USING A PRESSURE CHAMBER TO DETECT DAMAGE TO SEEDLINGS ACCIDENTALLY FROZEN DURING COLD STORAGE

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ABSTRACT: During cold storage, seedlings are sometimes accidentally frozen. A study to determine if a pressure-chamber device could be used to detect the extent of this type of injury indicated that the change in plant moisture stress of potted seedlings during the first week after freezing is a reliable measure for predicting seedling survival.

INTRODUCTION

Storage of bareroot seedlings is often a necessary step In the reforestation of conifers, as labor, geographic, and climatic constraints make it virtually impossible to plant seedlings immediately after they are lifted. It is well established that the temperature during storage can greatly affect seedling quality (Hocking and Nyland 1971). Currently, most conifer seedlings are stored between 0° and 3°C because cold temperatures reduce respiration and inhibit the development of harmful molds. But, despite improvements in the overall quality of refrigeration facilities, occasional equipment malfunctions result in seedlings being exposed to subfreezing temperatures. Such exposure can he especially injurious to root systems, which are more sensitive to freezing than shoots. Unfortunately we know little about the tolerance of roots to this type of injury, nor is there a simple and effective method of identifying its extent. When such a storage problem is discovered and it must be decided whether seedlings should be discarded or planted, there is little on which to base a decision. Consequently, in December 1982, as part of the Nursery Technology Cooperative at Oregon State University, we initiated a study to determine if a pressurechamber device could be effectively used to identify seedlings that were severely damaged by accidental freezing during storage.

METHODS

One hundred,2-year-old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings from a common seed source were randomly divided into 10 equal groups for 10 temperature treatments.
Each group was placed in a sealed plastic bag in a freezing chamber programmed to remain 1 hour at +1°C. The temperature was then lowered at the rate of 2°C per hour. We removed the first bag at -3°C and continued to remove one hag every half hour at each drop of 1°C until the temperature was -12°C. Immediately after removal from the freezing chamber, each bag was placed in a cold room (+1°C) and left overnight to thaw gradually.

The day after thawing, all seedlings were tagged with their freezing-treatment number and planted randomly in pots, one seedling from each treatment in each pot.

The following day, a small lateral branch from each seedling was removed and placed in a pressure chamber to determine its plant moisture stress (PPS). This procedure was repeated on the fourth and sixth days after potting. PMS was recorded as a positive number, so that an increase indicated greater water deficit within the seedlings. The night before each PMS determination, all pots were watered to field capacity to ensure similar soil moisture conditions for each pot on each evaluation date.

The seedlings were maintained for 2 months in a growth room under a 16-hour photoperiod and constant 22°C temperature. During this time, the pots were watered regularly and soil moisture remained fairly high. At the end of this period, we recorded the percentage of dead seedlings from each of the 10 freezing treatments and calculated the average PS per treatment for each assessment date. For each treatment, we calculated the average absolute increase and average percentage increase in PPS between the first and fourth and the first and sixth days after planting.

We then determined if there was a significant relationship between freezing temperature and PM.S on each date. Next we calculated correlation coefficients for the relationships between mortality and absolute and percentage changes in PPS over all treatments. Finally, we determined the average PMS for seedlings that lived and those that died and tested for significant differences. All reported differences were significant at P = 0.01 unless otherwise suited.

RESULTS

Twenty of the original 100 seedlings died during the 2-month assessment period. Figure 1 shows mortality percentages for each freezing treatment. Sixteen of the dead seedlings were from the two lowest temperatures, which indicates that among seedlings of the seed source used,

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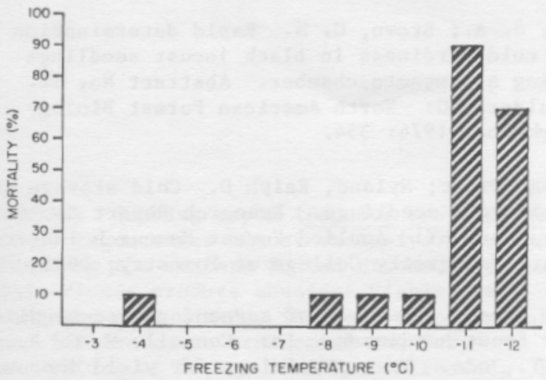

Figure 1.--Final mortality of seedlings, by freezing treatment.

