Cold-Hardiness Testing of Conifer Seedlings[1]

Karen E. Burr, Stephen J. Wallner, and Richard W. Tinus[2]

Abstract.--This paper briefly describes the results of preliminary experiments designed to test four objective methods of rapidly predicting cold hardiness of conifer seedlings; differential thermal analysis, ethylene evolution, and freeze-induced and heat-induced electrolyte leakage.

INTRODUCTION

We are testing four objective methods of rapidly predicting cold hardiness of conifer seedlings; differential thermal analysis, ethylene evolution, and freeze-induced and heat-induced electrolyte leakage. This information will be used as a research tool to optimize hardening regimes and as a management tool to reduce losses associated with the timing of removal of seedlings from the greenhouse, cold storage, and outplanting.

DIFFERENTIAL THERMAL ANALYSIS (DTA)

DTA of buds is one approach for those species that supercool, such as the spruces and Douglas-fir. The cold hardiness of these species is related to their capacity for supercooling, and the extent of that supercooling is measured by DTA.

The DTA profile of a cold hardy bud that supercools (fig. 1) has two peaks or exotherms which are formed when heat is released by the freezing of water within the bud. The first exotherm represents freezing of extracellular water which generally causes no injury to the bud. The, low temperature exotherm (LTE) represents freezing of supercooled intracellular water and is associated with lethal injury (Sakai 1978). The DTA profile of a cold hardy bud from a species that does not supercool, such as any of the pines, has a first exotherm but no LTE and is thus of no diagnostic value. The temperatures at which LTE's occur in buds that supercool are well correlated with bud acclimation and deacclimation to cold (fig. 2) and with concurrent changes in whole plant condition (fig. 3).

Concerns about bud sampling for DTA are eased by the relatively low variability of LTE temperatures among buds of individual cold hardy trees (figs. 4 and 5). However, extremely small buds do not provide reliable DTA data (fig. 6). Minimum fresh weight guidelines were set at 2.8 mg for Douglas-fir buds and 1.2 mg for Engelmann spruce buds. Position on the tree had no significant effect on LTE temperature for buds of adequate size on fully cold hardy trees.

Three other approaches for predicting cold hardiness are under consideration, since pines do not supercool and buds are not always present on those species that do.

ETHYLENE EVOLUTION

A seasonal pattern of ethylene production has been observed in white pine (Seibel and Fuchigami 1978). Ethylene levels were highest in spring

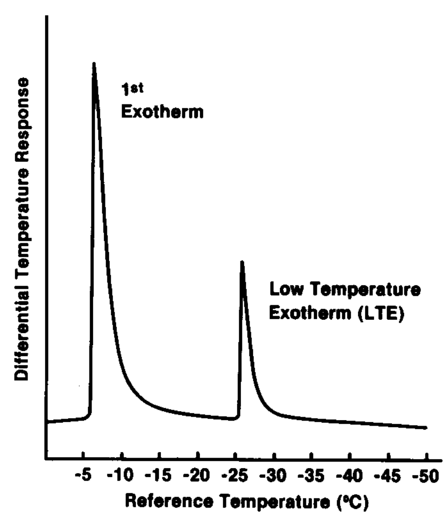

Figure 1.--DTA profile of a cold hardy bud that supercools.

---

[1]Poster presented at the Intermountain Nurseryman's Association Meeting. [Fort Collins, Colo. August 13-15, 1985].

[2]The authors are, respectively: Graduate Research Assistant, Department of Horticulture, Colorado State University, Fort Collins, Colo.; Professor of Plant Stress Physiology, Department of Horticulture, Colorado State University, Fort Collins, Colo.; Project Leader, USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Forestry Sciences Lab., Flagstaff, Ariz.

