

Cold Damage to Loblolly Pine Seedlings

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Cold temperatures during December 1983 and January 1984 caused extreme damage to loblolly pine crops in seedling nurseries. Damage levels were higher in southern provenance seed sources as had been observed over twenty years ago in Tennessee (Mignery 1963) . This paper will document our observations of the damage and effects on resulting seedling quality.

The physiological nature of cold damage in plants has been reviewed by Molich (1897, reissued in 1982), Franks (1983) and Steponkis (1984).

MATERIALS AND METHODS

Temperatures were recorded at depths noted using a Campbell Instruments CR-21 Microprocessor Weather Station. Data were recorded on audio tape, converted to digital output using an Omnidata Model 217 cassette reader, and graphed.

Estimation of root tissue damage was done by two methods, first visually and second using a vital stain. Visual estimation was done by separating the tap root into 2.5cm (1") depth zones. In each zone the tissues were scraped off down to the cambium at 4 positions at 90° intervals. Damage was assessed by the color of the cambial tissues and those layers immediately outside it. In healthy tissues only the outermost periderm tissues showed brown-orange coloration. In damaged tissues this color extended inward to include the cambium. Damage assessment in each root zone was based on the degree to which damage encircled the root potentially girdling it. Damage classes are summarized in Figure 1. Most damage was in zones 1 and 2.

The relationship between tissue browning and potential for growth at the cambium was determined by incubating tissues for 24 hours after either perfusion or vacuum infiltration of a 1% solution of 2, 3, 5-triphenyltetrazolium chloride (TTC) (Grano 1958) . This vital stain is converted from colorless to a red chromophore in the presence of respiring cells. After 24 hours, root segments were cut into freehand sections and examined at 40X.

Root growth potentials of damaged and undamaged seedlings were compared. Nursery beds were mapped into areas of high and low damage by subsampling seedlings, then equal populations of seedlings having high and low probabilities of damage were lifted and placed into root growth potential tests. Tests were conducted in a controlled environment chamber at 30°C day, 20°C night during a 16 hour photoperiod at 360 $\mu\text{mol photons m}^{-2}\text{sec}^{-1}$. Tests were run for 11 days and 28 days. New roots > 1cm in length were tallied, and root damage classes determined at the end of the test period.

Data on the effects of storage on the root growth potential of 16 loblolly families were drawn from a time of lifting-storage study in progress when the cold damage occurred. Lifting dates were Dec. 12, 1983 (370 chilling hrs i.e. hours of temp. between 0 and 8°C) , Jan. 9, 1984 (523 chilling hours) and Feb. 8, 1984 (752 chilling hrs.) . Storage was for 3 wks at 2°C. Root growth potential tests were done under greenhouse conditions with air temperature at approximately 20°C, soil temperature held at 20°C in a water bath, 12 hour photoperiod and a test period of 28 days. Roots > 1cm in length were tallied.

A field plantation was established with seedlings from damaged mapped nursery beds with 105 seedlings from each of two populations having either a high or a low probability of cold damage. Survival, height growth and bud condition were tallied on June 1.

RESULTS

Temperature:

Weather records at Magnolia Forest Regeneration Center indicate that air temperature at 20cm above the soil surface was below freezing for much of the last two weeks of 1983. The lowest temperature was reached in the early morning hours of December 25 when temperatures reached -14.8°C (5.4°F) (Figure 2). Soil temperature at 6mm (1/4") reached a low of -8°C (17.6°F) whereas at 15 cm (6") the temperature minimum was -4°C (24.8°F).

Root Regeneration and Tissue Damage:

The number of roots which had $> 1\text{mm}$ of new elongation after 11 days was proportional to amount of root damage (Figure 3A). TTC tests showed no staining in the cambial and phloem tissues at the beginning of the root regeneration tests (Figure 4A). At the end of eleven days, however, cambial and phloem tissues stained pink-red, indicating living tissues (Figure 4B). After 28 days, there was no difference between damaged and undamaged trees in the number of roots produced (Figure 3B) (Table 1).

