Increasing Speed, Accuracy, and Safety of Pressure Chamber Determinations of Plant Moisture Stress

John M. McGilvray and James P. Barnett

Biological technician and principal silviculturist, USDA Forest Service, Southern Forest Experiment Station, Pineville, LA

A newly developed paper-spot technique can increase the efficiency, accuracy, and safety of the pressure chamber method routinely used by many nursery specialists and silviculturists to evaluate seedling moisture stress. Tree Planters' Notes 39(3):3-4 ; 1988.

The pressure chamber provides a simple, accurate, rapid, and practical means of measuring plant moisture stress (PMS) (2, 3). PMS is a measure of the internal moisture stress that occurs in the wood vessels, or xylem, of plants. Forest scientists often use the term xylem water potential for PMS and express PMS as negative bars or megapascals (MPa), i.e., 1 MPa = 10 bars.

In addition to its use in diagnosing plant moisture stress, the measurement of PMS is particularly useful in analyzing the environmental conditions associated with stress in nurseries and established plantations and in trouble-shooting establishment problems (1). The pressure chamber is now being routinely used to evaluate seedling moisture stress by nursery personnel and regeneration specialists.

Methods

Standard methods. To use the pressure chamber, a small twig or, in the case of pines, a group of needles in a fascicle, is cut from the seedling and placed in the steel chamber with the cut end protruding from the lid (fig. 1). The chamber is then pressurized with nitrogen and the water column within the sample is forced up to the cut surface. The pressure required to do this is equal to the tension of the water column at the time the sample was cut. When water is first observed on the protruding cut surface, the chamber pressure is recorded (fig. 2).

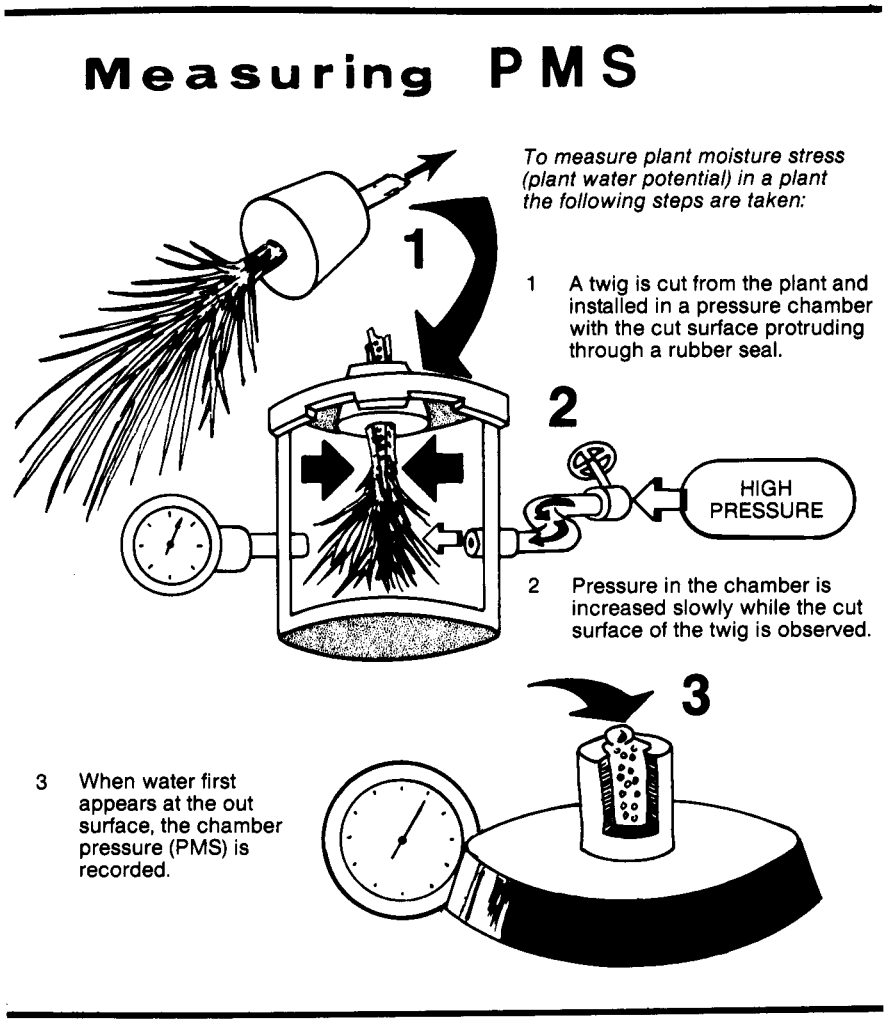

Figure 1—*Pressure chamber for monitoring plant water stress.*

In most conifers, resin bubbles appear before the water droplet and may indicate a false end point. Experience and the use of a hand lens are usually needed to determine when the true end point is reached. The determination of the true end point slows the measurements, is a source of error, and is sometimes unsafe. Because people using the pressure chamber tend to observe the cut twig or needle with a hand lens from directly above, they are at risk of being struck in the eye by needles ejected around the seal by the pressure.

New Steps. The following technique overcomes some of the current problems of determining the true end point in PMS measurements. Hold a small piece (about 1 by 1 inch) of *brown* paper hand toweling firmly against the end of the cut stem. As soon as a droplet of water reaches the cut surface and the true end point is reached, a readily visible, wet spot appears on the paper towel. The wet spot will appear darker than the dry toweling. Although the resin is released before the water, the resin exudation is not absorbed and therefore does not change the color of the paper. No hand lens is needed, and the wetting of the towel can be seen from a distance, so there is no need to bend directly over the seedling to observe the end point.

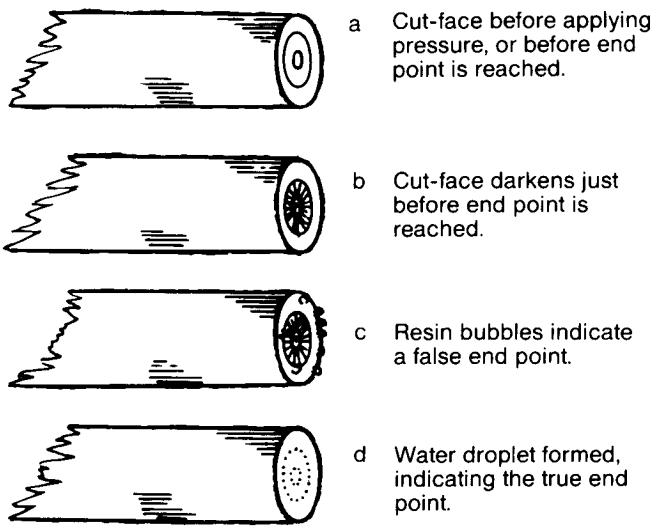

Figure 2—*Reading the end point on a cut seedling surface.*

When PMS measurements are made of fascicled needles (a more difficult operation), the moisture droplet is so small that ordinary paper toweling may not work. In this case, a finer textured paper such as cigarette paper can be used. Again, brown paper works best because the wet spot is more readily visible, and the use of a hand lens is not necessary.

Results

This paper-spot technique greatly simplifies the recording of PMS measurements. It results in more accurate determinations, greater speed in making measurements, and increases the safety of the process.

Literature Cited

1. Day, R.J.; Walsh, S.J. A manual for using the pressure chamber in nurseries and plantations. Silv. Rep. 19802. Thunder Bay, ON: Lakehead University School of Forestry; 1980. 49 p.
2. Scholander, P.F.; Hammel, H.T.; Hemmingson, E.A.; Bradstreet, E. D. Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. Proceedings of the National Academy of Sciences 52:119-125; 1964.
3. Scholander, P.F.; Hammel, H.T.; Bradstreet, E.D.; Hemmingson, E.A. Sap pressured in vascular plants. Science 148:229-246; 1965.

Hydraulic Conductance of Roots Present at the Time of Lifting and Newly Regenerated Roots of 2 + 0 Eastern White Pine Seedlings

Kurt H. Johnsen, Steven P. McConnell, Jon C. Regelbrugge, and John R. Seiler

Graduate assistants and assistant professor, Virginia Polytechnic Institute and State University, Blacksburg

Root hydraulic conductivity was measured on eastern white pine (Pinus strobus *L.) seedlings with varying proportions of newly regenerated roots. The surface areas of the tap root, old lateral roots, and newly regenerated roots were measured, and hydraulic conductance per seedling was calculated. Hydraulic conductance was significantly affected by the percent of the root system composed of newly regenerated roots as well as the temperature of the water used in the testing system. An increase from 0 to 25% new root surface is estimated to result in a 123% increase in hydraulic conductance.* Tree Planters' Notes 39(3): 5-8 ; 1988.

Root growth potential (RGP), the ability of a bareroot seedling to grow new roots when placed in a favorable environment, has been often correlated with high survival and increased growth (1, 2), making it a reliable physiological indicator of seedling vigor.

RGP is a good predictor of outplanting success for a number of reasons. First, a seedling with high RGP quickly grows new roots and establishes contact with the soil. Secondly, a seedling with high RGP is a vigorous one that has generally high overall growth potential. A third probable reason is that a seedling with high RGP quickly grows new roots, which may be much more efficient at water and nutrient uptake than the roots present at the time of lifting.

Root hydraulic conductance is a measure of the ability of a root to take up water. Root hydraulic conductance is typically measured by severing the top of a seedling, inserting the root system in a liquid-filled pressure chamber set at a standard pressure, and measuring the amount of liquid that is forced through the severed end of the stem in a standard time interval.

Hydraulic conductance is calculated by dividing the volume of measured liquid by the time interval used, the surface area or weight of the root system, and the pressure units. Roots with high hydraulic conductance provide less resistance to water flow and allow more water to move to the stem than roots with low hydraulic conductance.

It is still not clear if newly regenerated roots are more efficient at water uptake than roots present at the time of lifting (which typically are suberized) and if so, how much more. Chung and Kramer (3) demonstrated that removal of unsuberized roots from root systems on 1-year-old loblolly pine decreased hydraulic conductance but to a degree lower than the proportion of the root system removed. Sands et al. (4) showed that unsuberized roots have substantially higher hydraulic conductance than older roots.

This study was undertaken to determine the relative difference in hydraulic conductivity of roots present at the time of lifting (January) compared with newly regenerated roots. This information will be useful in assessing the relative importance of old versus new roots in seedling establishment after outplanting.

Materials and Methods

Two-year-old white pine seedlings were hand lifted from four locations (blocks) within the Virginia Department of Forestry Augusta State Nursery (Waynesboro, VA) on January 21, 1987. The seedlings were root pruned at 12 cm below the root collar and then placed in a hydroponic system as described by DeWald et al. (5). The blocks were maintained as from the nursery.

Testing of root hydraulic conductivity of the white pine root systems began on February 9, 1987, after the seedlings had time to initiate new roots. Tests were performed at 3- or 4-day

intervals to obtain a range in the proportion of new white roots.

On each test date, the root system of one seedling from each of the four original nursery locations was tested for hydraulic conductance. In order to remove potentially confounding age effects, at least one seedling that had no newly regenerated roots was tested on each test date. This resulted in a total sample size of 30.