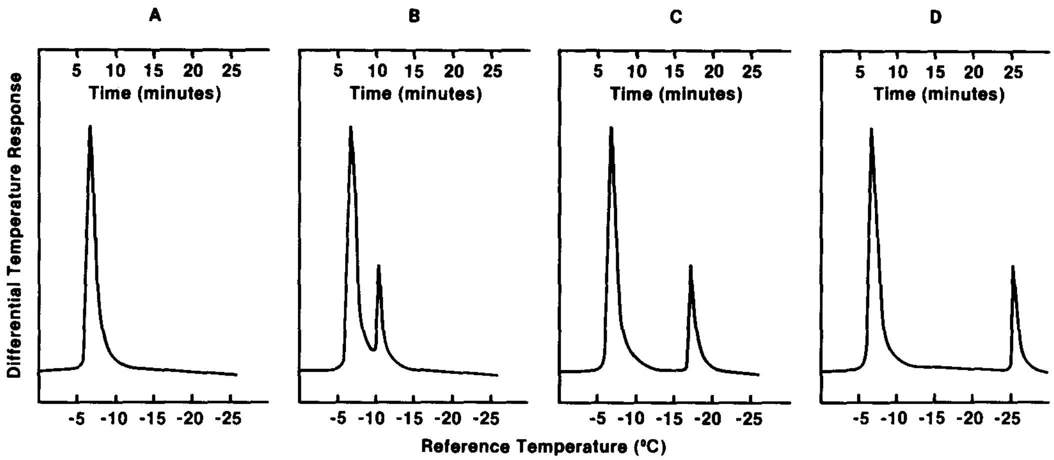

Figure 2.--Acclimation of buds that supercool, as indicated by LTE temperature. The reverse occurs with deacclimation. A. Non-acclimated. The single exotherm represents freezing of all tissue water which results in injury. B. Early acclimation. The capacity for supercooling has developed. C. Moderately cold hardy. LTE temperatures become progressively lower with increasing cold hardiness. D. Fully cold hardy. Bud LTE's at -25° C commonly occur in fully cold hardy Engelmann spruce grown in Colorado.

during active growth, declined to low levels in fall with vegetative maturity, and were not detectable during winter. Preliminary investigation using gas chromatography suggests this pattern may also exist in ponderosa pine, but it is not likely to occur in Douglas-fir or Engelmann spruce (fig. 7).

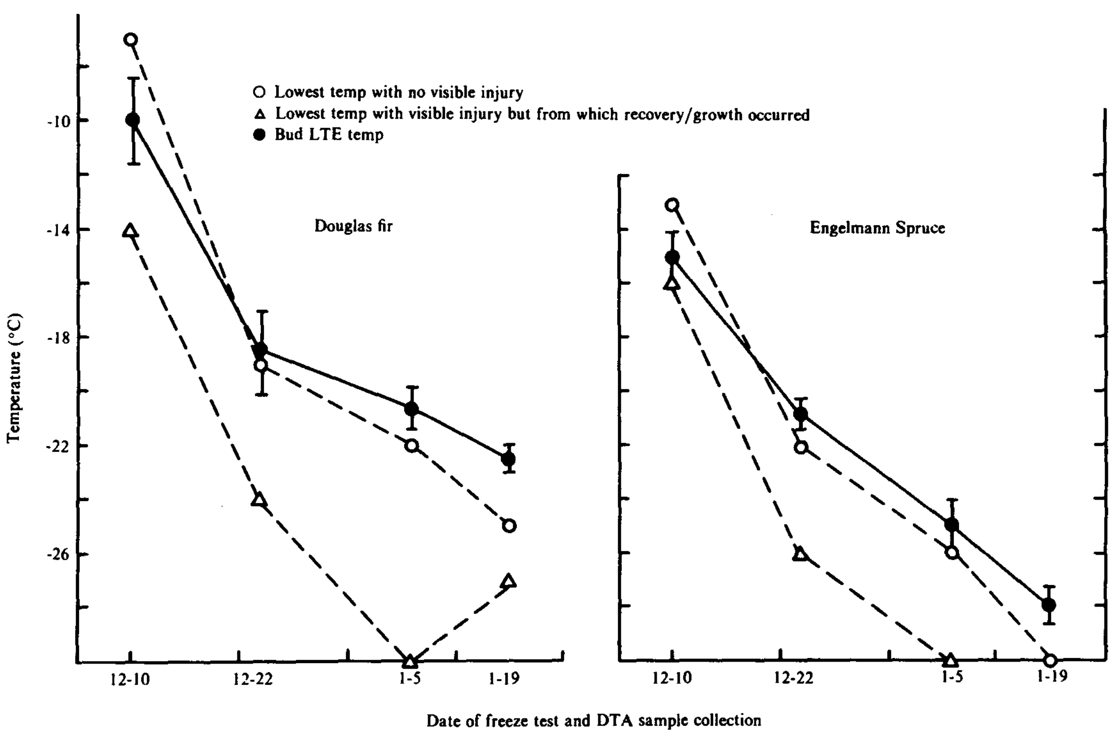

Figure 3.--Comparison of bud LTE temperatures and the results of whole plant freezing tests (Tinus et al. 1985).