Root Regeneration of Stored Trees:

Storage of stock lifted in January after the cold damage resulted in substantial depression in root growth potential (Figure 5A and B) . By February, storage in most cases improved root growth potential (Figure 5A and B) (Table 1).

Field Performance of Damaged Seedlings:

Survival of damaged stock was 7.2% below that of controls on June 1 (Figure 6A). An additional 20.7% of damaged trees had not yet broken bud on June 1 (Figure 6B), as compared with 2.2% of controls. Height growth was reduced 29% by damage, a result that, although significant ($\alpha = 0.0001$) may be of more consequence as an indication of vigor than a real volume loss since that represented a 3.3cm decrease (Figure 6C).

DISCUSSION

Cold weather damage to loblolly pine poses two problems to the owner of the seedlings: (1.) assessing the level of damage in the nursery crop and (2.) predicting the future performance of damaged stock following outplanting. The experience and research of this past winter allow us to suggest the following theory of damage and recovery in loblolly pine. First of all, the extreme cold weather led to injury of root tissues exterior to, and including the vascular cambium. These cell populations were not totally killed, but rather stained brown with phenols released from heavily damaged, ruptured cells. These tissues showed reduced uptake and/or conversion of TTC vital stain. During the first 11 days of recovery in a warm, moist, long day environment root regeneration was down probably due to damage

to phloem tissues causing a reduction in transport of necessary photosynthate and auxin. By the end of 11 days, the phloem and cambial tissues readily converted TTC stain, thus indicating a recovery of these tissues. Seedlings allowed 28 days in an ideal environment showed very good root growth potential . It is thus probable that recovery from damage was relatively rapid under these conditions.

Normally stock lifted too early exhibits decreased root growth potential after storage. Seedlings of 16 loblolly families lifted December 12 (370 chilling hours, i.e. hours between 0 and 8°C) showed improved root growth potential following 3 weeks storage, thus indicating that prior to the cold damage they were in proper physiological condition for high quality storage. Seedlings lifted January 9 (523 chilling hours) that would normally improve in storage, had very poor root growth potential after storage. Some of these families also had poorer root growth potential than expected without storage. The dramatic reduction in RGP following storage could indicate that normal recovery processes did not proceed in storage. Recovery of nonstored stock occurred during the root growth test, and stock lifted February 8 (752 chilling hrs.) had mostly recovered in the nursery beds prior to lifting.

Field performance of damaged loblolly seedlings would be expected to vary by family, date of lifting and duration of storage. Early mortality is lower than expected, however, slowed budbreak indicates that the damaged seedlings are entering the summer with reduced vigor. Fall survival counts will be necessary to fully assess the performance of damaged stock.

LITERATURE CITED

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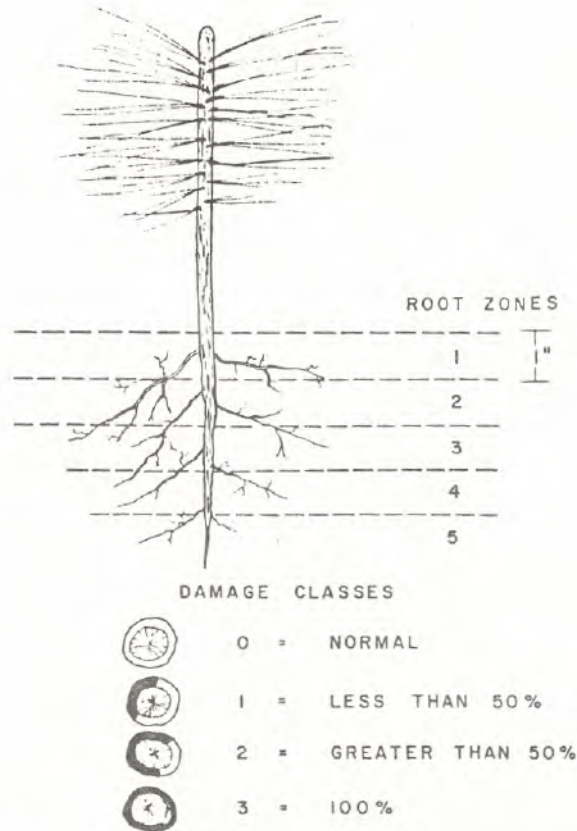


Figure 1. Seedling root damage classes were based on percentage of the circumference of the outer vascular tissues showing brown coloration. This was done in each of 5 depth zones. Most damage was in zones 1 and 2.