The top of each seedling was severed 4 cm above the junction of the highest lateral root. The stem surface was covered with high-vacuum grease and then inserted into a piece of rubber tubing. The tubing and stem were then inserted into the lid of a pressure chamber and a pipette was inserted into the tubing. Each root system was then placed in a plastic cylinder filled with tap water located in a pressure chamber.

After chamber pressure was raised to 5 bars, the volume of water flowing through the root systems was measured with the attached graduated pipette. Measurements were recorded at 10-minute intervals until the change in volume over the time interval remained constant for a minimum of four readings, indicating that the system had reached equilibrium. The change in volume at equilibrium was used for calculating hydraulic conductance. The temperature of the water in each chamber was also recorded.

Root surface area was estimated using a LICOR 3000 portable area meter. Surface area was estimated separately for the tap root, the old lateral roots (roots present at the time of lifting), and any newly regenerated lateral roots. Dry weights of the above categories of roots were measured to the nearest 0.001 g after the roots were dried to a constant moisture content at 60 °C.

Linear regression techniques were used in analyses, with root hydraulic conductance as the dependent variable. Independent variables used in the analysis were the percentage of the root system composed of new roots, tap root, and old lateral roots, water temperature at the time of testing, duration of time each seedling remained in the hydroponic system, and nursery block location. Root system proportions were analyzed on both a surface area and dry weight basis.

Results

Hydraulic conductance was significantly affected by both the percentage of the root system composed of newly regenerated roots and the water temperature during testing. The ratio of tap root to old lateral root, duration in the hydroponic system, and nursery block location were all nonsignificant predictors.

Because analyses using root dry weights provided comparable results, all further discussion will refer to analyses using root system surface area.

The best model resulting from linear regression analyses is:

Hydraulic conductance ($\times 10^6$) =
$-1.252 + 0.109$ (H_20 temp.)
$+ 0.036$ (% new roots)

Water temperature and percentage new roots are both highly significant ($P < 0.01$). Observed data and the regression line, using the mean water temperature (18.15 °C) from all test dates, are shown in figure **1.**

Discussion

Results indicate that newly regenerated roots are more efficient in water absorption than the root system present on the seedling at the time of lifting in late January. Using the regression model and the average temperature (18.15 °C) for all tests, a seedling with no newly regenerated roots is estimated to have a hydraulic conductance of 0.73×10^{-6} cm sec^{-1} bar^{-1}, while a seedling with 25% of the root system comprised of newly regenerated roots is estimated to have a hydraulic conductance of 1.63×10^{-6} cm sec^{-1} bar^{-1}. An increase from 0 to 25% new root surface area, therefore, results in

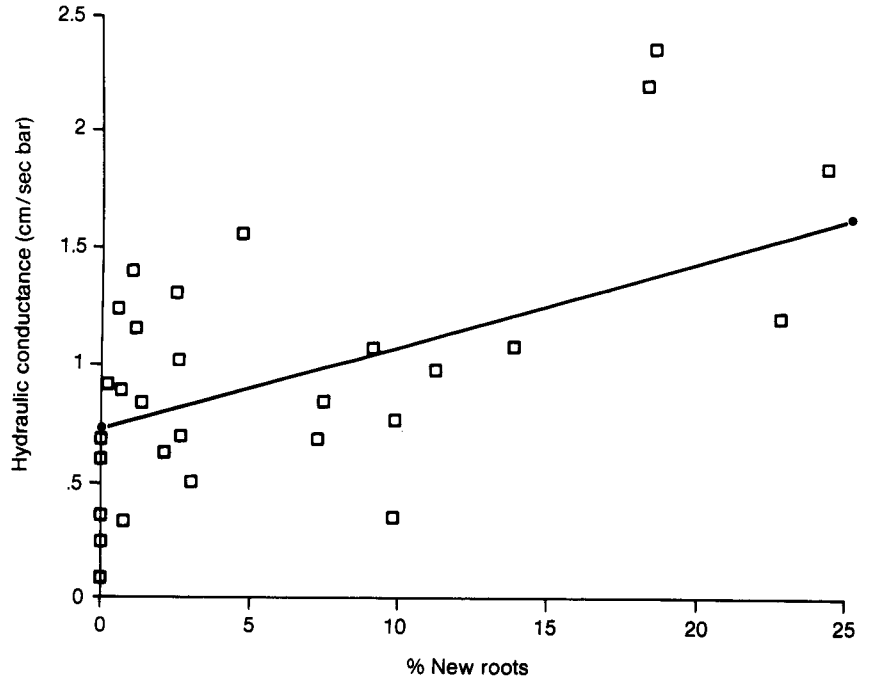

Figure 1—*Relationship of the percentage of the root system comprised of newly regenerated roots on hydraulic conductance of 2+0 eastern white pine. Regression line drawn using mean H_2O temp. of 18.15 °C with model: hydraulic conductance ($\times 10^6$) = −1.252 + 0.109 (H_2O temp.) + 0.036 (percentage new roots); N = 30, r^2 = 0.56.*

a 123% increase in hydraulic conductance. Extrapolation was used to calculate an estimate of the hydraulic conductivity of newly regenerated roots. A root system composed of all new white roots is estimated to have a hydraulic conductance of 4.35 x 10^{-6} cm sec^{-1} bar^{-1}. This would be a 493% increase in efficiency in water absorption in newly regenerated versus old roots. These estimates are comparable to those made by Sands et al. (4) and Chung and Kramer (3) using loblolly pine.

Water temperature of the testing system was a highly significant factor affecting root hydraulic conductance, and this confounds the plot of hydraulic conductance x the percentage new roots (shown in figure 1). This relationship has previously been shown by Sands et al. (4). Kramer (6) also demonstrated that root hydraulic conductance of eastern white pine was highly influenced by temperature and that the range of approximately 15 to 21 °C had the greatest effect. In this temperature range the relationship was approximately linear. The range of temperatures in this study was from 14 to 21.5 °C.

It should be understood that this study was not undertaken to examine the difference in hydraulic conductance between unsuberized and suberized roots, per se, but instead between root systems present at the time of lifting and newly regenerated roots. Newly regenerated roots were largely unsuberized, but the extent of suberization was dependent on length and age. Differences between suberized and unsuberized roots would be expected to be larger than differences estimated between new and old roots in this study.

Furthermore, hydraulic conductance has been shown to be greatly influenced by growing media (3). Because seedlings in this study were maintained in an artificial hydroponic system, estimates of hydraulic conductance may not be comparable to levels occurring in outplanted seedlings. Exact estimates are considered less important than the relative differences between old and new roots.

Root growth potential (RGP), the ability of a bareroot seedling

to grow new roots when placed in a favorable environment, has been found to be a useful predictor of field performance in several species in the Pinaceae (1, 2, 7, 8). Results of this study point to the importance of new root growth in increasing the capacity of a eastern white pine seedling to uptake water and thus points to the value of high RGP. Johnsen et al. (9) demonstrated that RGP was a useful predictor of first-year field performance, but that consistency in a seedling lot's ability to grow new roots was more important than average new root production. This relationship was stronger on non-irrigated than on irrigated sites.

Estimates obtained from this study indicate that new root growth does increase hydraulic conductivity but also indicate that roots present on the seedling at the time of lifting can conduct water. In fact, at early planting dates when soil temperatures are not yet conducive to new root growth, old roots must provide all water absorptive surface area. This may also be concurrent with times of lower transpirational demand. As environmental temperatures increase, transpiration increases, old root hydraulic conductivity increases, and more efficient new roots are produced. The relative importance of new and old roots may be dependent on soil temperature, soil moisture levels, degree of contact between old roots and soil, and vapor pressure deficits between needles and the atmosphere.

Literature Cited

1. Feret, P.P.; Kreh, R.E. Seedling root growth potential as an indicator of loblolly pine field performance. Forest Science 31:1005-1011; 1985.
2. Ritchie, G.A. Root growth potential: principles, procedures, and predictive ability. In: Duryea, M.L., ed. Proceedings, Evaluating seedling quality: principles, procedures, and predictive abilities of major tests; 1984 Sept. 16 18; Corvallis, OR. Corvallis, OR: Oregon State University, Forest Research Laboratory; 1985.
3. Chung, H.; Kramer, P.J. Absorption of water and '2P through suberized and unsuberized roots of loblolly pine. Canadian Journal of Forest Research 5:229-235; 1975.
4. Sands, R.; Fiscus, E.L.; Reid, C.P.P. Hydraulic properties of pine and bean roots with varying degrees of suberization, vascular differentia tion and mycorrhizal infection. Australian Journal of Plant Physiology 9:559-569; 1982.
5. Dewald, L.E.; Feret, P.P.; Kreh, R.E. A 15-day hydroponic system for measuring root growth potential of stored loblolly pine seedlings. In: Gen. Tech. Rep. SO-54. Proceedings, Third Biennial Southern Silviculture Research Conference; 1984 November 7-8; Atlanta, GA. New Orleans, LA: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station; 1985: 4-10.
6. Kramer, P.J. Species differences with respect to water absorption at low soil temperatures. American Journal of Botany 29:828-832; 1942.
7. Jenkinson, J.L.; Nelson, J.A. 1-0 Douglas-fir: a bare-root planting option. In: Proceedings, Western Forest Nursery Council Western Nurserymen's Conference; 1982 August 10-12; Medford, OR. Ashland, OR: Southern Oregon State College, Southern Oregon Regional Services Institute; 1983: 63-76.
8. Brissette, J.C.; Roberts, T.C. Seedling size and lifting date effects on root growth potential of loblolly pine from two Arkansas nurseries. Tree Planters' Notes 35:34-38; 1984.
9. Johnsen, K.H.; Feret, P.P.; Seiler, J.R. Root growth potential as a predictor of first-year field performance for non-irrigated and irrigated eastern white pine seedlings. In: Gen. Tech. Rep. SE-42. Proceedings, Fourth Biennial Southern Silviculture Research Conference, 1986 November 4-6; Atlanta, GA. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station; 1987: 245-250.

A BASIC Computer Program to Calculate Daily Potential Evapotranspiration

Douglas M. Stone

Research forester, USDA Forest Service, North Central Forest Experiment Station, Grand Rapids, MN

A BASIC microcomputer program was developed to calculate daily potential evapotranspiration (PET) by the method of Thornthwaite and Mather. Daily and cumulative PET values are calculated and displayed as rapidly as daily air temperatures are entered. In example, PET is used as an index of actual evapotranspiration where soil water is maintained at low tensions. Tree Planters' Notes 39(3): 9-12; 1988.

In 1948, Thornthwaite (3) introduced the concept of potential evapotranspiration (PET)—the water lost from a fully vegetated site that is well supplied with soil water. Although PET is determined by many climatic factors, it can be estimated from average air temperature and day length using a method developed by Thornthwaite and Mather (4). This method has been applied over a wide range of forest environments. Zahner (7) first used it to calculate monthly and growing season soil water deficits of southern pine sites across the mid-South.