ELECTROLYTE LEAKAGE

Plant hardiness with respect to temperature stresses which, when injurious, cause disruption of cell membranes and the subsequent leakage of cell contents, can be quantified by measuring the amount of electrolytes which leak from the plant tissue following exposure to a given stress. Electrolyte leakage is reported as percent index of injury,

calculated by the formula

$$1 - \frac{1-(T_1/T_2)}{1-(C_1/C_2)} \times 100$$

where T_1 and T are the conductivity of the treatment solution before and after boiling, respectively, and C_1 and C_2 are the conductivity of the control solution before and after boiling, respectively (Flint et al. 1967). A lower percent index of injury indicates greater hardiness.

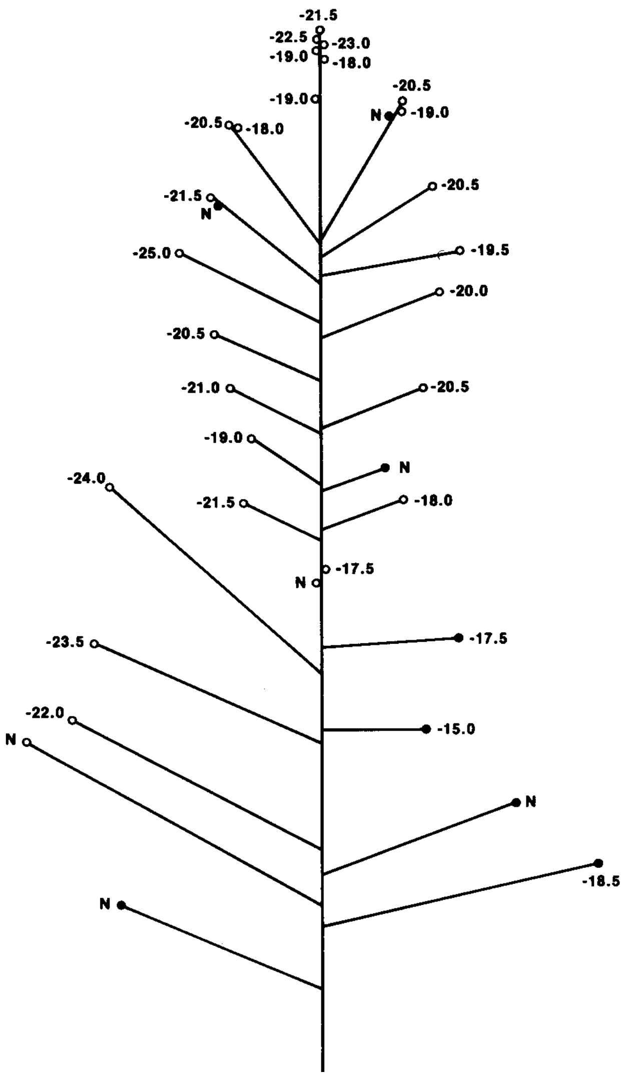

Figure 4.--Scale drawing of a fully cold hardy, 2-year-old, container grown Douglas-fir seedling 33.2 cm tall. LTE temperatures are in °C for each bud. Mean LTE temperature, ± 1 standard deviation, is -20±1.5° C. 'N' indicates no reliable LTE detected.

