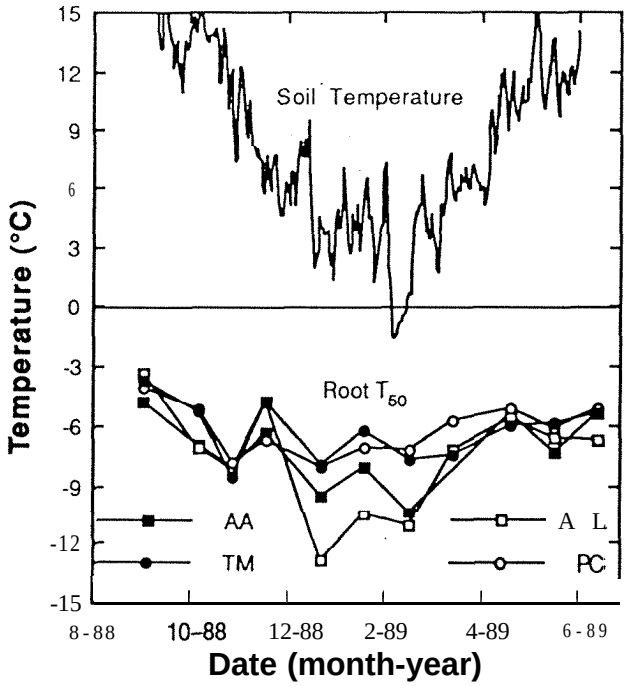

FIG. 3. Seasonal progression of soil temperature and root tissue T_{50} of seedlings growing at the 275 m elevation site. Species codes are the same as in Fig. 2. Each point is the mean of two seedlings. The average standard error of the mean for all species and measurement times was 0.429°C.

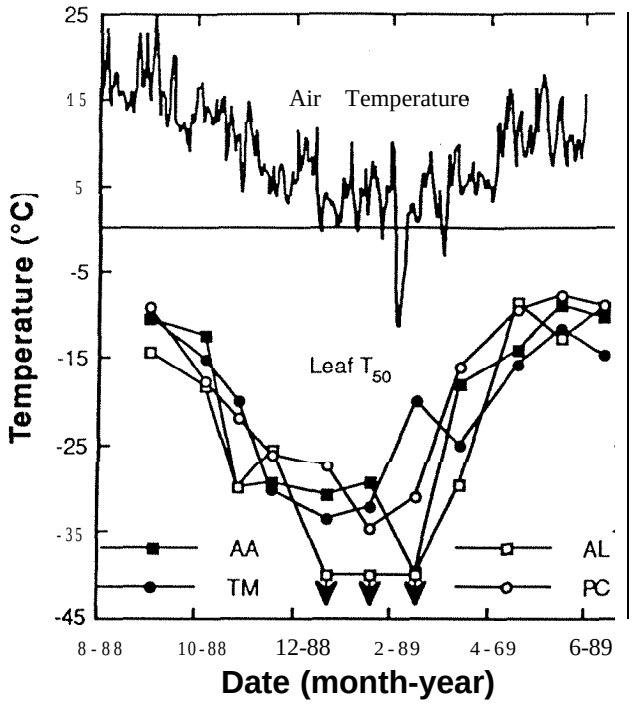

FIG. 4. Seasonal progression of air temperature and needle tissue T_{50} from seedlings growing at the 275 m elevation site. Species codes are the same as in Fig. 2. Arrows indicate that cold hardiness exceeded the minimum test temperature of -40°C. Each point is the mean of two seedlings. The average standard error of the mean for all species and measurement dates was 1.57°C.

break, cold hardiness is less than half of the winter maximum and at the time of shoot elongation, cold hardiness is at a seasonal low (Glerum 1973).

The dynamics of root growth and cold hardiness are somewhat different from those of the shoot, but as in shoots, active growth and a high degree of cold hardiness do not occur simultaneously (Smit-Spinks et *al.* 1985; S. Colombo, personal communication). After shoot growth ceases before the end of summer, roots show one of two annual growth peaks in autumn (Krueger and Trappe 1967; Ritchie and Dunlap 1980). Following this autumn peak, as temperatures decline, root growth ceases and cold hardiness increases to a maximum during the coldest weather (Mityga and Lanphear 1971; Wildung et al. 1973; Alexander and Havis 1980). In early spring the larger of the two annual peaks in root growth occurs just prior to shoot growth in sapling and mature trees, while in seedlings, the peak in root growth immediately follows shoot growth (Ritchie and Dunlap 1980; Teskey and Hinckley 1981).