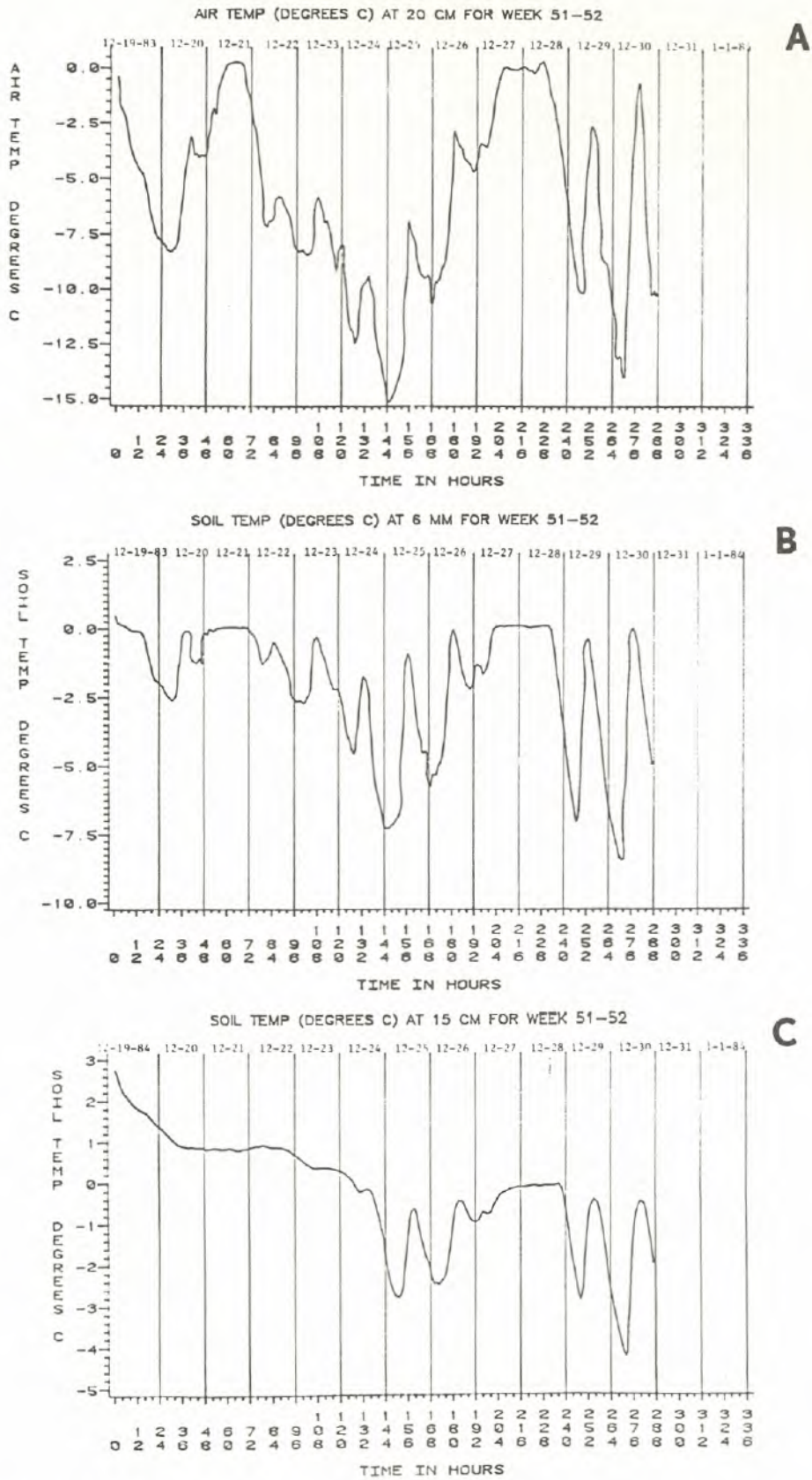


Figure 2. Temperature profiles from Dec. 19 to Dec. 30, 1983 at Weyerhaeuser Forest Regeneration Center, Magnolia, Arkansas. (A) Air temperature at 20 cm. (B) Soil temperature at 6mm and (C) Soil temperature at 15 cm.

