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A Self-Contained Freezing Chamber for Tree Ecophysiological Studies in the Field

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ABSTRACT. A self-contained freezing chamber was constructed for use in tree ecophysiological studies in the field. The chamber has two layers, an outer layer of polystyrene board to provide both physical support and insulation, and an inner chamber of plastic to protect the twig and foliage from mechanical damage from the packs of freezing medium. The freezing medium is a NaCl:ice mixture which can provide chamber temperatures as low as −20.3°C. The mean temperature at seven locations within the chamber was −4.1°C with a standard deviation of ±0.3°C when a NaCl:ice mixture of 16(gm):1.65(l) was used. In field trials, it took two people about 3 minutes to set up the apparatus on a twig. The technique was found to be both practical and economical for field freezing experiments, especially in handling large sample sizes. FOR. SCI. 37(3):924–930.
ADDITIONAL KEY WORDS. Frosts, freezing damage, low temperature stress.

LOW TEMPERATURE IS ONE FACTOR limiting the distribution and productivity of trees. Frosts occur during the growing season in some areas at high latitudes or high elevations (Christersson 1985, Christersson et al. 1987, Rothwell and Lieffers 1988). An understanding of the responses of trees to freezing events is critical for success in tree breeding programs and for frost protective measures in forestry. Equipment has been developed to conduct freezing experiments in the field (e.g., Holbert 1933, Johansson and Torssell 1956). However, most of this equipment is heavy, expensive, lacks the capacity for large sample sizes, and requires electric power.

Due to the limitations of equipment, most freezing experiments are conducted in the laboratory on greenhouse grown seedlings (Pharis et al. 1970, Christersson et al. 1987, Lindström and Mattsson 1989) or on twigs transported from the field (Sakai and Weiser 1973, DeLucia and Smith 1987). Observations of frost damage in the field after the occurrence of natural frosts are also common (Strand and Lundmark 1987, Lundmark et al. 1988). While the above methods provide insight into the mechanisms of trees responses to frosts, they have limitations. Laboratory experiments cannot provide information on the integrated effects of natural environments during and after frosts. Experiments using cut twigs also make it impossible to examine post-freezing injury and recovery processes. Even though observations after natural frosts may be ideal, such events are sporadic and capricious, and this makes it difficult to plan experiments.

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The freezing chamber described here provides an inexpensive, practical technique to achieve freezing conditions in the field.

MATERIALS AND METHODS

Chamber Construction and Assembly

The apparatus consists of a freezing chamber, two sets of wooden clamps, and a wooden post. The freezing chamber is held by the clamps and is mounted on the post by a single bolt so that the post-chamber angle α can easily be adjusted to fit the twig angle to minimize the tension on and bending of the twig (Figure 1).

The freezing chamber has three parts: an outer insulation chamber, a freezing medium package, and an inner protection chamber. The outer chamber consists of two half-chambers, each cut from a 30 × 28 × 6 cm polystyrene board. The central portion of each board was hollowed out to produce a cavity of 20 × 18 × 2.5 cm, and a twig channel was cut at one end of the chamber (Figure 2). The apparent thermal conductivity, material density, and specific heat of the polystyrene board are 0.0361 W/m/°C, 24 kg/m^3, and 1.14 kJ/kg/°C, respectively (Croy and Dougherty 1984).

