# COLD HARDINESS TESTING OF CONTAINER SEEDLINGS

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Successful greenhouse production of containerized forest tree seedlings requires high rates of transplant survival. Low temperature is one of the environmental stresses that the seedling must be able to tolerate, so cold acclimation is an important aspect of the production cycle. Unfortunately, there are currently no convenient methods for determining when a particular lot of seedlings has attained the level of hardiness that will allow survival. The rate and extent of hardening vary between species and probably between some seed sources of the same species; certain production variables also influence the hardening process. Many nursery management decisions are made more difficult by this uncertain status of tree seedlings. Researchers and nurserymen need a rapid, convenient means of accurately measuring the cold hardiness of nursery-grown tree seedlings.

We are interested in the possibility that differential thermal analysis (DTA) may provide a convenient, rapid estimate of tree seedling cold hardiness and dormancy. DTA is a method of measuring patterns of freezing in plant tissue. To understand its potential value as a measure of hardiness, it is necessary to briefly review relevant mechanisms of cold resistance.

## COLD HARDINESS

The various ways that plants resist the effects of cold stress have recently been reviewed by Burke et al. (1976) and described in more detail by Levitt (1980). To avoid injury, a plant must avoid the freezing of intracellular water in crucial tissues, since such freezing is fatal to the affected cell. North American tree species avoid the freezing of water in living cells by

1. supercooling to temperatures as low as -40°C (water in xylem and extracellular spaces does freeze), or

2. movement of freezable water from the cell to extracellular ice (such plants tolerate extreme cellular dehydration and survive very low temperatures).

As trees harden, their capacity to supercool and/or to withstand the presence of extracellular ice and cell dehydration increases. Although cell water in some tissues may supercool, other tissues of the same plant exhibit the second hardiness mechanism. Tree tissues that are known to supercool include buds and xylem ray parenchyma.

Hardiness limits are imposed by the killing point of the most sensitive tissue. In supercooling species, hardiness limits are -40°C (or slightly lower), since this is the theoretical limit for supercooling (of pure water). When water crystallizes after extensive supercooling, it freezes very rapidly and is instantly fatal. The hardiness limit imposed by the supercooling mechanism is well correlated with native plant distributions (Becwar 1980, George et al. 1974). Buds do not typically supercool as much as do stems;

the killing point for buds of fully acclimated trees is usually no lower than -30°C (Sakai 1978).

Trees that deep supercool include commercially important conifer and hardwood species. There have been essentially no studies of supercooling in containerized nursery stock. The characteristic is of special interest here because the DTA technique provides a precise measure of the freezing temperature of supercooled water and thus the cold hardiness of the tissue.

## DIFFERENTIAL THERMAL ANALYSIS

DTA is a calorimetric method based upon measuring the heat evolved as water freezes; the freezing of water is an exothermic process. To characterize freezing patterns in plant tissue pieces, two thermocouples are used in series (Burke et al. 1976). The first thermocouple is enclosed in a small chamber, adjacent to the tissue, while the other serves as a reference. A small aluminum block holds the thermocouples, and the whole system is cooled at a known rate. The temperature difference between two thermocouples (sample and reference) is continuously recorded. As water freezes, a sharp increase (exotherm peak) occurs because sample temperature rises while reference temperature remains the same. Typically, a large initial peak is observed; this represents the freezing of extracellular water, and except in very tender tissue, does not cause injury. The freezing of supercooled water results in a second exotherm at progressively lower temperatures in hardening plants; this is called the low temperature exotherm (LTE) and is associated with injury (Fig. 1).

The advantages of DTA for measuring freezing patterns and, potentially, for applied practical determinations of seedling hardiness include close correlation between LTE and low temperature killing points; simplicity; speed--a run can be made in less than an hour; small sample size; and large possible sample number.

## DTA AS A NURSERY TOOL

Several observations from the literature suggest that efforts to develop DTA as a practical technique can succeed. These include the fact that DTA provides an objective, precise measurement (the temperature of the LTE) that closely matches the tissue killing point (Becwar 1980, Burke and Stushnoff 1979). In addition, the LTE shifts and occurs at a progressively lower temperature as woody plants acclimate (Sakai 1978, Becwar 1980). There is a precedent for considering the practical application of DTA in the work of Proebsting and Sakai (1979). They measured peach flower bud hardiness and evaluated DTA as a tool in orchard management.