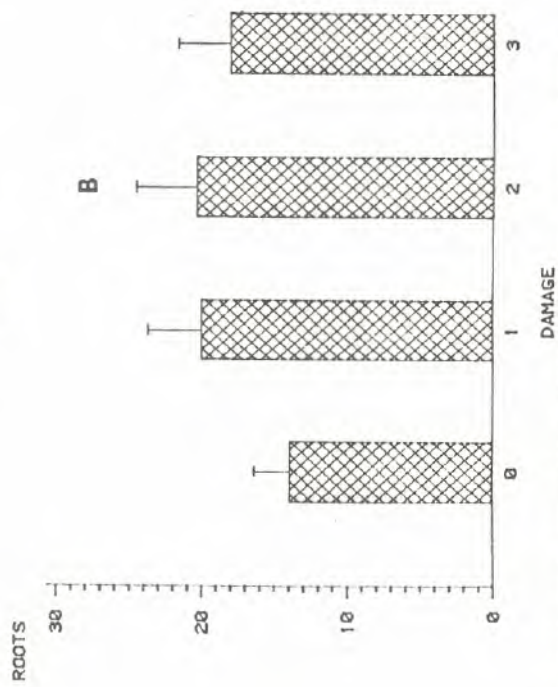
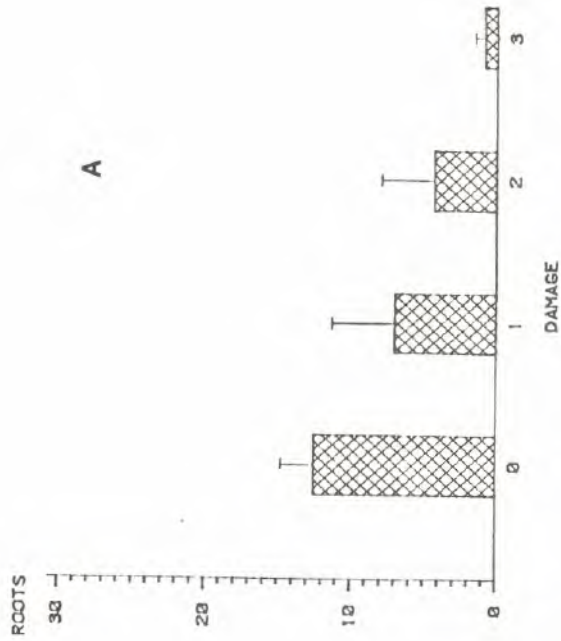


Figure 3. Root growth potential by damage class for seedlings in warm, moist, long day environment in growth chamber for (A) 11 days and (B) 20 days.