In Alaska, where tree growth is frequently limited by temperature, Patric and Black (2) reported that PET reflected forest distribution better than temperature alone. Verry and Timmons (6) found that the PET of a complex upland-peatland watershed in northern Minnesota approximated the water balance value within 8% on an annual basis and within 3% over a 3-year period of average to wet years. First-year survival of pine seedlings planted from 1929 to 1976 on droughty sands in Michigan was significantly related to growing season soil water deficits calculated as the difference between monthly precipitation and PET (1).

A BASIC microcomputer program to estimate PET by the Thornthwaite and Mather method was developed as part of a study to determine nutrient leaching in a sandy forest soil treated with simulated acid rain. Two-year-old white spruce *(Picea glauca* (Moench) Voss) seedlings are being grown in 15 x 30-cm soil columns equipped with porous polyethylene filters to collect leachate passing through the root zone. The seedlings are covered at night and during periods of rain to exclude dew and natural rainfall. The soil cores are treated weekly; the leachate is collected, weighed, and analyzed for 20 chemical characteristics. About 100 ml of leachate is required for the analyses. We use weekly PET values to estimate how much water to apply to the soil columns to obtain a sufficient sample and to avoid excessive leaching due to overwatering.

Data Files

To calculate PET, two data files are required—one for unadjusted PET and one for daylength. Values for each are given in Thornthwaite and Mather (5). The average monthly temperature for the location must be known to determine the proper values. Figure 1 lists a BASIC program to enter the unadjusted PET values into a data file called "UPET." Comments following apostrophes provide information for the user but are ignored by the computer. The statement at line 150 opens the file and the loop at lines 160-230 enters the data for temperatures from 34 to 80 °F in 0.5° increments.

PET at temperatures below 34 °F is negligible for most areas; values for average daily temperatures above 80°F are available in Thornthwaite and Mather (5). It is quicker to simply enter the numbers and let the computer place the decimal (line 180); the values are displayed at line 190. The optional check at line 200 requires an answer for each entry; however, this may be quicker than manually checking and correcting errors after the file is created.

Figure 2 lists a program to generate the day-length file; it works in the same way as the previous one. Day-length values in 12-hour units (without the decimal) are entered for each

```
100  'TEMPET.BAS          D. M. STONE          12-2-86
110  '
120  'TO LOAD TEMP & PET DATA IN "UPET" FILE
130  '(THORNTHWAITE & MATHER 1957, TABLE 3.)
140  '
150  OPEN "O", #1, "UPET"
160  FOR T = 34 TO 80 STEP .5
170      INPUT "ENTER THE PET VALUE";PE
180      P = PE/100
190      PRINT, T,P
200      INPUT "IS THE PET CORRECT, (Y OR N)"; A$
210      IF A$ = "N" THEN 170
220      PRINT #1, T;P
230      NEXT T
240  CLOSE #1
250  END
```

Figure 1—*A BASIC program to create the unadjusted PET (UPET) file.*

```
100  'SUNLITE.BAS          D. M. STONE          12-2-86
110  '
120  'TO LOAD DAY LENGTH DATA IN "DAYLT" FILE
130  '(THORNTHWAITE & MATHER 1957, TABLE 8.)
140  '
150  OPEN "O", #1, "DAYLT"
160  FOR J = 1 TO 365          '(J = JULIAN DATE)
170      INPUT "ENTER THE DAY LENGTH"; DL
180      D = DL/100
190      PRINT, J,D
200      INPUT "IS THE DAY LENGTH CORRECT, (Y OR N)"; A$
210      IF A$ = "N" THEN 170
220      PRINT #1, J;D
230      NEXT J
240  CLOSE #1
250  END
```

Figure 2—*A BASIC program to generate the day-length (DAYLT) file.*

day of the year. The latitude of the site must be known to enter the appropriate values for duration of sunlight. For sites in northern latitudes, this program could be modified to exclude the winter months, provided the appropriate Julian dates are used. If, for example, you wish to use an April through October season, statement 160 should read "FOR J = 91 to 304." This shortened season would require less memory, and its file would load more quickly. We used the data for the entire year so that we could evaluate the PET program under greenhouse conditions during the winter.

It takes about an hour to create the unadjusted PET and the day-length files; they then can be copied onto a backup disk and used indefinitely. The two files combined occupy less than 6,000 bytes of memory.

The Program

The program calculates and displays daily PET values for 7 days in just over a minute or values for 1.4 days in less than 2 minutes. The calculations are performed as fast as the data are entered. It takes about 6 seconds to load the two data files into the memory. The program and files combined require less than 8,000 bytes.

Figure 3 is a listing of the program. The dimension statements (DIM) on lines 330 and 340

```
100 '"PET"          D. M. 'STONE          12-2-86
110 '
120 'TO CALCULATE DAILY POTENTIAL EVAPOTRANSPIRATION (PET)
130 '       BY THE THORNTHWAITE & MATHER (1957) METHOD.
140 '
150 '
160 '    ********************************
170 '    *                              *
180 '    *        VARIABLE NAMES:       *
190 '    *                              *
200 '    *  J = JULIAN DATE             *
210 '    *  N = NUMBER OF DAYS          *
220 '    *  P = UNADJUSTED PET          *
230 '    *  T = AVERAGE DAILY TEMPERATURE (F)
240 '    *  DL = DAYLENGTH              *
250 '    *  TN = MINIMUM DAILY TEMPERATURE
260 '    *  TX = MAXIMUM DAILY TEMPERATURE
270 '    *  PET = DAILY POTENTIAL EVAPOTRANSPIRATION
280 '    *  TPET = TOTAL PET FOR THE PERIOD
290 '    *  UPET = UNADJUSTED PET FILE NAME
300 '    *  DAYLT = DAY LENGTH FILE NAME
310 '    ********************************
320 '
330 DIM A(99)
340 DIM B(370)
350 OPEN "1", #1, "UPET"
360 WHILE NOT EOF (1)
370     INPUT #1, T,P
380     A(T) = P
390     WEND
400 '
410 OPEN "I", #2, "DAYLT"
420 WHILE NOT EOF (2)
430     INPUT #2, J,DL
440     B(J) = DL
450     WEND
460 '
470 LET TPET = 0
480 INPUT "ENTER THE JULIAN DATE OF THE FIRST DAY AND NO. OF DAYS.";J,N
490 PRINT
500 FOR I = 1 TO N
510     INPUT "ENTER THE MAX. & MIN. TEMP"; TX,TN
520     PRINT
530     PRINT "DAY NO."I,"MAX.",TX,"MIN.", TN
540     INPUT "ARE ALL ENTRIES CORRECT, (Y OR N)";A$
550     IF A$ = "N" THEN 510
560     T = (TX + TN)/2
570     PET = A(T) * B(J)
580     TPET = TPET + PET
590     PRINT,"PET FOR DAY";I;"=";PET,"TOTAL FOR THE PERIOD IS:";TPET
600     PRINT
610     B(J) = B(J + 1)
620     NEXT I
630 '
640 PRINT
650 PRINT "THE ESTIMATED PET FOR THIS";N;"-DAY PERIOD IS:";TPET;" In."
660 PRINT
670 INPUT "DO YOU WANT TO MAKE ANOTHER RUN, (Y OR N)"; A$
680 IF A$ = "Y" THEN 470
690 CLOSE #1 : CLOSE #2
700 END
```

Figure 3—*Listing of a BASIC program to calculate daily PET.*

reserve space in arrays A and B for the two data files; lines 350 and 410 open them for input to the program. The loop on lines 360-390 loads the temperature and unadjusted PET values from the "UPET" file into memory; that on lines 420-450 loads the Julian dates and corresponding day-length values. The PET accumulator is set to zero in 470. At 480 the beginning date and number of days in the run are entered; they must be separated by a comma. The main loop begins at 500; in 510 the maximum and minimum temperatures for the first day are entered (separated by a comma). Print statements display a blank line on the monitor and make it easier to read. Line 530 displays the day number and corresponding temperatures; 540 is an optional check to ensure that the correct values were entered. If they were, the "Y" key is pressed to continue; if not, the "N" is pressed and the program returns to 510 so that the correct values can be entered. At 560 the average temperature for the day is calculated, and in 570 the PET (the product of the values in the two arrays). The daily PET is added to the total in 580, and both are displayed at 590. The Julian date is incremented in 610, and 620 transfers control back to 510 to enter the temperatures for the next day. After the loop is completed for

the number of days specified in 480, the results are displayed in 650. If additional calculations are desired, pressing the "Y" key at 670 transfers control back to 470; otherwise, the data files are closed and the program ends.

Discussion

PET data have been used mainly in conjunction with precipitation measurements to estimate water balances and/or soil water deficits for monthly (7), growing season (1), annual (6), or longer periods (2). PET also can be used to estimate actual evapotranspiration (AET) for short intervals because the two agree closely as long as soil water is readily available; that is, at tensions up to about 2 atmospheres (8). For the spruce seedlings described earlier, we can usually estimate weekly AET within 50 ml, or about 0.1 inch. PET is thus a useful index of AET, enabling us to predict the water needed to recharge the soil columns and obtain the required volume of leachate for analyses.

Equally important, it helps avoid overwatering.

Thornthwaite's method of calculating PET has been widely applied because it provides good estimates, requires only average daily air temperatures, and is easily calculated. Daily or periodic PET estimates could be useful in forest nurseries or in other environments where soil water is maintained at low tensions. Estimates of PET may be especially useful where overwatering could cause excessive leaching or other problems and where pumping costs must be minimized.

Copies of the program may be obtained by sending a formatted 360-K, 5¼-inch diskette to Douglas M. Stone, USDA Forest Service, Forestry Sciences Laboratory, 1831 Highway 169 East, Grand Rapids, MN 55744.

Literature Cited

1. Cleland, D.T.; Johnson, J.E. Pine plantation survival related to calculated moisture deficits on the Huron National Forest (1929-1976). Tree Planters' Notes 37:17-22; 1986.
2. Patric, J.H.; Black, P.E. Potential evapotranspiration and climate in Alaska by Thornthwaite's classification. Res. Paper PNW-71. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station; 1968. 28 p.
3. Thornthwaite, C.W. An approach toward a rational classification of climate. Geographical Review 38:55-94; 1948.
4. Thornthwaite, C.W.; Mather, J.R. The water balance. Drexel Institute of Technology, Laboratory of Climatology, Publications in Climatology 8(1): 1-86; 1955.
5. Thornthwaite, C.W.; Mather, J.R. Instructions and tables for computing potential evaporation and the water balance. Drexel Institute of Technology, Laboratory of Climatology, Publications in Climatology 10(3): 1-311; 1957.
6. Verry, E.S.; Timmons, D.R. Waterborne nutrient flow through an upland-peatland watershed in Minnesota. Ecology 63:1456-1467; 1982.
7. Zahner, R. Evaluating summer water deficiencies. Occ. Paper 150. New Orleans, LA: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station; 1956. 18 p.
8. Zahner, R. Refinement in empirical functions for realistic soil-moisture regimes under forest cover. In: Sopper, W.E.; Lull, H.W., ed. Forest hydrology. New York: Pergamon; 1967: 261-274.