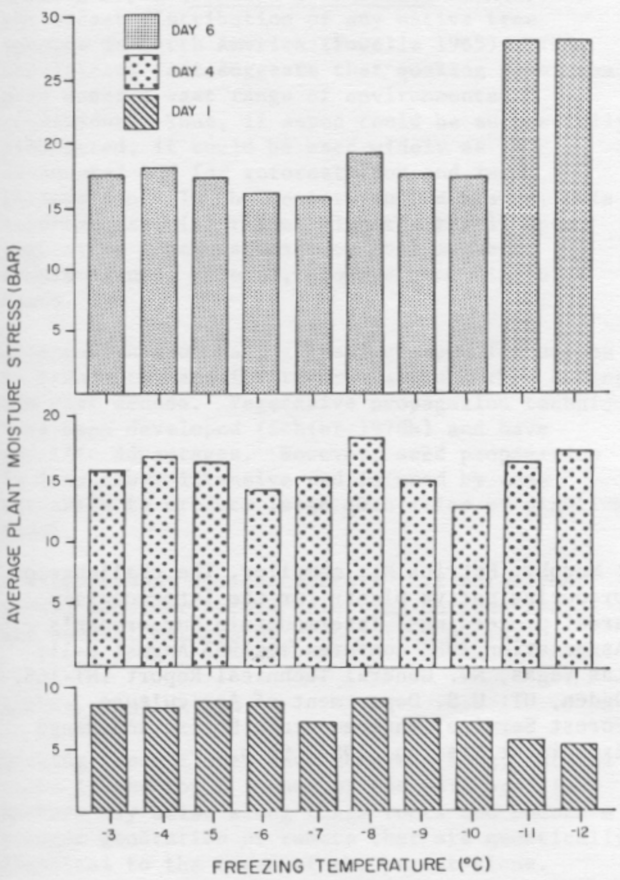

Figure 2.--Average plant moisture stress of seedlings, by treatment and day of evaluation.

the threshold temperature for lethal damage (-11°C) was quite uniform. Figure 2 shows

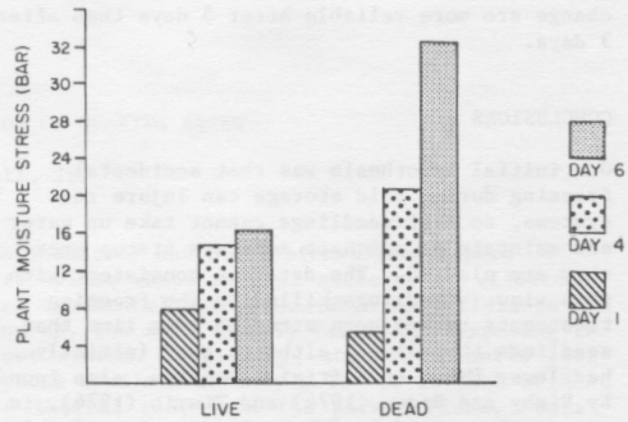

Figure 3.--Average plant moisture stress of surviving and dead seedlings.

average PMS by treatment for each assessment date. There are three interesting things to note: first, that average PPS for all treatments increased over time; second, that firstday PPS tended to be lower in the colder treatments (freezing temperature and PMS were significantly and positively correlated); and third, that this initial trend dramatically reversed during the following 5 days. Seedlings from the two coldest treatments had the highest average PPS values on the sixth day after planting, and freezing temperature and PPS were significantly (P = 0.05) and negatively correlated.

The relationships between lethal freezing injury and PPS (fig. 3) show that seedlings that died had significantly lower initial PMS values that then rose precipitously. Seedlings that lived had higher initial PPS values that increased gradually between the first and fourth days and then remained relatively unchanged. PMS on the sixth day, and the percentage difference between the first and sixth days, were significantly higher for those seedlings that eventually died.

As might be expected from this discussion, the percentage of dead seedlings from a given freezing treatment was closely correlated with the absolute and percentage increase in PPS for that treatment. There was a strong correlation between mortality and both absolute and percentage increases in PMS for both measurement intervals (days 1 to 4, days 1 to 6). Significant correlation coefficents were:

Percentage mortality x absolute increase in PPS

Days 1 to 4	r = 0.80
Days 1 to 6	r = 0.98

Percentage mortality x percentage increase in PMS

Days 1 to 4	r = 0.85
Days 1 to 6	r = 0.96

Although all correlations were significant, the larger coefficents for the longer time intervals indicate that predictions of mortality from PPS change are more reliable after 5 days than after 3 days.

CONCLUSIONS

Our initial hypothesis was that accidental freezing during cold storage can injure root systems, so that seedlings cannot take up water and maintain an adequate moisture status once they are planted. The data are consistent with this view. Seedlings killed by the freezing treatments became more stressed over time than seedlings that lived, although they initially had lower PMS. An initial reduction, also found by Bixby and Brown (1974) and Timmis (1976), is apparently caused by internal rupturing of cells and release of water into the xylem. Over time, the transpirational demand probably depletes the available water in the seedlings, and PMS rises rapidly as the water is not replenished by the injured root system.

Because we found considerable variability in the initial PMS values of seedlings receiving the same freezing treatment, and because the change in PMS was so closely correlated with lethal injury, we believe that the procedure outlined-measuring seedlings once soon after planting and once 5 days later--is a more reliable technique for predicting injury than a single PMS measurement. The exact magnitude of change in PMS that indicates severe freezing damage, however, is not clear. In this study, a 4-fold increase between the first and sixth days reliably indicated seedling mortality; those with less than a 4-fold increase in PMS lived. The 4-fold separation value predicted the final survival status of 97 percent of the seedlings. In preliminary results from another trial, however, a 3-fold increase during the first week after planting indicated mortality. In this second trial, there was little or no change in the FMS values over time for most surviving seedlings, in contrast to the rough doubling of PMS between the first and sixth days for surviving seedlings in the study reported here.

Although some calibration must be done to perfect the technique, the data clearly suggest that a pressure chamber can be a very useful tool in identifying seedling injury caused by unintentional freezing during cold storage. The assessment procedure outlined is simple, requiring only a pressure chamber and a small amount of greenhouse or growth-room space, and it can be completed within a week after the suspected injury occurs.

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