Measurements of cold hardiness collected on the four subalpine conifer species examined followed the aforementioned seasonal patterns. Figures 3 and 4 show that cold hardiness increased (T_{50} declined) in both roots and needles collected from the low-elevation site as temperatures decreased between September and December. Similar patterns have been reported in coniferous shoots (Maronek and Flint 1974; Glerum 1973; Colombo et *al.* 1984; Burr et al. 1989) and roots (Mityga and Lanphear 1971; Smit-Spinks *et al. 1985;* S. Colombo, personal communication). Identical autumn cold acclimation trends were found for seedlings located at the high-elevation site.

During the later part of winter, trends indicated that cold hardiness of *P. contorta* and *T. mertensiana* foliage no longer changed in response to changes in air temperatures, and this occurred prior to changing sensitivity of root cold hardiness. Between January 3 1 and February 7, a period of unseasonably cold weather occurred causing soil temperatures at the low-elevation site to fall below freezing for 10 days (Figs. 3 and 4). Despite the cold temperatures, needle cold hardiness for *P. contorta* and *T. mertensiana* decreased. In contrast, root cold hardiness in all four species and needle cold hardiness of A. *amabilis* foliage increased in response to the period of cold temperatures. Roots increased in hardiness between January and February at an average rate of $0.051°C \cdot day^{-1}$, which was similar to the average rate in autumn of $0.050°C \cdot day^{-1}$ (September to December). Thus it appears that by early February needle cold hardening of *P. contorta* and *T. mertensiana* no longer increased in response to cold temperatures, but cold hardiness in *A. amabilis* did, as did root cold hardiness in all four species. By early March, cold hardiness of these latter tissues were also insensitive to cold periods, suggesting that they had lost their ability to respond to temperature stimuli by this time.

Controlled-environment studies were conducted to decrease variation created by natural fluctuations in temperature. The short-term response to a decrease in temperature from 15 to 3°C is evident (Fig. 5), and as mentioned above, the level of hardiness achieved was statistically equal to that found for field-preconditioned material. Under constant cool temperatures all tissues measured, except *T. mertensiana* roots, tended to deharden over time (Fig. 5), apparently not

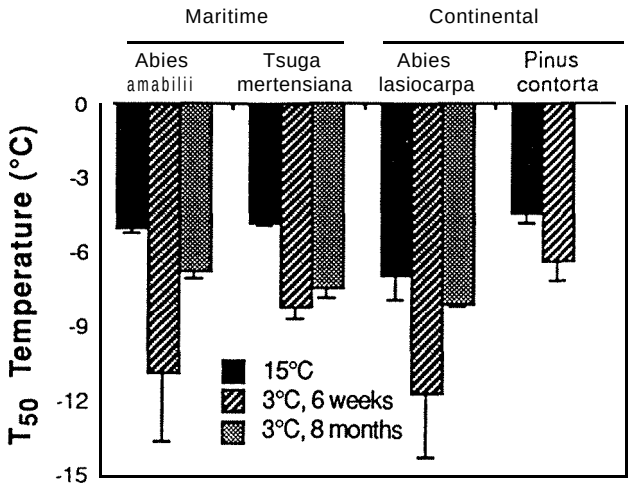

FIG. 5. Root tissue T_{50} for seedlings grown in a controlled-environment chamber at either 15 or 3°C for 6 weeks (n = 2 seedlings) or at 3°C for 8 months (n = 4 seedlings).

able to sustain the level of hardiness achieved in the first 6 weeks at 3°C. A similar response has been reported for agronomic crops and was attributed to preparation for spring growth (Levitt 1980).