- • Bud fresh weight <2.8 mg
- o Bud fresh weight >2.8 mg

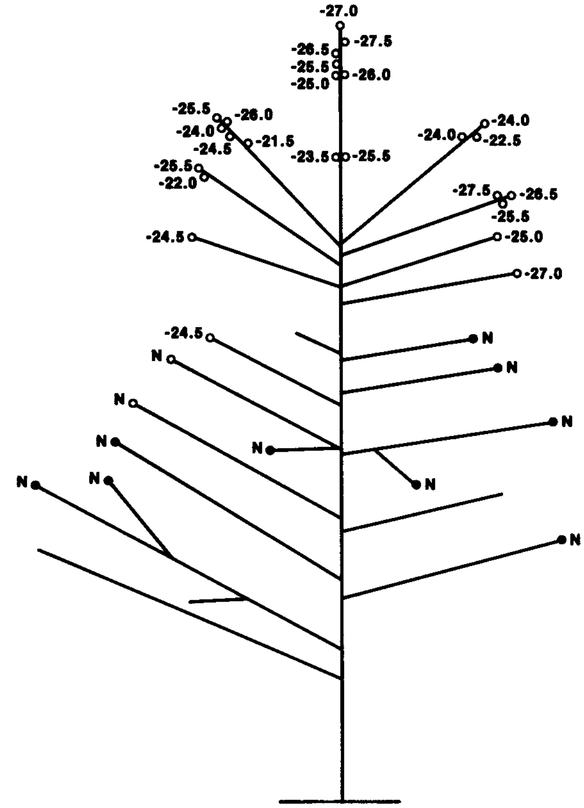

Figure 5.--Scale drawing of a fully cold hardy, 2-year-old, container grown Engelmann spruce seedling 24.5 cm tall. LTE temperatures are in °C for each bud. Mean LTE temperature, ± 1 standard deviation, is -25±2.8° C. 'N' indicates no reliable LTE detected.

- • Bud fresh weight <1.2 mg
- o Bud fresh weight >1.2 mg

Freeze Induced

Electrolyte leakage from Douglas-fir needle tissue following in vitro freezing stress was measured on samples taken at 16 intervals throughout a 152-day, growth chamber controlled, cold hardening and deacclimation regime to produce the series of curves in figure 8. Seven test temperatures were selected at each interval to produce each individual curve. Precise testing procedures enable the detection of fairly small changes in cold hardiness over time. Similar series of curves have been produced for ponderosa pine and Engelmann spruce.

Heat Induced

The changes which confer cold hardiness also result in greater heat tolerance for certain species (Levitt 1980). Electrolyte leakage from needle tissue following in vitro heat stress was measured to assess the possibility of this occurring in conifers. Results for ponderosa pine were opposite those for Douglas-fir and Engelmann spruce (fig. 9).

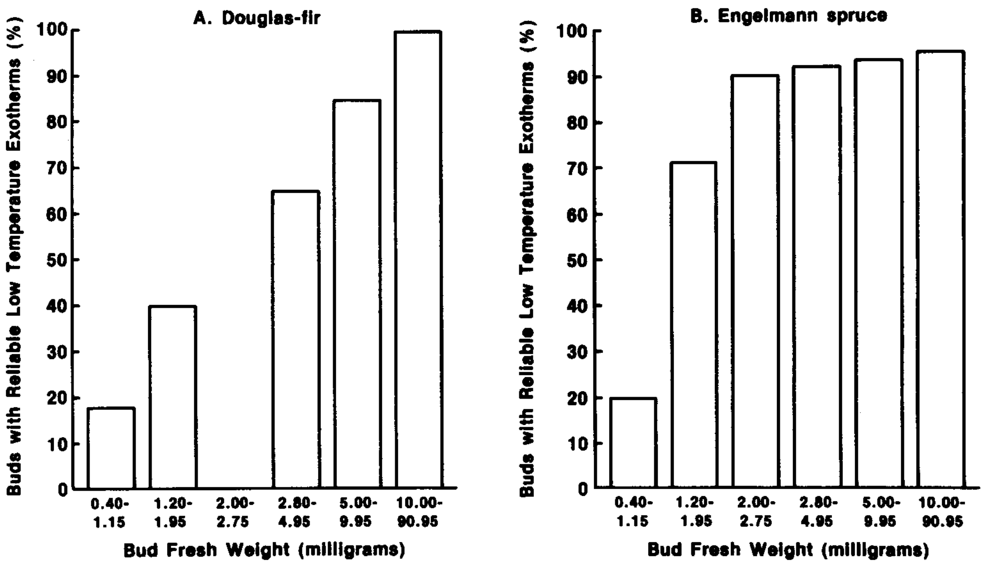

Figure 6.--Percentage of buds from cold hardy seedlings with reliable LTE's by bud fresh weight.

FUTURE RESEARCH

Extensive whole plant freezing tests are being conducted to calibrate each of the four quick tissue tests and to determine how well these tests predict cold hardiness. These results are also being compared with measurements of dormancy and root growth capacity.

LITERATURE CITED

Flint, H. L., B. R. Boyce, and D. J. Beattie. 1967. Index of injury--A useful expression of freezing injury to plant tissues as determined by the electrolytic method. Canadian Journal of Plant Science 47:229-230.

Levitt, J. 1980. Responses of plants to environmental stresses. Volume 1. Chilling, freezing and high temperature stresses. Academic Press, N.Y.