The objective of our work is to obtain data needed to develop DTA as a method of measuring cold hardiness (and dormancy) of forest tree seedlings. We plan to compare the DTA profiles of selected tissues from species of commercial importance and to observe changes correlated with conditions that alter hardiness. Terminal buds appear to be especially promising as test tissue, mainly because of sampling ease/uniformity and their well-defined DTA profiles (Sakai 1978). In preliminary experiments we observed that the bud LTE of containerized Engelmann spruce occurred at lower temperatures as hardening progressed. We did not find supercooling in bud or stem tissues of several pine species tested; however, the needles of certain pines have been reported to show an LTE in DTA profiles (Becwar 1980).

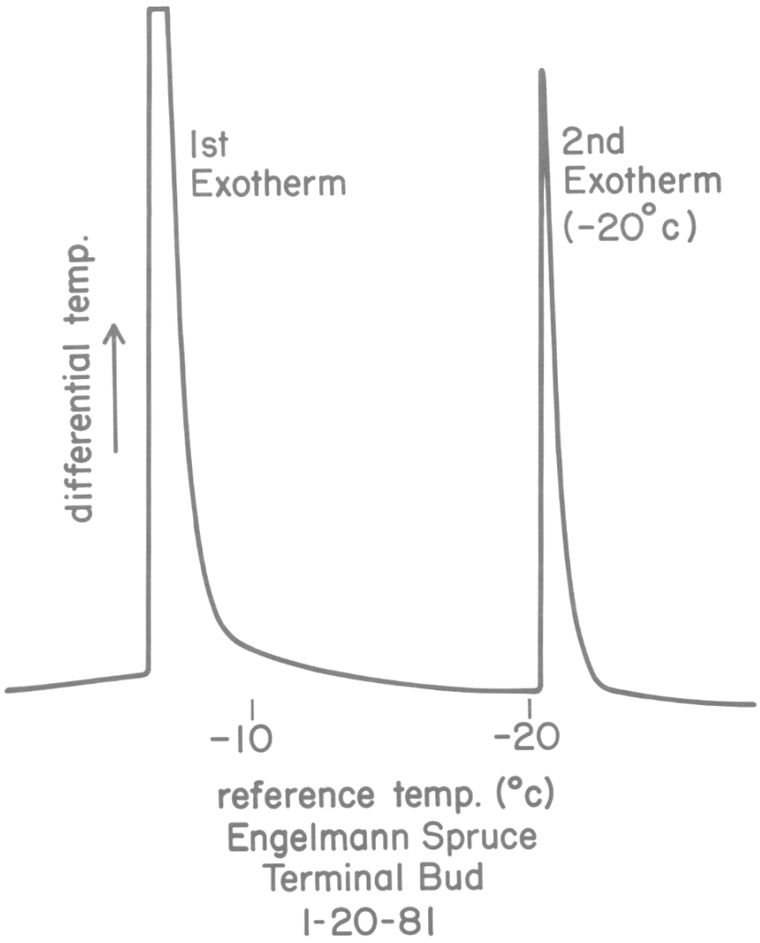


Figure 1. Differential thermal analysis (DTA) of a terminal bud excised from an Engelmann spruce seedling in January 1981. The low temperature exotherm *(LTE) occurred when the bud reached -20$^0$ C. The LTE represents the freezing* point of supercooled water and the low temperature killing point of the bud.

As pointed out earlier, supercooling is not a part of frost resistance in all woody plants. Clearly, the absence of this feature imposes a potentially serious restriction upon the widespread use of DTA as a nursery tool. Supercooling must occur if DTA data are to be used in this way. The major limitation evident from the literature is the apparent absence of supercooling (and thus LTE) in most species of Pinus. On the other hand, tissues of many conifers do contain supercooled water when in the hardened state. These include essentially all Abies and Picea species that have been examined, as well as members of *Juniperus, Larix,* and other genera. In addition, the buds of Douglas fir are known to exhibit well-defined peaks (LTE) on DTA profiles.

There are many practical situations in which decision making would be simplified by the "on the spot" measure of hardiness that DTA might provide (Table 1). The central idea of this project is to determine if DTA can predict--quickly and easily-the temperature seedlings can be expected to tolerate at any point in the production cycle.

Table 1. Examples of possible applications of differential thermal analysis (DTA) data as a nursery management tool.

1. Determination of lifting schedules
   Knowing that seedlings are hardened enough to withstand subfreezing storage would be valuable information.

2. Evaluation of seedling hardiness for fall plantings.
   Species/seedlot differences make arbitrary "calendar date" decisions difficult.

3. Measurement of hardiness as a guide to moving seedlings between greenhouse and unprotected environments.

4. Monitoring changes in hardiness (and dormancy) during cold storage.

5. Permitting "certification" of seedling hardiness for marketing purposes.

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