Loblolly Pine Seedling Morphology and Production at 53 Southern Forest Nurseries

James N. Boyer and David B. South

Research associate and associate professor and director, Auburn University Southern Forest Nursery Management Cooperative, Alabama Agricultural Experiment Station, Auburn

*Seedling morphology of loblolly pines (*Pinus taeda *L.) was surveyed at 53 southern nurseries. Selected cultural and soil data are also given. A range of conditions were sampled at each nursery. Morphology varied widely among samples. Seedbed density and sowing date influenced seedling size.* Tree Planters' Notes 39(3):13-16; 1988.

Loblolly pine (*Pinus taeda* L.) is the most commonly planted tree in this country, with well over a billion seedlings produced each year. Loblolly pine seedling morphology can vary considerably, depending upon genotype, cultural practices, soil conditions, and numerous other environmental factors. As an example, Burns and Brendemuehl (2) found that seedling morphological grade varied considerably among three Florida State forest nurseries. Most importantly, seedling morphology can affect survival and growth upon outplanting (7, 9). Morphological measurements of seedlings have been reported in many research studies, but there is little information available on the morphology of loblolly pine seedlings produced operationally at forest nurseries across the South.

This paper reports the results of a survey of loblolly pine seedling production throughout the South. The objectives of this survey were to determine:

1. the range and distribution of loblolly pine seedling morphology produced at southern forest nurseries; and
2. any significant correlations of certain soil factors or cultural practices with seedling production and morphology.

Materials and Methods

During the second and third weeks in December 1982, four separate crews visited a total of 53 forest tree nurseries in 13 Southern States (fig. 1). Most of these nurseries were on the Coastal Plain. Three different sites within each nursery were chosen in order to examine a range of conditions. (In several nurseries, fewer than three samples were taken.) Each sample

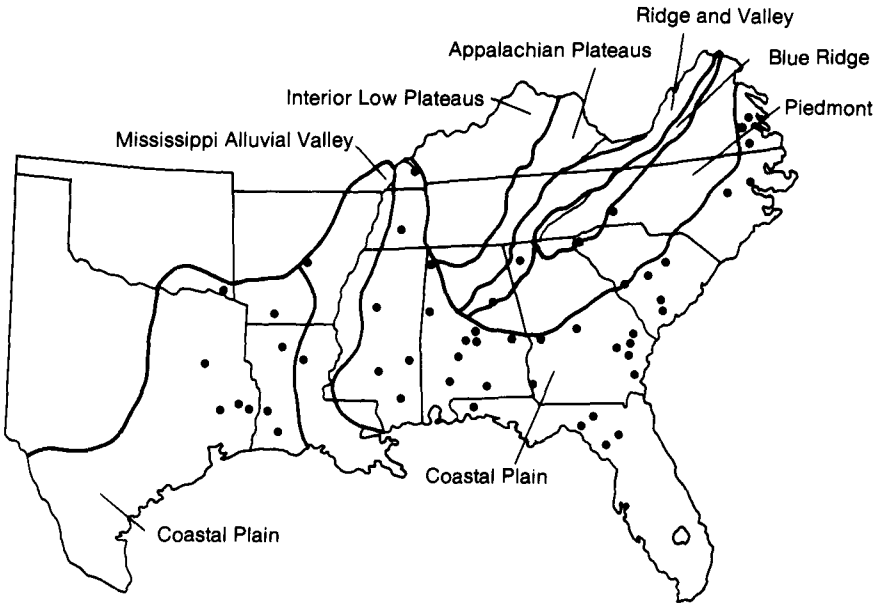

Figure 1—*Locations of sampled nurseries.*

Table 1—*Cumulative percentile distributions for 153 nursery samples in the Southern United States*

Seedling variable	Minimum value	Values for percentile					Maximum value
		10th	25th	50th	75th	90th	
Median sample height (cm)	7.0	15.3	19.0	21.0	25.0	28.0	31.0
Median sample diameter (mm)	1.1	2.5	2.8	3.3	3.7	4.0	4.5
Height/diameter (mm/mm)	38.1	51.4	60.9	70.0	79.5	87.5	113.6
Percent in quality grade[a]							
Grade 1	0.0	0.0	0.8	4.3	10.2	16.9	32.4
Grade 2	0.0	18.8	30.1	46.2	60.9	69.5	81.7
Lg. cull	5.4	15.3	24.0	35.7	49.2	61.4	82.7
Sm. cull	0.0	1.7	3.2	7.2	15.4	23.8	91.7
Weight (g DW/sample)							
Shoot	9.1	109.8	154.3	198.0	247.5	319.3	457.0
Root	1.9	35.4	45.0	54.5	68.0	101.0	157.1
Total	11.0	147.8	201.8	255.4	317.5	406.3	576.2
Shoot wt./root wt.	1.5	2.5	2.8	3.4	4.2	4.9	6.0
Total sample (g/g)							
Grade 1	0.8	2.1	2.5	3.1	3.9	5.0	6.8
Grade 2	1.4	2.4	2.9	3.4	4.1	4.9	6.0
Lg. cull	1.1	2.5	3.0	3.7	4.7	5.5	7.7
Sm. cull	0.3	1.8	2.8	4.1	5.7	7.9	31.0
Quality index[b]	0.03	0.12	0.15	0.21	0.27	0.34	0.52
Basal area (cm^2/sample)	0.4	5.7	8.0	10.7	13.4	16.1	21.1
Density (no./sample)	36	77	98	122	140	168	234
Sowing date (mo./day)	3/26	4/5	4/8	4/19	5/1	5/6	6/7

[a]Grade 1 = ≥ 4.8 mm diameter, grade 2 = 3.2–4.7 mm, large culls = 2.0–3.1 mm, small culls = < 2.0 mm.
[b]Quality index equals the average seedling weight in grams (shoot plus root) divided by the sum of the shoot–root ratio and 0.1 of the height–diameter ratio.

Table 2—*Cumulative percentile distributions for average seedling weights*

Seedling variable	Minimum value	Values for percentile					Maximum value
		10th	25th	50th	75th	90th	
Weight (g DW/seedling)							
All							
Shoot	0.25	1.03	1.33	1.67	2.16	2.56	3.37
Root	0.05	0.30	0.37	0.50	0.59	0.78	1.29
Total	0.31	1.41	1.67	2.14	2.74	3.35	4.17
Grade 1							
Shoot	0.70	2.48	3.23	4.10	4.80	5.81	9.90
Root	0.30	0.70	1.00	1.25	1.53	1.90	4.02
Total	1.20	3.40	4.43	5.40	6.24	7.47	13.80
Grade 2							
Shoot	1.17	1.63	1.85	2.19	2.51	2.77	3.33
Root	0.31	0.48	0.52	0.61	0.73	0.89	1.55
Total	1.62	2.16	2.47	2.88	3.16	3.56	4.28
Lg. cull							
Shoot	0.35	0.73	0.88	1.09	1.32	1.53	2.02
Root	0.09	0.19	0.23	0.28	0.37	0.47	0.79
Total	0.44	0.97	1.14	1.37	1.68	1.97	2.42
Sm. cull							
Shoot	0.05	0.21	0.30	0.43	0.52	0.70	1.20
Root	0.01	0.04	0.07	0.10	0.16	0.20	0.90
Total	0.09	0.27	0.40	0.53	0.69	0.86	1.60

Table 3—*Cumulative percentile distributions for selected soil variables*

Soil variable	Minimum value	Values for percentile					Maximum value
		10th	25th	50th	75th	90th	
≥ 75% Sand (60 samples)							
Organic matter (%)	0.5	0.6	0.8	1.2	1.5	2.2	2.5
CEC (meq/100 g)	1.0	1.2	1.5	1.9	2.3	2.7	3.4
pH	4.5	5.0	5.2	5.4	6.0	6.1	6.4
Bulk density (g/cm³)	1.2	1.3	1.3	1.4	1.5	1.6	1.6
50–75% Sand (68 samples)							
Organic matter (%)	0.8	1.2	1.3	1.6	2.1	2.6	3.2
CEC (meq/100 g)	1.3	1.9	2.5	3.0	3.5	3.8	4.6
pH	4.8	5.1	5.3	5.5	6.0	6.4	6.9
Bulk density (g/cm³)	1.0	1.2	1.3	1.4	1.5	1.6	1.7
<50% Sand (25 samples)							
Organic matter (%)	1.1	1.3	1.4	1.7	2.0	2.5	2.8
CEC (meq/100 g)	2.4	3.3	4.5	5.3	6.2	7.0	7.8
pH	5.0	5.1	5.5	5.8	6.4	7.1	7.2
Bulk density (g/cm³)	1.0	1.2	1.2	1.3	1.4	1.5	1.7

consisted of the seedlings lifted from 1 linear foot of seedbed.

Seedlings were lifted by hand, and each sample was labeled *good, poor*, or *average* according to the nursery manager. At each seedling sample location, soil from the surface 15 cm was collected for determination of sand content, pH, cation exchange capacity (CEC), and organic matter and nutrient content. Soil bulk density and infiltration rate were also measured at these locations. For each sample, density (seedlings per linear foot of seedbed) and sowing date were recorded. Soil samples were analyzed by A&L Laboratories, Memphis TN.

Stem height and root collar diameter were measured on individual air-dried seedlings. Seedlings were separated into four grades (based on root collar diameter) and ovendried at 70 °C. Seedlings from each grade were combined for measurement of total shoot and root weights.

Calculations were then made of height-diameter ratio (5), shoot-root ratio, average seedling weight (shoot, root, and total), total sample biomass, percentage of the sample in each seedling grade (as measured by diameter), and a "quality index." The quality index is equal to the average seedling dry weight (shoot plus root) divided by the sum of the shoot-root ratio and 0.1 of the height-diameter ratio (3).

Distributions of the seedling variables are shown as percentiles (tables 1, 2, and 3). The value given for a particular percentile is higher than the values for that percentage of the samples. For instance, in the first entry in table 1, 25% of the samples had a median height below 19 cm. Correlation coefficients were calculated between seedling data and soil and cultural data to determine which factors influence seedling production most strongly.

Results and Discussion

Seedling production and morphology among the 53 nurseries varied considerably (table 1). Most nurseries were producing very few grade 1 trees. For example, 50% of the samples contained 4.3% grade 1 seedlings or less. Table 2 shows that, even within grades, morphology (seedling weight) can vary widely.

For instance, average root weight for grade 1 trees ranged from 0.3 to 4 g. As seedling grade decreased (from grade 1 to cull), shoot-root ratio tended to increase (table 1). This illustrates that grade 1 seedlings are larger by weight and direct more of their energy into root development than grade 2 or cull seedlings.

Correlations between soil variables and seedling morphology were generally poor, with correlation coefficients less than 0.27. Seedling size decreased slightly with higher levels of potassium, calcium, and manganese. It may be that these results are confounded with other factors. Bulk density was the only other soil factor that was correlated with seedling variables; high bulk densities were associated with poorer seedlings.

Of the samples taken from nurseries with at least 50% sand, 25% had a bulk density of greater than 1.5 g/cm^3. Distributions for bulk density, CEC, organic matter, and pH are given in table 3. For a more complete description of the distributions of soil nutrient levels in southern forest nurseries, see South and Davey (6). Likewise, the reader is referred to Boyer and South (1) for distributions of loblolly pine seedling nutrient levels.