Sakai, A. 1978. Low temperature exotherms of winter buds of hardy conifers. Plant and Cell Physiology 19:1439-1446.

Seibel, J., and L. Fuchigami. 1978. Ethylene production as an indicator of seasonal development in red-osier dogwood. Journal of the American Society for Horticultural Science 103(6):739-741.

Tinus, R. W., J. E. Bourque, and S. J. Wallner. 1985. Estimation of cold hardiness of Douglas-fir and Engelmann spruce seedlings by differential thermal analysis of buds. Annals of Applied Biology 106:393-397.

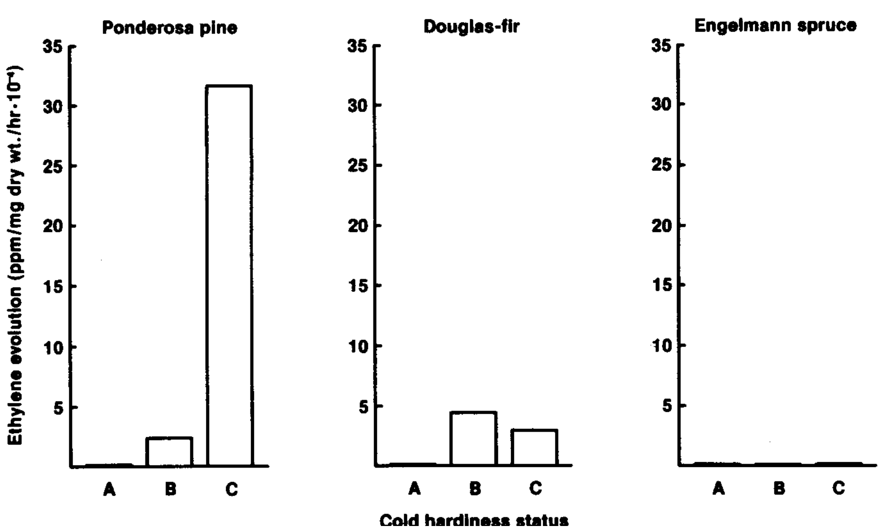

Figure 7.--Ethylene evolution from needle samples of three species at varying levels of cold hardiness: (A) fully cold hardy, (B) early cold deacclimation, (C) actively growing.