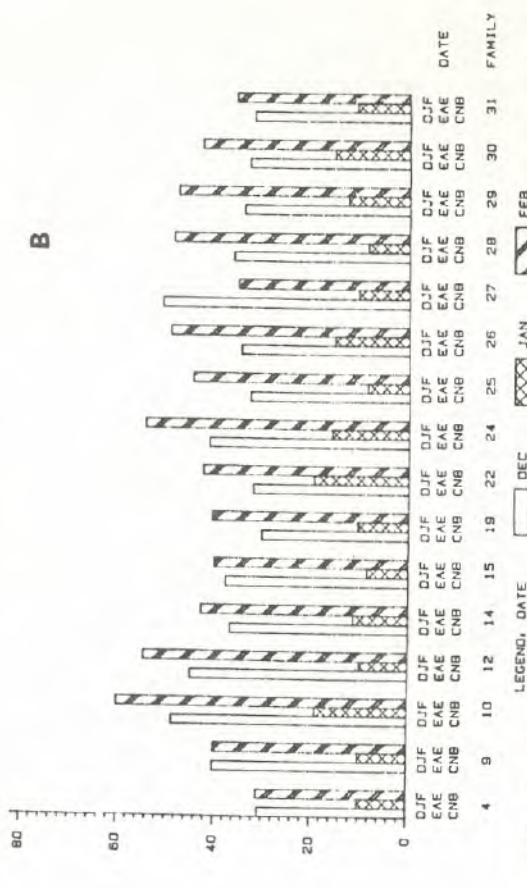
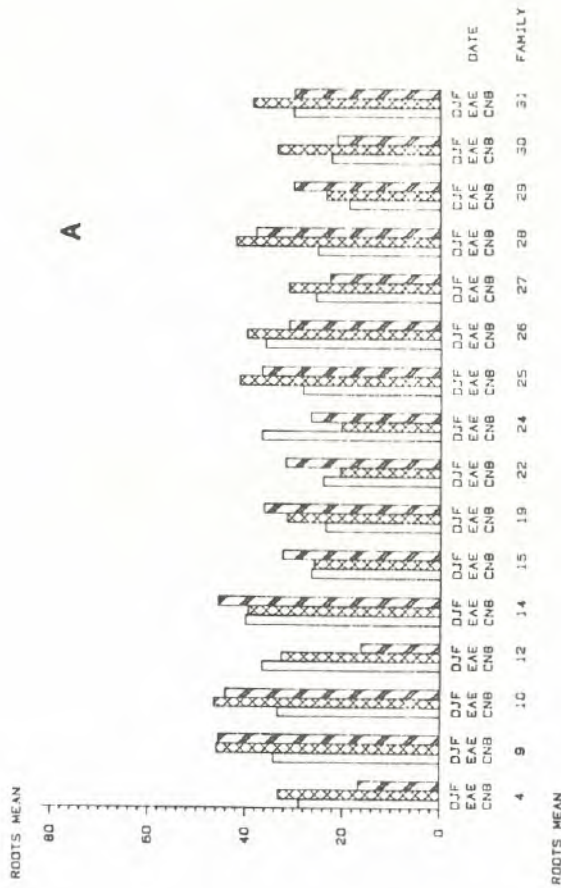
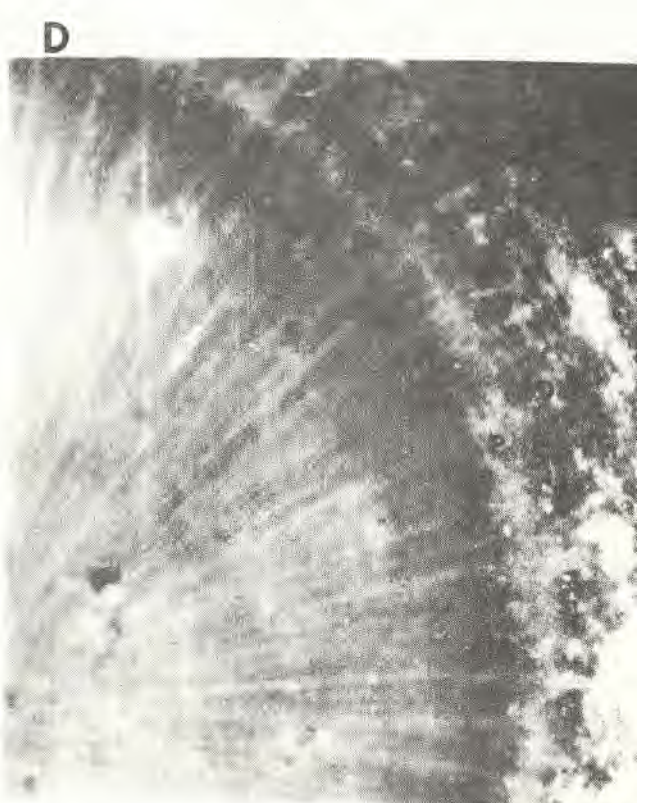