The highest correlations were between seedbed density and total sample biomass (r = 0.54), basal area (r = 0.55), and height-diameter ratio (r = 0.49). Correlations between seedbed density or sowing date and most morphological measures were highly significant. When these correlations were examined, it was found that lower seedbed density and earlier sowing date resulted in larger and heavier seedlings with a higher quality index. Dry weight and shoot-root ratio of loblolly pine seedlings have been shown to be related to seedbed density, with weight decreasing and shoot-root ratio increasing with increasing density (4). Burns and Brendemuehl (2) found that seedling size decreased with increasing density, with the proportion of large high-grade seedlings reduced and the proportion of small cull seedlings increased at greater seedbed densities.

Van den Driessche (8) reported that not only do seedling dry weight and stem diameter decrease as seedbed density increases, but the tree percent, or number of seedlings obtained from a given amount of seed, also decreases as density increases. He also stressed the importance of sowing as early as possible in the spring. A week or two gained in the spring has a disproportionately large effect on seedling morphology at the end of the season because of the exponential nature of seedling growth.

Literature Cited

1. Boyer, J.N.; South, D.B. Nutrient content of nursery-grown loblolly pine seedlings. Circ. 282. Auburn, AL: Auburn University, Alabama Agricultural Experiment Station. 1985.
2. Burns, R.M.; Brendemuehl, R.H. Nursery bed density affects slash pine seedling grade and grade indicates field performance. Res. Paper SE-77. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station; 1971.
3. Dickson, A.; Leaf, A.L.; Hosner, J.F. Quality appraisal of white spruce and white pine seedling stock in nurseries. Forestry Chronicle 36:10-13; 1960.
4. Harms, W.R.; Langdon, O.G. Competition-density effects in a loblolly pine seedling stand. Res. Paper SE-161. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station; 1977. 8 p.
5. Hodgson, T.J.; Donald, D.G.M. Research measurements of conifer seedlings in South Africa. South African Forestry Journal 113:1-5; 1980.
6. South, D.B.; Davey, C.B. The southern forest nursery soil testing program. Circ. 265. Auburn, AL: Auburn University, Alabama Agricultural Experiment Station; 1983. 38 P.
7. South, D.B.; Boyer, J.N.; Bosch, L. Survival and growth of loblolly pine as influenced by seedling grade: 13year results. Southern Journal of Applied Forestry 9:76-81; 1985.
8. Van den Driessche, R. Forest nursery handbook. Res. Note 48. Victoria, BC: British Columbia Forest Service; 1969. 44 p.
9. Wakeley, P.C. Planting the southern pines. Monogr. 18. Washington, DC: U.S. Department of Agriculture; 1954. 233 p.

Germinability of Cook Pine (Araucaria columnaris) Seeds Under Different Storage Conditions

Paul G. Scowcroft

Research forester, USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, Institute of Pacific Islands Forestry, Honolulu, HI

Up to 25 metric tonnes of seeds of Cook pine, Araucaria columnaris *(Forst. f.) Hook., are exported from Hawaii in abundant seed years. Excess seeds cannot be stored and used to fill orders in poor seed years because the seeds quickly lose their ability to germinate. The effects of storage temperature, seed moisture content, and nitrogen enrichment of storage containers on short-term and long-term seed germinability were studied in the laboratory. Results confirm the need for storage at near-freezing temperatures and seed moisture contents between 15 and 25%. Filling storage containers with nitrogen gas failed to prolong storage life of seeds and even air-filled storage containers failed to prolong storage of physiologically immature seeds. Tree Planters' Notes 39(3):17-25; 1988.*

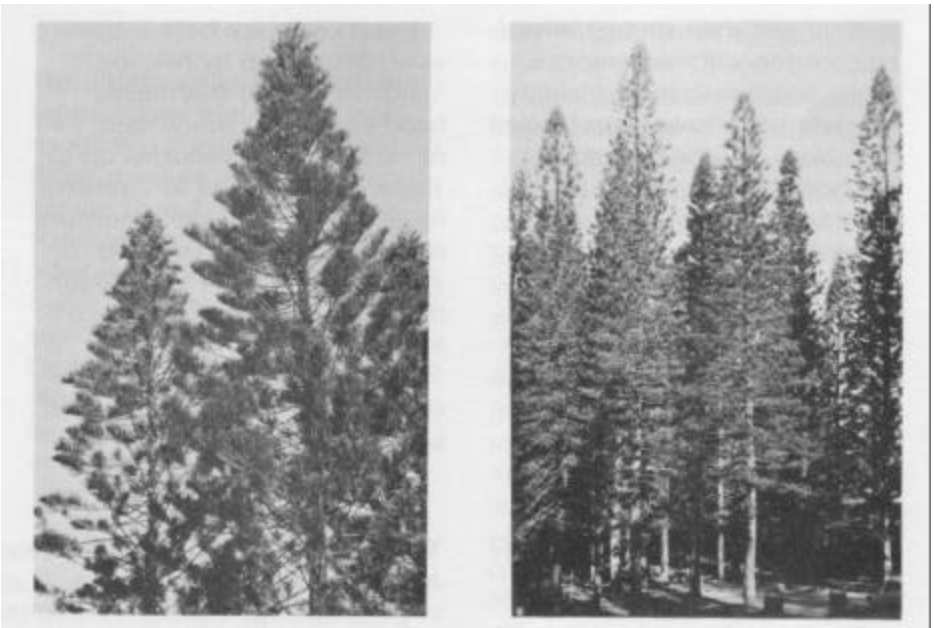

Figure 1—*A native of New Caledonia, Polynesia, and the Isle of Pines, Cook pine* (Araucaria columnaris) *is an evergreen coniferous tree that has been introduced to Hawaii and Florida. Although the tree is not a pine, its pine-tree-like shape and symmetry make it desirable as a Christmas tree and as an ornamental. In Hawaii, scattered stands of pure Cook pines make ideal recreation areas.*

Cook pine—*Araucaria columnaris* (Forst. f.) Hook.—is the most abundant Araucaria species in the Hawaiian Islands (fig. 1). It is commonly, but incorrectly, locally referred to as Norfolk Island pine (*A. heterophylla* [Salisb.] *Franco* [syn. *A. excelsa* (Lamb) R. Br.]), a species sparsely represented in the State.

Demand for *A. columnaris* seeds from Hawaii is great: up to 25 metric tonnes (wet weight basis) have been exported in abundant seed years, which usually occur every 4 or 5 years (9, 23). This quantity represents the harvest from about 130 hectares of public and private plantations statewide. Stands on Oahu alone yielded about 13 metric tonnes of seed in 1985 (6).

Suppliers of *A. columnaris* seeds face two problems: rapid loss of viability during shipment and inability to store seeds for more than a few months without significant loss of viability. This loss is particularly disturbing because suppliers do not have reserves for filling orders in years of poor seed production.

These problems are common to many large-seeded tropical tree species (18, 24). Their seeds are shed in a moist condition and are short lived. Seeds can be successfully stored for only a few weeks or months and must be kept at relatively high temperatures and high seed-moisture contents. Seeds with these viable characteristics have been labeled *recalcitrant* (15).

In contrast, seed viability of most temperate zone trees species can be retained for years by first drying to about 5% moisture

content and then storage in sealed containers at low temperatures. Seeds with these viability characteristics have been labeled *orthodox. Araucaria columnaris* has been provisionally classified as intermediate in seed storage physiology (21).

The interaction of seed moisture content and storage temperature, including freezing, on longevity of *A. columnaris* seeds and the effect of storage under nitrogen gas were examined.

Materials and Methods

Seeds were supplied by Hurov Seeds from cones collected in Nuuanu Valley, Oahu, HI, in August 1972 and September 1973. In 1972, the cones were beginning to disintegrate, so cones and loose seeds were packed in polyethylene bags for shipment. In 1973, the cones were still closed, so each cone was wrapped separately in plastic wrap. Both seed lots were shipped to the USDA Forest Service's National Tree Seed Laboratory, Macon, GA, for testing.

Moisture content (wet weight basis) of both lots was estimated when seeds arrived 4 days later by using four 3-g subsamples ovendried for 16 hours at 105 °C and cooled for 2 hours over desiccant before final weighing (10). Fresh seed moisture contents were 27% in 1972 and 68% in 1973. There were about 1,235 (± 40 SD) seeds per kg fresh weight in 1973.

Intact cones for both lots were easily broken up by twisting. Seeds were then thoroughly hand mixed and subdivided. Part of each lot was ovendried up to 32 days at 22 °C and 40% relative humidity to the desired moisture contents. Moisture contents after drying were 21 and 7% for the 1972 seed lot and 26 and 6%, for the 1973 seed lot.

Seed groups at each moisture content were further subdivided and assigned at random to different storage treatments (table 1). There were not enough seeds supplied to test all possible treatment combinations.

Seeds were stored in 0.5-liter air-tight glass jars. The loosely packed seeds occupied about 65 to 80%, of each jar. Where used, nitrogen gas was slowly injected through a tube lowered to the bottom of each jar before sealing. An estimated 90% of the air was thus displaced by nitrogen gas.

Table 1—*Seeds of Cook pine* (Araucaria columnaris) *subjected to combinations of initial seed moisture content, storage period, composition of gas in storage containers, and storage temperature*

Initial moisture (%)	Storage period (mo)	Air				Nitrogen			
		24 °C	15 °C	3 °C	−7 °C	24 °C	15 °C	3 °C	−7 °C
1972 seed lot									
27	0	*							
	3	*	*	*			*	*	*
	6	*	*	*	*				
21	0								
	3	*	*	*					
	6	*	*	*	*				
7	0								
	3	*	*	*					
	6	*	*	*	*				
1973 seed lot									
68	0	*							
	6								
	12								
	24								
26	0	*							
	6			*	*			*	*
	12				*				*
	24				*				*
6	0	*							
	6				*				*
	12				*				*
	24				*				*

After seeds were stored, they were tested for germination in walk-in germination chambers programmed for 30 °C and 2,150 lux for 8 hours and 20 °C and darkness for 16 hours. Seeds were placed on crepe cellulose germination paper (Kimpak) moistened with 100 ml of tap water, fitted into shallow 12.7 by 17.8 cm plastic boxes, and covered. No additional water was added during the germination period. For each replication, 50 or 100 seeds were used. Each treatment combination was replicated 4 to 16 times.

A germinated seed was one whose radicle had emerged enough that the seed coat lifted off the germination paper. Abnormal germination included conditions described in seed testing rules (10). At the end of the 4-week test period, seeds were x-rayed to determine the number of full ungerminated seeds.