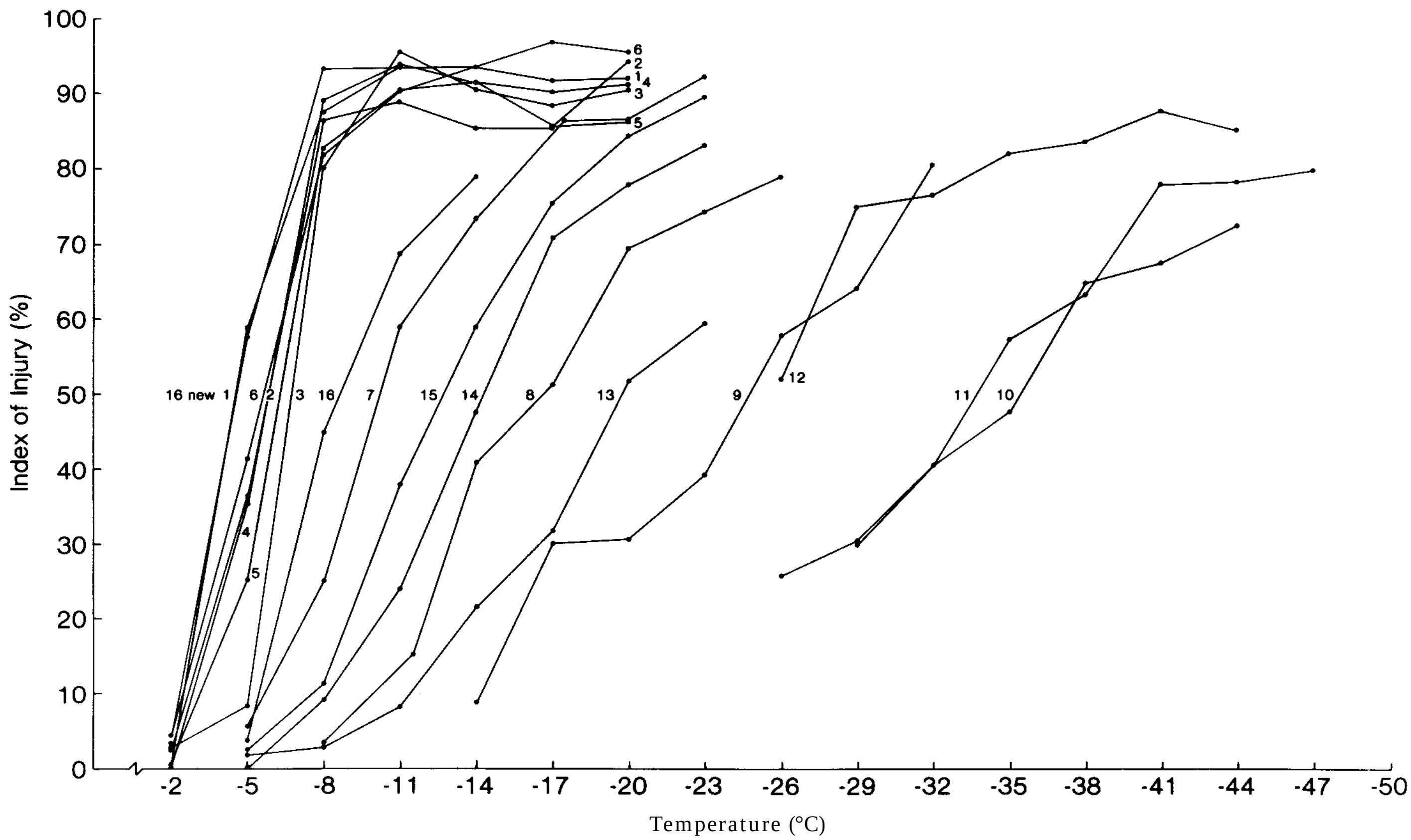

Figure 8.--Index of injury following in vitro freezing stress of Douglas-fir needles sampled at intervals throughout a cold hardening and deacclimating regime. The hardening portion of the regime began with actively growing seedlings represented by curve 1 and ended 111 days later with fully cold hardy seedlings represented by curve 11. The deacclimation period began on the 112th day and includes curves 12 through 16. The previous season's growth and the new growth were both tested on the completely deacclimated, actively growing seedlings on the 152nd day and are represented by curves 16 and 16 new, respectively.

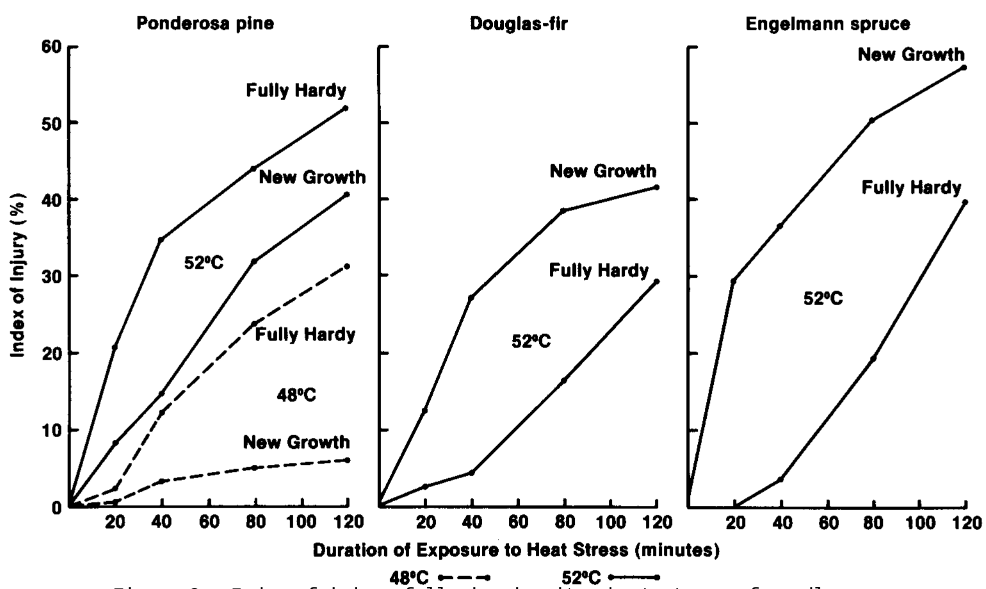

Figure 9.--Index of injury following in vitro heat stress of needle samples from actively growing and fully cold hardy seedlings of three species.