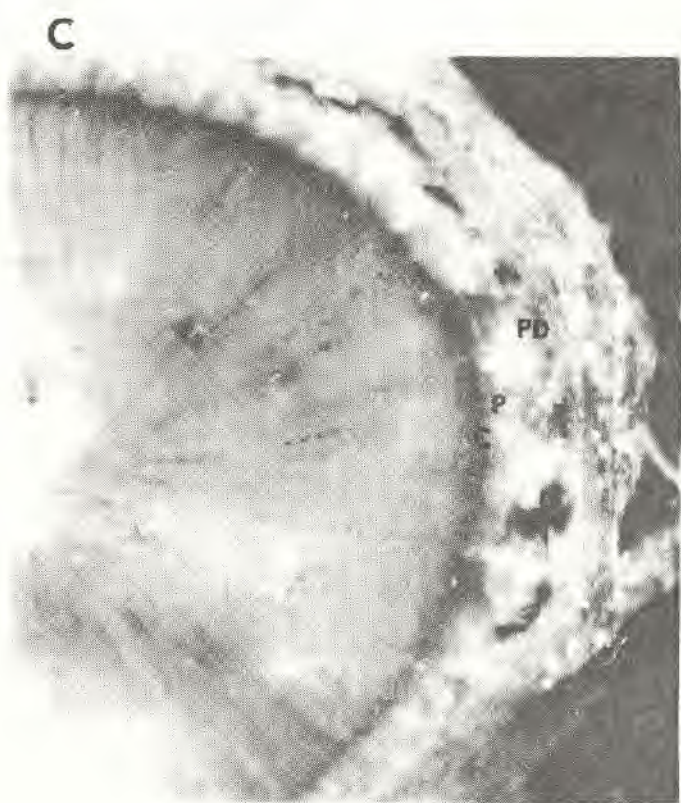
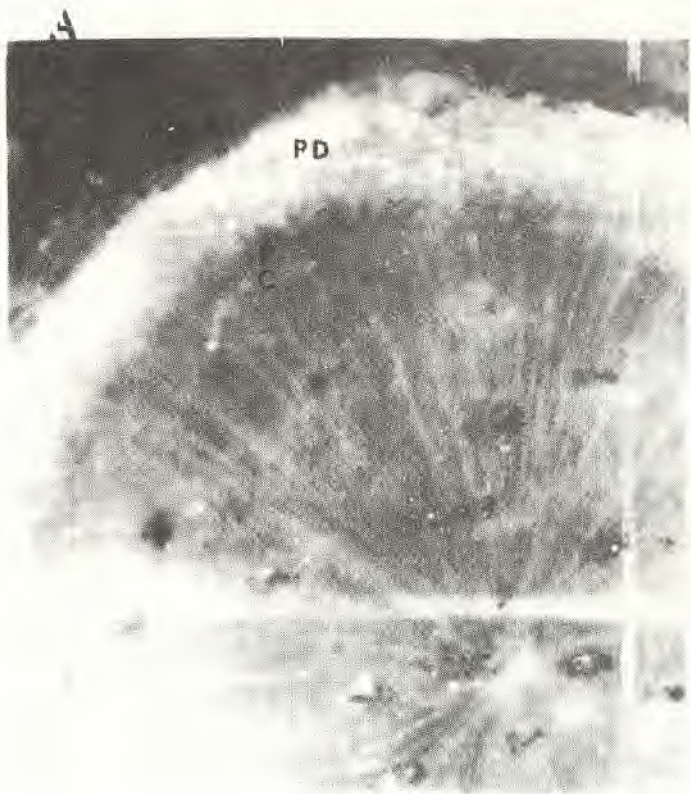