The results were analyzed by untransformed and arcsin $\sqrt{x}$ transformed data. Treatment effects and interactions could not be properly assessed by usual statistical methods, in part because the assumption of equal variances among means was invalid, even when germination data were first transformed using arcsin $\sqrt{x}$. Instead, I compared treatment means by using the approximate *t* statistic (5) computed as follows:

$$t = (\bar{X}_1 - \bar{X}_2)/[s_1^2/n_1) - (s_1^2/n_2)]^{0.5}$$

The difference between means for each pair of different treatments was considered significant if the absolute value of *t* was greater than *T*, where *T* is the Bonferroni critical value for the number of pairwise comparisons being considered with an overall significance level of at most 0.05. Degrees of freedom for selecting the Bonferroni *T* were estimated by using Satterthwaite's approximation (13),

$$df = (s_1^2/n_2 + s_2^2/n_2)^2 / \{s_1^4/[n_1(n_1-1)] + s_2^4/[n_2(n_2-1)]\}$$

Graphic and tabular summaries and statistical tests are based on untransformed data. I chose to present untransformed data because they are readily interpretable and because results of all statistical comparisons were the same as obtained using arcsin $\sqrt{x}$ transformed data.

Results and Discussion

Germination of the 1972 lot of *A. columnaris* seeds averaged 78% (± 2.6 SE) on receipt at the National Tree Seed Laboratory. Significantly lower germination was achieved by the 1973 seed lot (61% ± 1.1 SE). Lower germination may have been due to seed immaturity because cones in 1973 had not begun to break apart as had cones collected in 1972. The 1973 seed lot had a much higher fresh moisture content than the 1972 seed lot—68 to 27%. Furthermore, immature seeds collected in 1976 showed higher moisture contents (47%) and lower germination rates (41%) than mature seeds collected a few weeks later in the season (40% moisture and 70% germination).

Another indicator of immaturity of the 1973 seed lot was the large increase in number of empty seeds over time (table 2). After just 6 months' storage, there were significantly more empty seeds than initially. This contrasts with results for the 1972 seed lot, which showed no appreciable increase in empty seeds after 6 months' storage (table 2).

Two other factors could have contributed to lower germination for seed collected in 1973: climatic effects on viability and intraspecific variability, but neither was evaluated.

Storage temperature. Clearly, *A. columnaris* seeds did not retain viability when stored at room temperature (table 3). Even storage at 15 °C did not prevent germinability decreasing from 78% (fresh seeds) to 40% in 3 months. Loss of germinability during warm storage was accentuated for seeds with high moisture content (fig. 2). After 6 months' storage, such seeds collected in 1972 and stored at 24 or 15 °C did not germinate.

Table 2—*Average number of empty* Araucaria columnaris *seeds per 100 sown as determined by x-ray examination after germination tests, by seed moisture content and storage period*

Initial seed moisture (%)	Storage period (mo)	Average no. of empty seeds /100	
		1972	1973
26–27	0	15.8 a ± 1.6	2.25 a ± 0.85
	6	13.8 a ± 2.6	35.84 b ± 2.50
6–7	0	—	1.50 a ± 1.50
	6	14.3 a ± 3.1	33.76 b ± 3.24

Within column means followed by the same letter do not differ significantly ($P > 0.05$). Values are means ± standard errors.

Table 3—*Average percent germination in seed lots of* Araucaria columnaris *harvested in 1972 and 1973 and stored at different temperatures for 3 and 6 months*

Storage temperature (°C)	Average percent seed germination		
	1972		1973
	3 months	6 months	6 months
− 7	—	59.1 a ± 3.4	8.4 a ± 1.3
3	61.8 a ± 3.3	50.2 a ± 2.0	23.8 b ± 2.6
15	39.5 b ± 3.0	13.5 b ± 3.6	—
24	8.8 c ± 4.0	7.1 b ± 3.4	—

Within column means followed by the same letter do not differ significantly ($P > 0.05$). Values are means ± standard errors.

The adverse effect of warm storage on germinability of seeds with high moisture content has been attributed to respiratory heating or microbial proliferation or both (4, 7). Heat may reach lethal levels and storage microorganisms may partially or wholly decay seeds in storage. Even if such seeds are not destroyed, saprophytic fungi may proliferate so rapidly when seeds are removed from storage and placed in conditions favorable for germination that they decay before germinating. Decay during and after storage was evident in this study. Half of the seeds stored at 24 °C and at moisture contents of 27 and 21% decayed before 3 months elapsed. Mold was a serious problem during germination for some hydrated seeds stored at 24 and 15 °C. At lower temperatures, neither problem was apparent.

Seeds must be stored at temperatures just above or possibly just below freezing, so as to minimize loss of germinability (fig. 2). However, even cold storage failed to prevent statistically significant losses of germinability for most treatments. The only cold storage treatments that prevented significant loss of viability were a) 3 months' storage at 3 °C and with seed moisture of either 27 or 21 % and b) 6 months' storage at -7 °C and with seed moisture of either 27 or 21% (fig. 2).

The data from the 2 collection years differ regarding the effect of below-freezing temperature on seed storage life. Seeds collected in 1972 and stored at -7 °C for 6 months germinated as well as those stored at 3°C (table 3). Both cold storage treatments resulted in significantly greater germination than the two warmer storage treatments. In contrast, seeds collected in 1973 and stored at -7 °C for 6 months germinated more poorly than seeds stored at 3 °C (table 3).

The beneficial effect of low-temperature storage on prolonging viability of recalcitrant *Araucaria* seeds has been observed by others (3, 14, 19). Akamine (1, 2) reported that 7 °C was the optimum storage temperature for *A. excelsa* (syn. *A. heterophylla*), provided seeds are kept under 60 to 75% relative humidity. Slightly lower temperatures

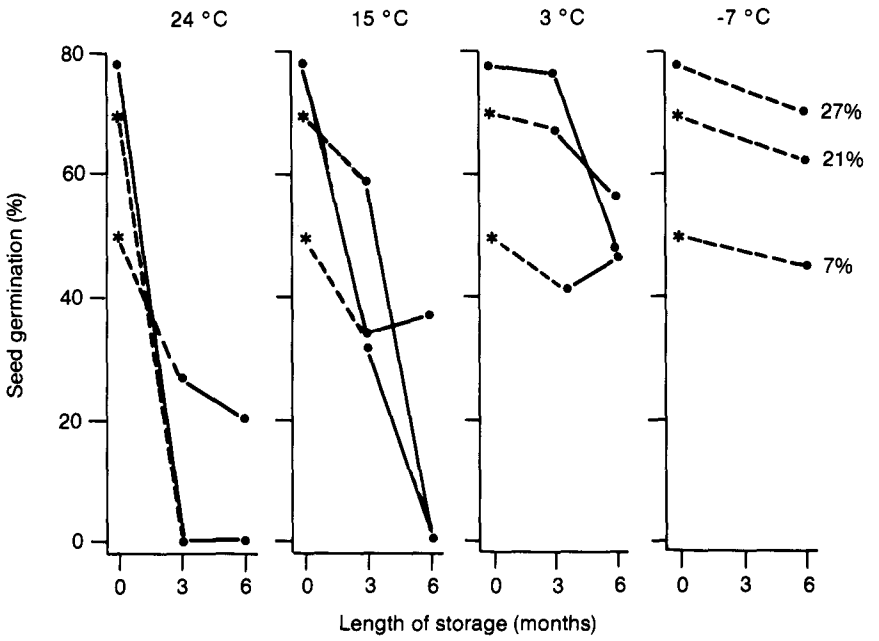

Figure 2—*Percentage germination of the 1972 lot of* Araucaria columnaris *seeds before and after storage at 24, 15, 3, and −7 °C and at seed moisture contents of 27, 21, and 7 percent (fresh weight basis). Asterisks (*) denote estimated percentage germination. Broken lines connect estimated and measured values. Vertical bars are standard errors of the means.*

(2 to 4 °C) have also been recommended for moist *A. excelsa* seeds (12) cited in King and Roberts (11). Tompsett (22) cautiously suggested that long-term storage (>18 months) of A. columnaris seeds might be achieved at -18 °C if seed moisture content was first lowered to 7%.

Seed moisture content. Data for the 1972 seed lot indicated that the effect of seed moisture content on germination depends on storage temperature (fig. 2). Seeds stored at 24 or 15 °C for 6 months germinated best if first dried to 7% moisture. Seeds stored at 3 °C for 6 months germinated equally well at all three moisture contents. Seeds stored at -7 °C for 6 months germinated best if they were not dried.

Although germination of dried but unstored seeds was not determined for the 1972 seed lot, I suspected that ovendrying reduced viability (see estimated initial values in figure 2). Test results for the 1973 seed lot (fig. 3) showed a decline of seed viability as a result of ovendrying. Germination of fresh, undried seeds at 68% moisture content averaged 62%. Drying seeds to 26 and 6% moisture reduced germination to 50 and 11 %, respectively. The latter germination percent was significantly lower than the other two percentages. Either high temperature or desiccation or both contributed to the loss of viability.

Ignoring for the moment the effect of heating, the length of drying time alone could account for loss of germination ability. It took 28 days of drying at 22 °C followed by 4 additional days at 32 °C for the 1973 seed lot to reach 6% moisture content. It took 10 days of drying at 22 °C and constant 40% relative humidity for seeds to reach 26% moisture content. Akamine (1) found that *A. excelsa* seeds exposed to ambient temperature (21 to 27 °C) lost about half of their germinability in 1 month, regardless of relative humidity (ambient to constant 90%). Havel (8) reported an even greater loss for *A. hunsteinii* after 1 month's exposure to ambient temperatures (13 to 35 °C) and relative humidities in New Guinea.

Desiccation, not heat, was probably the time-dependent

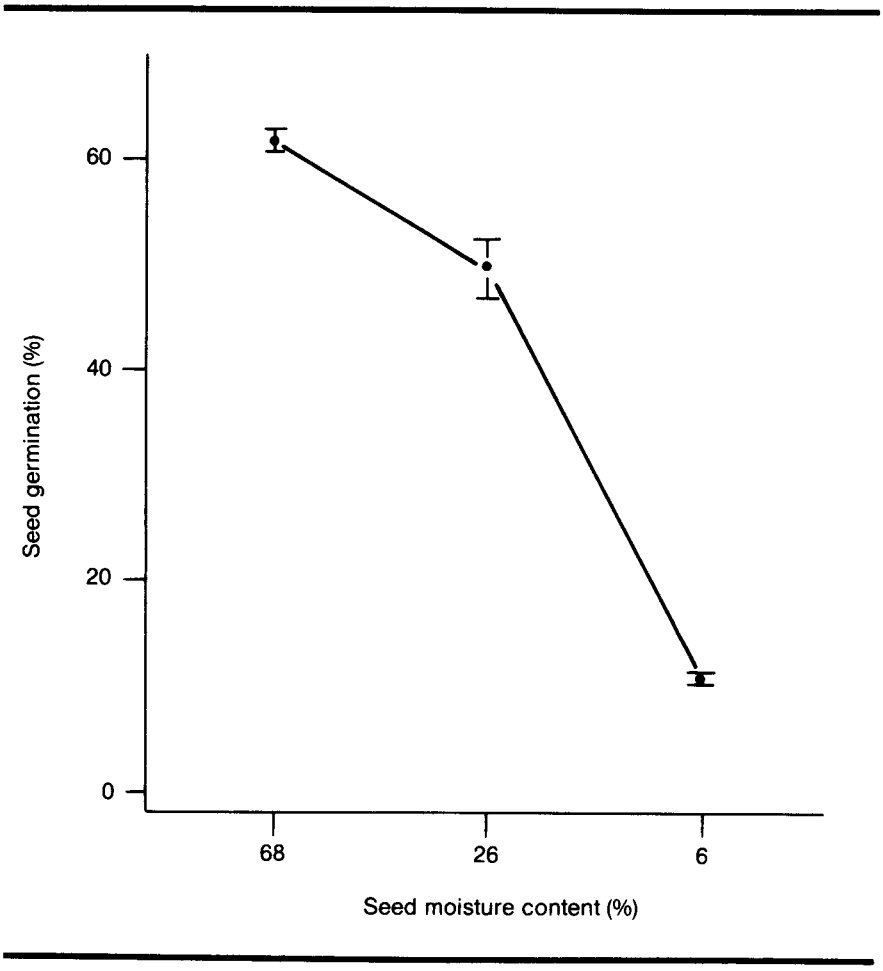

Figure 3—*Percentage germination of the 1973 seed lot before storage as a function of seed moisture content. Vertical bars are standard errors of the means.*

factor contributing most to loss of seed viability during ovendrying. Tompsett (21) reported that initial germination of A. columnaris seeds dried to 7% moisture content was about 30% or half the value achieved by fresh seeds at 36% moisture content.