A NaCl-ice mixture was used as the freezing medium. Ice chips were obtained

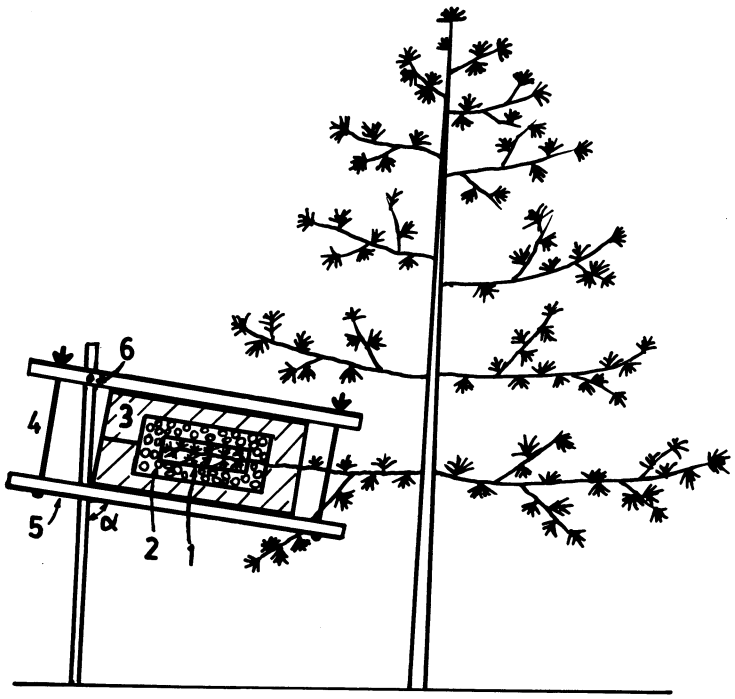

FIGURE 1. A cutaway view of the field setup of the freezing chamber. The chamber is mounted to the pole with a single bolt. The chamber can be slided forward or backward within the wooden clamps so that the angle α is adjusted to fit the twig angle and minimize the physical stretch on the twig (1—inner chamber, 2—freezing medium, 3—outer chamber, 4—bolt, 5—wooden clamp, 6—mounting bolt).

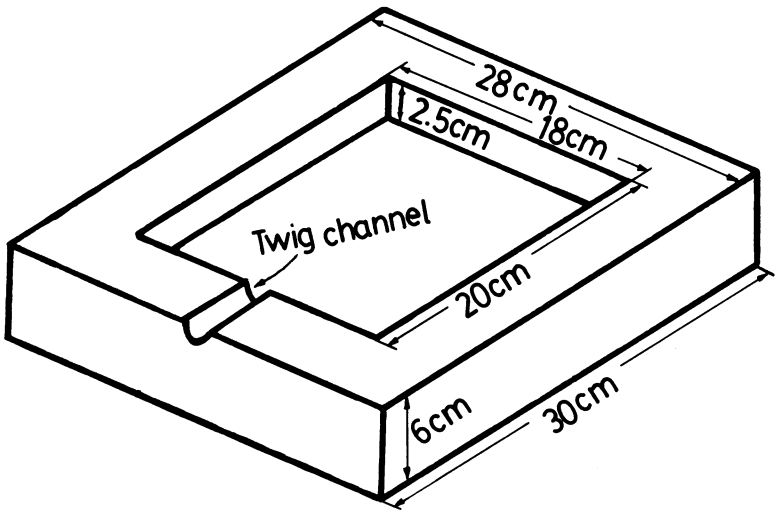

FIGURE 2. One-half of the outer freezing chamber. The material is polystyrene board.

from an ice-making machine (ICE-O-MATIC, A Welbilt company, NSF Testing Laboratory, Ann Arbor, MI). "Sifto" coarse salt (NaCl) (Domtar Inc., Sifto NaCl Division, Mississauga, Canada) was used. Salt and ice mixtures were used as a freezing medium in orchards (West and Edlefsen 1917), but the associated apparatus is neither easily replicated nor suitable for use in the forest.

The inner protective chamber, to protect the twig from mechanical damage caused by the pressure of the freezing medium, was made from a petri dish (90 × 90 × 9 mm). Holes (about 1 cm apart) were drilled through the petri dish walls to facilitate heat exchange during the cooling process, and a twig channel was also cut in one end.

A sponge gasket was used around the perimeter of the two half outer chambers and around the twig channel. The two half-chambers were pressed and held together by the wooden clamps held parallel to the twig.

The procedures for applying the apparatus *in situ* were as follows:

1. Install the wooden post about 15 cm from the tip of an experimental branch and mount one set of the wooden clamps onto the post by a single bolt (Figure 1).
2. Weigh NaCl and ice chips (ice chips can also be measured by volume).
3. Mix ice chips and NaCl by shaking them together vigorously in a large covered container.
4. Quickly put the mixture into two separate 18 × 20 cm zip-lock plastic bags and seal the bags.
5. Hold one of the half outer chambers horizontally and place in sequence in the hollow space: a bag of freezing medium, the inner chamber containing the tip of an experimental branch and the second bag of the freezing medium.
6. Insert the sponge gasket and cover with the second half of the outer chamber.
7. Press and loosely clamp the two half-chambers together using the wooden clamps on the post.
8. Slide the chamber along the clamps to fit the twig angle, put on the second set of clamps and tighten the clamps.