During winter in maritime subalpine environments, roots of conifers are subject to nearly constant temperatures of 2°C. Such conditions are similar to those provided in the controlled-environment experiment. It is known that fine-root biomass (Vogt *et al.* 1980) and root growth in rhizotron windows (Keyes 1982) increase for A. *amabilis* early in the spring under snowpack despite low soil temperatures. Root cold hardiness appears to be inversely correlated with root growth in other conifers (Smit-Spinks *et al.* 1985; S. Colombo, personal communication). In the present study, a decline in root cold hardiness occurred under constant low temperature conditions, during the period when roots have been observed to begin growing in the natural environment. Perhaps when an appropriate quantity of some factor such as hours of chilling is met, the roots deharden and begin to grow despite continued low temperatures.

Mechanisms of cold tolerance

Differential thermal analysis can be used to' identify exotherms resulting from the heat of fusion liberated during freezing (Burke *et al.* 1976). High-temperature exotherms can be viewed as the freezing of intercellular water in tissue (Quamme *et al.* 1972; Burke *et al.* 1976) and can have one sharp peak or multiple peaks. Figure 6 shows the exotherm patterns for roots of *T. mertensiana.* These patterns are similar to those found for roots of the other species tested. The mean temperature at which the roots of all species froze at the low-elevation site from September to June was -4.9 ± 0.6°C. The consistent temperature of intercellular freezing was expected, and resulted from the use of a synthetic ice nucleator, fluorophlogopite (Rajashekar *et al.* 1983; Tinus *et al.* 1985). Use of ice nucleators in laboratory tests is important for avoidance of unnaturally large amounts of injury caused by sudden freezing of water supercooled to artificially low temperatures (Burke *et al. 1976).*

Freezing injury to root tissue, as indicated by T_{50} in the four species examined, occurred at the initiation point of the high-temperature exotherm in unhardened tissue and near the end

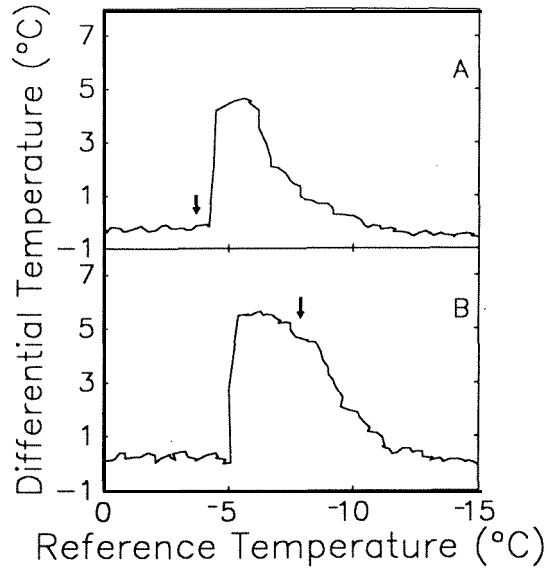

FIG. 6. Freezing events as measured by differential thermal analysis for *Tsuga mertensiana* seedlings growing at the tow-elevation site. (A) Unhardened roots measured in September. (B) Hardened roots in December. The arrows indicate the T_{50} point of injury.

of this exotherm in hardened tissue (Figs. 6A and 6B). These results suggest that unhardened root tissue is injured during freezing of intercellular water, whereas hardened roots appear to tolerate freezing of intercellular water.

Conclusions

Differences in root cold hardiness were found among the different species, but these differences did not reflect the patterns of species distribution. *Abies amabilis* and *A. lasiocarpa* had equal root cold hardiness, yet *A. amabilis* occurs only in mild wet maritime climates. *Pinus contorta* is distributed in continental environments where soil temperatures drop well below freezing, yet its roots were the least cold hardy of those tested. Based on these results the hypothesis that root cold hardiness influences distribution was rejected.

Root cold hardiness increased during autumn along with declining temperature; however, differences in the maximum level of root cold hardiness among preconditioning environments could not be detected when measured in December.

The maximum level of root cold hardiness was not sustained for *A. amabilis* and *A. lasiocarpa* even when seedlings were maintained under constant 3°C conditions. In contrast, *T. mertensiana* was able to maintain maximum cold hardiness for up to 8 months.

Intercellular freezing in roots occurred at the same temperature regardless of the species tested, the season of the year, or the preconditioning environment. Injury in unhardened roots occurred at the initiation of freezing, but hardened roots were injured after freezing.

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