However, he found that seeds dried to 22, 15, and 12% moisture content germinated nearly as well as fresh seeds. In contrast, fresh seeds of *A. hunsteinii*, a recalcitrant seed species, could be dried from 53 to 32% moisture content with no loss of germination ability (19). Below 32% moisture, germination declined until at 14% moisture seeds failed to germinate.

Immaturity of the 1973 seeds may have also contributed to the loss of germination ability upon desiccation. Harrington (7) noted that immature seeds in general have a shorter life-span than do mature seeds and are likely to lose viability upon drying.

The elevated temperature need to bring seeds from the 1973 seed lot down to 7% moisture could have been another factor leading to the significant loss of germinability. Roberts (16) suggested that if seeds are dried by heating, the higher the initial seed moisture content the greater their probability of damage-a suggestion supported by results of the current test, in that, unstored 1972 seeds dried from 27 to 7% moisture had estimated germination above 45%, whereas, unstored 1973 seeds dried from 68 to 6% had germination of only 10%.

Nitrogen enrichment of storage jars. For undried seeds from the 1972 seed lot, storage in a nitrogen-enriched environment at above freezing temperatures adversely affected germinability. After 3 months' storage at 3 and 15 °C, germination was about 20% lower in high-nitrogen (low-oxygen) atmospheres than in air (fig. 4). This result agrees with the observation by Roberts (17) that, in general,

oxygen benefits seeds stored at high moisture contents and adversely affects seeds stored at low moisture contents.

Similar results were reported by Tompsett (20), who found that germination of recalcitrant, hydrated *A. hunsteinii* seeds decreased as oxygen concentration was reduced from 21 to 0%. He suggested that lower seed respiration accompanying lower oxygen concentrations may inhibit repair of cellular damage and thereby reduce seed longevity.

Three-month germination of frozen (-7 °C) seeds stored in a high-nitrogen environment was not significantly different from that of undried, unstored seeds (fig. 4)-80 and 78%, respectively. Although 3-month data for frozen seeds in air were not available because of the limited seed supply, germination probably would not have been significantly different from that for frozen seeds in a high-nitrogen environment or for undried, unstored seeds. I suspect this would have been the case because all biological processes, including respiration and degradation processes, would be slower in frozen seeds. So reduced oxygen concentration would have less effect than it would on unfrozen seeds.

Additional testing of below-freezing temperature in combination with high nitrogen concentration was done using

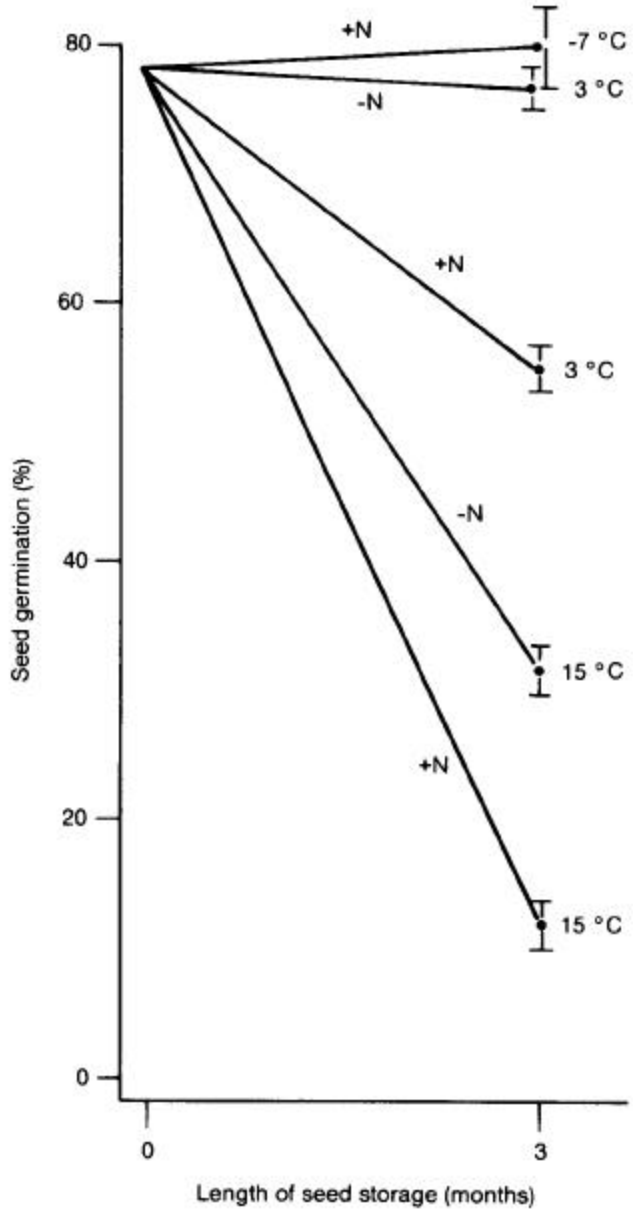

Figure 4—*Percentage germination of the 1972 seed lot after 3 months of storage in jars with (+ N) and without (− N) nitrogen gas and at −7, 3, and 15 °C. All seeds were initially at 26% moisture content. Vertical bars are standard errors of the means.*

the 1973 seed lot. In contrast to results with the 1972 seed lot, adding nitrogen to storage containers did not prolong germinability of frozen seeds from the 1973 seed lot (fig. 5).

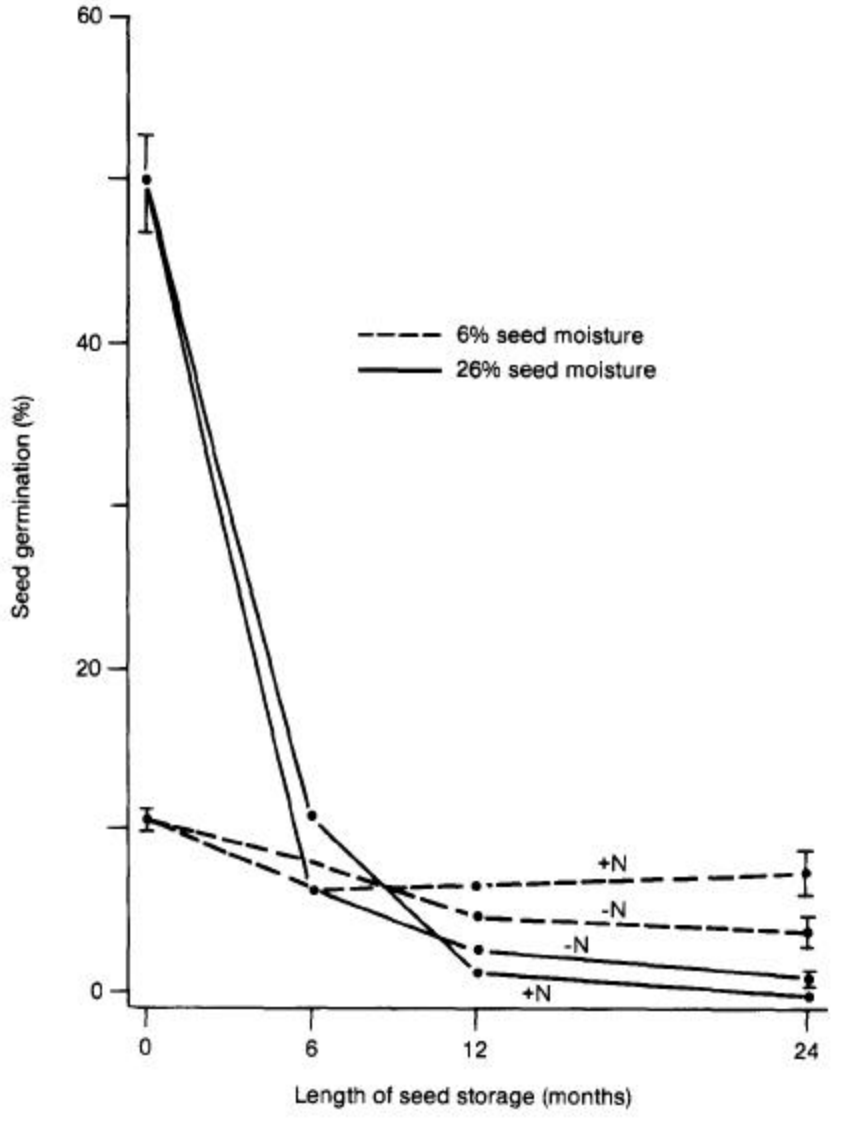

Figure 5—*Percentage germination of the 1973 seed lot after 6, 12, and 24 months of storage as a function of initial seed moisture content and with (+ N) and without (– N) nitrogen gas added to storage containers. Vertical bars are standard errors of the means.*

Summary

The implicit objective of this research and that of Akamine (1, 2) and Tompsett (22) was to provide seed suppliers and nursery workers with short-term and long-term storage criteria for maintaining high seed viability. These experiments did not fully satisfy this objective. The best germination achieved after short-term storage (<1 year) was 70% in the current study. The best germination achieved after long-term storage was 20% (22). These relatively low germination values are probably unacceptable to seed suppliers.

Integrating the results of this study with those of Tompsett (22), yields several general recommendations:

1. Store seeds in air-tight containers at temperatures near or below freezing. Controlled desiccation of seeds before storage may be desirable.
2. Dry seeds quickly, but not at oven temperatures above about 24 °C. Seed moisture content should be about 15 to 25% when storage is done at temperatures near freezing.

3. Do not store seeds in nitrogen gas—especially if storage temperature is above freezing.

Adherence to these recommendations does not guarantee long-term maintenance of high germinability. Success at prolonging viability of *A. columnaris* seeds appears quite variable. Part of the variability may be attributed to collection of physiologically immature seeds, as was the case in this study. Collectors should harvest only fully ripened seeds, a condition indicated by disintegration of cones on the trees and relatively low seed moisture contents (<40%).