CHAMBER TESTING

The following tests were conducted on the apparatus:

1. *Temperature calibration.* Temperature calibration experiments were conducted at 5°C

ambient temperature in a growth chamber for the following NaCl:ice ratios: 0:600, 10:600, 20:600, 40:600, 60:600, 80:600 and 100:600. Each NaCl:ice mixture had three replicates. The temperature in the freezing chamber was sampled at 10-sec intervals using teflon coated copper-constantan thermocouples (the diameter of the sensor wire was 0.12 mm) attached to a CR21X data-logger (Campbell Scientific Canada Corp.). A temperature calibration curve was developed using minimum cooling temperatures (average of three replicates) in the freezing chamber.

2. *Cooling rate, temperature sustainability and the effects of ambient temperatures.* The freezing experiments for the NaCl:ice ratios noted above were also conducted at 20°C ambient temperature. The differences in minimum cooling temperatures which were obtained at the two different ambient temperatures were tested by ANOVA. The cooling rate and temperature sustainability over 2 hr for the two different ambient temperatures were examined.
3. *Spatial temperature variation in the chamber.* The NaCl:ice ratio for this test was 16(g):1.65(l). The test was conducted at 20°C. A black spruce twig was inserted along the diagonal of the inner chamber. Three copper-constantan thermocouples were attached to needles on the main stem. Four thermocouples were attached to separate lateral shoots. The three probes on the main stem were located about 5 cm apart at the tip, the midpoint, and the base of the stem. The lateral shoots were selected on both sides of the main stem, and thermocouples were positioned about 3 cm from the main stem. The minimum temperatures over a 2-hr period from the seven thermocouples were recorded on the data-logger.
4. *Protective efficiency of the inner chamber.* Mature black spruce and tamarack trees (six of each species) were selected for assessing the protective efficiency of the inner chamber on the foliage. Photosynthesis, transpiration rate, and stomatal resistance of foliage on the selected twigs from these trees were measured using a portable gas exchange system (The Analytical Development Co. Ltd., England) at 10:00 A.M. on the day prior to placement in the chambers (temperature = 24°C, photosynthetic radiation density = 1400 − 1800 $\mu E/m^2/s$). The same trees and the same twigs were reused for the following experiment. At 7:00 A.M. on the next day, freezing chambers containing a freezing medium substitute (a similar volume of polystyrene beads to simulate the pressure from the NaCl:ice mixture) were put on branch tips of three trees of each species. Branch tips of the remaining trees were placed into freezing chambers without either freezing medium (substitute) or inner chambers (i.e., no pressure on the foliage), and were used as controls. The freezing chambers were left on the trees for 2 hr. The branch tips were inspected visually for mechanical damage immediately after the freezing chambers were removed. At 10:00 A.M., photosynthesis, transpiration, and stomatal resistance on the same twigs were remeasured. The weather conditions on that day were similar to those of the previous day (temperature = 25°C, photosynthetic radiation flux density = 1460 − 1850 $\mu E/m^2/s$).

   The differences in these physiological parameters between the two treatments (i.e., with and without pressure) were tested by ANCOVA using the measurement of the previous day as a covariant. Also, a paired t-test was used to test if the same trees performed similarly before and after the chamber (with pressure) was applied.
5. *Field trials.* The technique was tested in the field on 9 mature black spruce and 9 tamarack. The temperature of the foliage in the chamber was monitored as noted above.

RESULTS

Temperature Calibration

Minimum cooling temperatures between 0° and −20.3°C were obtained at 5°C ambient temperature as NaCl:ice ratios increased from 0:600 to 100:600 (gram: gram) (Figure 3).

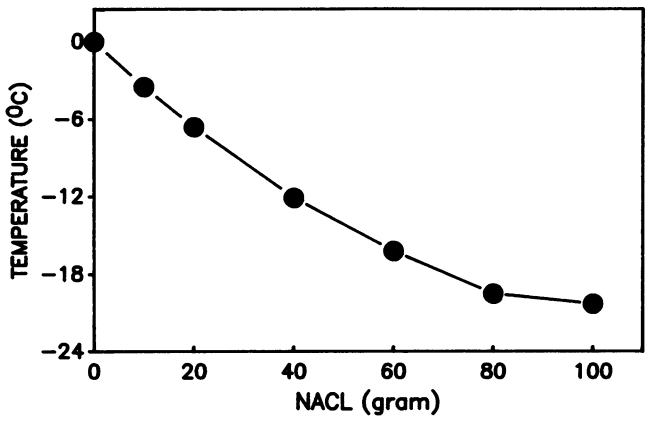

FIGURE 3. Calibration curve for the relationship between minimum cooling temperature and NaCl:ice mass ratio. The amount of ice is 600 g.