Figure 5. Root growth potential of 16 open pollinated loblolly pine families lifted December 12 prior to cold damage or January 9 or February 8 after damage: (A) immediately after lifting and (B) after 3 weeks storage at 2°C.

Figure 4. Cross sections of tap root 24 hours after perfusion with 1% tetrazolium chloride (TTC) . TTC forms the red chromophore, formazan, in the presence of respiring tissues. (A) and (C) are seedling taken directly from the nursery, whereas (B) and (D) were incubated in a warm (30°C) moist, long day (16 hr. photoperiod) environment for 11 days. A and B are undamaged seedlings whereas C and D were damage class 3 in the root zones sampled. In healthy trees (A and B) the vascular cambium (C) and phloem tissues (P) stained bright red, whereas the periderm (PD) remained white. In photo C, the vascular cambium (C) phloem (p) and periderm (PD) were orange-brown. After 11 days incubation (photo D) the vascular cambium (C) and phloem (p) tissues stained pink-red whereas the periderm was a darker stage of orange-brown.



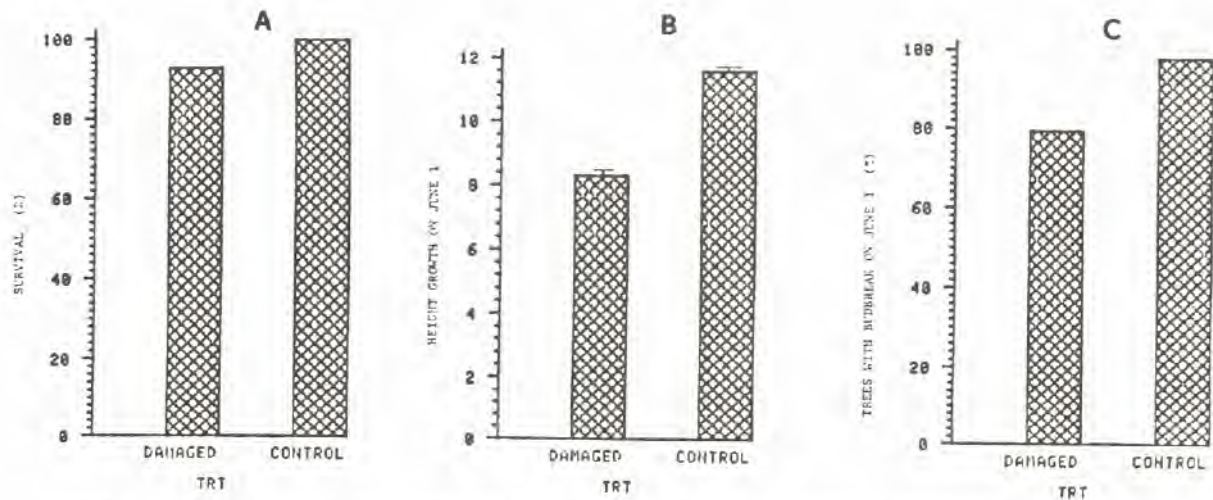


Figure 6. Field performance characteristics for damaged and undamaged seedlings lifted February 9 and planted February 22. (A) survival, (B) height growth (cm) of survivors and (C) proportion of live seedlings having broken buds by June 1. Assessment was made June 1.

Table 1. Analysis of Variance for data presented on comparison of cold damaged trees with controls.