COOLING RATE, TEMPERATURE SUSTAINABILITY, AND THE EFFECTS OF AMBIENT TEMPERATURES

The temperature in the chamber generally reached the minimum value within 4 to 28 min. depending on the NaCl:ice ratios and ambient temperatures. The higher the ambient temperature or the higher the NaCl:ice ratio, the longer time it took for the temperature in the chamber to reach the minimum. For example, it took 4 and 12 min. for a 10:600 NaCl:ice mixture to cool the chamber to a minimum temperature of -3.4°C at ambient temperatures of 5° and 20°C, respectively. Salt and ice mixtures of 10:600 and 100:600 at an ambient temperature of 20°C required 12 and 28 min., respectively, to produce minimum temperatures. The cooling rate was the greatest during the first 8 min. for all the NaCl:ice mixtures.

There was no significant difference between the minimum cooling temperatures at 5° and 20°C ambient temperatures ($P \leq 0.05$). The temperature in the chamber increased gradually after the minimum was reached. A higher ambient temperature produced a faster temperature increase. For example, the temperature increments over a 2-hr period for a NaCl:ice mixture of 10:600 was 0.4°C at 5°C and 0.6°C at 20°C.

SPATIAL VARIATION IN TEMPERATURE IN THE CHAMBER

The minimum cooling temperatures in the chamber from the seven probes ranged from -3.7°C to -4.6°C and had a mean of -4.1°C and a standard deviation of 0.3°C.

PROTECTIVE EFFICIENCY OF THE INNER CHAMBER

No visual mechanical damage to the foliage was observed from the pressure of the freezing medium substitute. No significant difference ($P \leq 0.05$) in photosynthesis, transpiration, and stomatal resistance was detected between the two groups of trees treated by freezing chambers with and without the freezing medium substitute (i.e., polystyrene beads). There was also no significant difference ($P \leq$

0.05) in photosynthesis, transpiration, and stomatal resistance of the branch tips after treatment (i.e., freezing chamber containing polystyrene beads) compared to pretreatment (i.e., the previous day).

Field Trials

In field trials, it generally took two people about 3 min. to set up the apparatus on a twig. The course of temperature changes in the chamber was similar to our laboratory results.

DISCUSSION

The freezing chamber described here is efficient, economical, and practical under field conditions. The technique can be applied to branches of trees, aboveground portions of seedlings, or other plants. Depending on the NaCl:ice ratio of the freezing mixture, relatively stable temperatures of 0° to −20.3°C can be obtained inside the chamber. The technique itself is nondestructive to foliage being studied. Therefore, the response of the trees and recovery processes can be monitored continuously for as long as desired.

Ice chips and NaCl form an imbalance system in which solid water transforms into liquid phase quickly. This phase change draws thermal energy from the nearest environment, lowering its temperature until equilibrium is reached (provided the system is reasonably adiabatic). Within a certain range, the greater the amount of NaCl added to a fixed mass of ice, the lower is the final temperature. The minimum temperature which can be obtained equals the eutectic point (−21°C) of the freezing mixture (Lewis 1932). The process is also dependent on the amount of plant tissue in the chamber and the insulating properties of the outer chamber. The insulating material and the ambient temperature are especially important in determining the sustainability of the chamber temperature as both of them affect the heat flux into the chamber.

Several modifications to the chamber to accommodate different freezing experiments can be envisioned. First, fast cooling rates may be inappropriate for many physiological studies, particularly when incipient injury is of interest (Steffen and Palta 1987). Slower cooling rates of foliage can be achieved by using an insulated inner chamber and/or coarser salt. For example, a cooling rate of 4.5°C/hr (0.075°C/min.) for the final 10°C drop in temperature was obtained by insulating the inner chamber with a 2 cm thick armaflex sheet insulator (Armstrong, Canada). Still slower cooling rates could be achieved by adding more insulation. Second, larger foliage samples may be handled by increasing the size of the chamber and the volume of the freezing medium. In our experiments the foliage was less than 1% of the mass of the ice. If the mass of the foliage is large relative to the mass of the freezing medium, however, the thermal energy from the foliage may slow the cooling process. Finally, if longer periods of stable low temperature are required, more insulation could be added to the outer chamber.

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