| DEPENDENT VARIABLE | MS | | DF | | α | SIGNIFICANCE DECISION at α=0.05 |
|--|-----------|--------|-----------|-------|--------|---------------------------------|
| | TREATMENT | ERROR | TREATMENT | ERROR | | |
| Number of roots between damage classes: | | | | | | |
| . After 11 days in growth chamber | 427.4 | 58.8 | 3 | 42 | 0.0005 | S |
| . After 28 days in growth chamber | 209.1 | 4670.5 | 3 | 32 | 0.7001 | NS |
| Field Plantation Damaged vs Control: | | | | | | |
| . Height Growth | 100.0 | 18.9 | 9 | 194 | 0.0001 | S |
| . Survival | 0.263 | 0.037 | 1 | 202 | 0.0081 | S |
| Family Root Growth Potential before and after Storage by Month: | | | | | | |
| 25 Dec | 160.0 | 165.4 | 1 | 31 | 0.3330 | NS |
| Jan | 8796.4 | 279.4 | 1 | 31 | 0.0001 | S |
| Feb | 586.0 | 560.2 | 1 | 34 | 0.3211 | NS |
| 4 Dec | 160.0 | 165.4 | 1 | 31 | 0.3330 | NS |
| Jan | 4340.7 | 170.5 | 1 | 31 | 0.0001 | S |
| Feb | 1849.0 | 204.3 | 1 | 34 | 0.0049 | S |
| 26 Dec | 12.44 | 371.2 | 1 | 31 | 0.8559 | NS |
| Jan | 5377.8 | 300.4 | 1 | 34 | 0.0002 | S |
| Feb | 2988.4 | 565.9 | 1 | 34 | 0.0278 | S |
| 9 Dec | 256.8 | 359.1 | 1 | 28 | 0.4049 | NS |
| Jan | 10499.2 | 374.3 | 1 | 31 | 0.0001 | S |
| Feb | 272.3 | 453.1 | 1 | 34 | 0.4436 | NS |
| 10 Dec | 2146.8 | 308.1 | 1 | 34 | 0.0124 | S |
| Jan | 6696.7 | 292.4 | 1 | 34 | 0.0001 | S |
| Feb | 2088.7 | 1128.7 | 1 | 31 | 0.1835 | NS |
| 12 Dec | 470.4 | 424.1 | 1 | 25 | 0.3024 | NS |
| Jan | 4183.1 | 296.8 | 1 | 31 | 0.0007 | S |
| Feb | 13263.4 | 417.6 | 1 | 34 | 0.0001 | S |
| 27 Dec | 3867.6 | 326.4 | 1 | 25 | 0.0021 | S |
| Jan | 3342.9 | 288.4 | 1 | 30 | 0.0019 | S |
| Feb | 1444.0 | 355.6 | 1 | 34 | 0.0519 | NS |
| 14 Dec | 297.6 | 93.4 | 1 | 34 | 0.5789 | NS |
| Jan | 7140.3 | 160.3 | 1 | 34 | 0.0001 | S |
| Feb | 75.1 | 342.5 | 1 | 34 | 0.6426 | NS |
| 15 Dec | 1065.4 | 527.6 | 1 | 31 | 0.1653 | NS |
| Jan | 2738.8 | 145.4 | 1 | 34 | 0.0001 | S |
| Feb | 552.3 | 487.5 | 1 | 34 | 0.2947 | NS |
| 28 Dec | 940.8 | 219.4 | 1 | 28 | 0.0477 | S |
| Jan | 10100.3 | 157.9 | 1 | 34 | 0.0001 | S |
| Feb | 1067.1 | 546.4 | 1 | 34 | 0.1713 | NS |
| 19 Dec | 280.2 | 143.7 | 1 | 25 | 0.1749 | NS |
| Jan | 3623.5 | 125.8 | 1 | 31 | 0.0001 | S |
| Feb | 157.6 | 423.6 | 1 | 31 | 0.5463 | NS |
| 29 Dec | 1914.9 | 231.2 | 1 | 30 | 0.0073 | S |
| Jan | 944.5 | 184.1 | 1 | 31 | 0.0306 | S |
| Feb | 2809.0 | 701.5 | 1 | 34 | 0.0534 | NS |
| 30 Dec | 499.9 | 262.2 | 1 | 19 | 0.1833 | NS |
| Jan | 2950.2 | 197.2 | 1 | 35 | 0.0005 | S |
| Feb | 4246.7 | 358.8 | 1 | 34 | 0.0016 | S |
| 31 Dec | 24.0 | 390.3 | 1 | 25 | 0.8062 | NS |
| Jan | 2950.2 | 197.2 | 1 | 35 | 0.0005 | S |
| Feb | 279.5 | 385.2 | 1 | 35 | 0.4009 | NS |
| 22 Dec | 542.7 | 317.5 | 1 | 31 | 0.2007 | NS |
| Jan | 6.8 | 3019.2 | 1 | 28 | 0.8035 | NS |
| Feb | 1034.7 | 381.5 | 1 | 34 | 0.1088 | NS |
| 24 Dec | 145.8 | 437.6 | 1 | 28 | 0.5684 | NS |
| Jan | 6.8 | 107.8 | 1 | 28 | 0.8035 | NS |
| Feb | 1034.7 | 381.47 | 1 | 34 | 0.1088 | NS |