Suppliers should also be aware that successful storage is only half of their problem; delivering viable seeds to buyers is the other half. Rapid deterioration after removal from storage and during shipment and germination is likely (3). Studies addressing the effect of shipping methods on germinability of stored Araucaria seeds remain to be done.

Acknowledgments

I thank Earl W. Belcher for conducting the germination tests; Douglas J. C. Friend, Robert A. Merriam, C. Eugene Conrad, and Roger G. Skolmen for reviewing earlier drafts of this manuscript; and James A. Baldwin for statistical guidance.

References

1. Akamine, E.K. Germination and viability studies of forest tree seeds. In: Report of the Hawaii Agriculture Experiment Station 1945-46. Honolulu: University of Hawaii; 1947: 116-118.
2. Akamine, EX Viability of Hawaiian forest tree seeds in storage at various temperatures and relative humidities. Pacific Science 5(1):36-46; 1951.
3. Arentz, F. Some factors affecting the viability of Klinkii pine (*Araucaria hunsteinii)* in storage. Seed Science and Technology 8:277-282; 1980.
4. Baker, K.F. Seed pathology. In: Kozlowski, T.T., ed. Seed biology, vol. II. New York: Academic Press; 1972: 317-416.
5. Games, P.A.; Howell, J.F. Pairwise multicomparison procedures with unequal N's and/or variances: a Monte Carlo study. Journal of Educational Statistics 1 :113-125; 1976.
6. Hall, P. Personal communication; Dow Seeds Hawaii, Ltd; 1986.
7. Harrington, J.F. Seed storage and longevity. In: Kozlowski, T.T., ed. Seed biology, vol. III. New York: Academic Press; 1972: 145-245.
8. Havel, J.J. Plantation establishment of Klinkii pine (Araucaria *hunsteinii)* in New Guinea. Commonwealth Forestry Review 44(3):172-187; 1965.
9. Hurov, R. Personal communication; Hurov Seeds; 1978.
10. International Seed Testing Association (ISTA). International rules for seed testing. In: Proceedings of the International Seed Testing Association 31:1-152; 1966.
11. King, M.W.; Roberts, E.H. The storage of recalcitrant seeds: achievements and possible approaches. Rome: FAO International Board for Plant Genetic Resources; 1979.
12. Magini, E. Forest seed handling, equipment, and procedures. II. Seed treatments, storage, testing, and transport. Unasylva 16:20-35; 1962.
13. Milliken, G.A.; Johnson, D.E. Analysis of messy data: designed experiments, vol. I. Berkeley, CA: Lifetime Learning; 1984.
14. Ntima, 0.0. Fast growing timber trees of the lowland tropics: 3. The Araucarias. Oxford: Oxford University, Department of Forestry, Commonwealth Forestry Institute; 1986.
15. Roberts, E.H. Predicting the storage life of seeds. Seed Science and Technology 1:499-514; 1973.
16. Roberts, E.H. Seed deterioration and loss of viability. In: Thomson, J.R., ed. Advances in research and technology of seeds, part 4. Wageningen, The Netherlands: Centre for Agricultural Publishing and Documentation (Pudoc); 1979: 25-42.
17. Roberts, E.H. Loss of seed viability during storage. In: Thomson, J.R., ed. Advances in research and technology of seeds, part 8. Wageningen, The Netherlands: Centre for Agricultural Publishing and Documentation (Pudoc); 1983: 9-34.
18. Sasaki, S. The physiology, storage, and germination of timber seeds. In: Chin, H.F.; Enoch, I.C.; Raja Harun, R.M., eds. Seed technology in the tropics. Kuala Lumpur, Malaysia: Art Printing Works Sdn. Bhd.; 1977: 111-115.
19. Tompsett, P.G. The effect of desiccation of the longevity of seeds of *Araucaria hunsteinii and A. cunninghamii.* Annals of Botany 50: 693-704; 1982.
20. Tompsett, P.G. The influence of gaseous environment on the storage life of Araucaria seed. Annals of Botany 52:229-237; 1983.
21. Tompsett, P.G. Desiccation studies in relation to the storage of Araucaria seed. Annals of Applied Biology. 105:581-586; 1984.
22. Tompsett, P.G. The effect of moisture content and temperature on the seed storage life of Araucaria columnaris. Seed Science and Technology 12:801-816; 1984.
23. Walters, G.A. Araucaria. In: Seeds of woody plants in the United States. Agric. Handb. 450. Washington, DC: U.S. Department of Agriculture; 1974: 223-225.
24. Wang, B.S.P.; Pitel, J.A.; Webb, D.D. Environmental and genetic factors affecting tree and shrub seeds. In: Thomson, J.R., ed. Advances in research and technology of seeds, part 7. Wageningen, The Netherlands: Centre for Agricultural Publishing and Documentation (Pudoc); 1982: 87-135.

Hybrid Pines (Pitch Pine x Loblolly Pine) Studied in the Appalachian Region of Maryland

John G. Genys

Professor, University of Maryland, Center for Environmental and Estuarine Studies, Appalachian Environmental Laboratory, Frostburg, MD

Hybrids of pitch pine x loblolly pine (Pinus rigida x taeda) *originating from a cross of pitch pine* (P. rigida) *of unknown origin with loblolly pine* (P. taeda) *pollen from Georgia were studied in Garrett County, MD. At 17 years after planting, 53% of the hybrid trees survived; their heights averaged 7.8 m and their diameters (DBH) were 15.5 cm. Pitch pines and loblolly pines native to Maryland planted for comparison had similar heights and diameters, but the loblolly pines showed a high mortality rate-only 19% of the planted specimens survived.*
Tree Planters' Notes 39(3):26-27; 1988.

Pitch pine (*Pinus rigida* Mill.) and loblolly pine (*P. taeda* L.) are two common forest trees of the eastern United States that are native to Maryland. Pitch pine tolerates a cold climate but is a relatively slow growing tree and has only a moderate commercial value. Loblolly pine is an outstanding tree in the southern parts of the State.

In 1962, the hybrids of these two species *(P. rigida x taeda)* were planted in various parts of the State, with an objective to compare them with the parent species, loblolly and pitch pines. The first results of this research were reported by Genys in 1970 (1). At that time, the 7-year-old hybrids in the Appalachian region were larger than loblolly pines, and all three types of specimens showed an equally good survival. This present report is based on measurements of the same three groups of trees at age 17.

Contribution 1885-AEL.

Materials and Methods

The seed lot of *P. rigida x taeda* was supplied to the author by Dr. S. K. Hyun of South Korea in 1962. The hybrid seeds were harvested from female parent trees *P. rigida* of uncertain origin that had been pollinated by *P. taeda* pollen from Georgia. Seed of *P. rigida* used for comparison were collected at Harmans, MD (near Baltimore). Loblolly pine seed used for the same reason was also of Maryland origin, but its exact provenance was not known.

One-year-old (1 + 0) seedlings of these three sources were produced at the Buckingham State Forest Tree Nursery in Harmans, MD, during the growing season of 1962. Next spring (in 1963) the specimens were planted on 11 sites in various parts of Maryland, including the Appalachian region in Garrett County, near Friendsville, MD. The plantation that is the subject of this report is a former farm field at an elevation of 773 m surrounded by northern hardwood trees. The soil is rocky and relatively shallow. The growing season in this area lasts only 120 to 140 days.

The research sources were arranged in eight randomized blocks. Plots were 4-tree squares with trees spaced at 2.1 by 2.1 m. Heights and diameters (DBH) of 17-year-old trees were measured in spring 1980. A record was kept of survival rates. In four blocks, all *P. taeda* trees died.

Consequently, the analysis of variance of heights and diameters was based on data from only four blocks, with 3 degrees of freedom (df) for "blocks," 2 df for "varieties," and 6 df for "interaction." Survival analysis included data from all eight blocks. Student's ranges were used for estimation of the least significant differences (LSD).

Results

Survival and growth rates of *P. rigida x taeda*, loblolly pine, and pitch pine were studied for 1 year in the nursery and 16 years in western Maryland (table 1).

Survival. Pitch pine and the hybrids showed similar survival rates, 66 and 53%, respectively. Of the loblolly pines, from 32 trees planted only 6 (19%) survived. This low survival rate of P. taeda was significantly (0.05 level) different than that of pitch pine or the hybrids (LSD = 31 %).

Table 1—*Data on survival and growth rates of pitch pine* (Pinus rigida), *loblolly pine* (P. taeda), *and hybrids* (P. rigida × taeda) *at 16 years after planting in Appalachian region of Maryland at Savage River State Forest, Garrett County*

Seed ID	Biotype and origin	16-yr Survival (%)	17-yr Height (m)	17-yr DBH (cm)
119	*P. rigida* (Maryland)	66	8.18	15.3
80	*P. taeda* (Maryland)	19	7.92	14.1
156	*P. rigida* × *taeda* F^2	53	7.77	15.5
LSD at 0.05 level		31	NA	NA
F–value		11.8**	0.10	0.3

**Significant at 0.01 level.
DBH = diameter at breast height.

Height. On the average, the three types of pines at 16 years atter planting were about 8 m high. *P. rigida x taeda* hybrids , were about 7.8 m tall, and pitch pines were the tallest (8.2 m) high. However, the heights of these three sources were not significantly different at 0.05 level. Apparently, at this age, the height of hybrids was similar to that of the other sources, and their superiority of growth rate observed at the age of 7 years was no longer evident (1).

Diameter (DBH). The diameters of the three types of pines ranged from 14.1 to 15.5 cm but did not differ significantly at 0.05 level. It is noteworthy, however, that loblolly pines, which were about the same height as the hybrids and pitch pines, had the smallest diameters. Their stems appeared more slender and their limbs smaller than among the specimens of *P. rigida* or *P. rigida x taeda.*

Discussion

Excellent results with *P. rigida x taeda* hybrids were obtained in South Korea (2, 3). In Maryland, these hybrids grew more slowly than *P. taeda* within the *P. taeda* range, but they may be valuable in areas of colder climate (1, 4).

The results of this study in Maryland's mid-Appalachian region showed that 17-year-old trees representing pitch pine, loblolly pine, and *P. rigida x taeda* hybrids grew in height at the same rate and had similar diameters. The major difference among the sources was in their ability to survive in the Appalachian region. Although pitch pines and hybrids showed a satisfactory survival, loblolly pines did not (only 19% survived).

Planting this particular source of hybrids (a cross of *P. rigida* of unknown origin pollinated by *P. taeda* from Georgia) in the mid-Appalachian region promises no economic advantages. There is, however, a strong possibility that hybrids resulting from crosses of selected Maryland sources would be more outstanding.

Literature Cited

1. Genys, J.B. Hybrids of pitch pine x loblolly pine, *Pinus rigida* x *taeda,* studied in Maryland. Chesapeake Science 11(3):191-198; 1970.
2. Hyun, S.K. Forest tree breeding work in Korea. Inst. Paper 1. Suwon, Korea: Institute of Forest Genetics; 1956. 16 p.
3. Hyun, S.K.; Ahn, K.Y. Principal characteristics of x *Pinus rigitaeda*. Res. Rep. 1. Suwon, Korea: Institute of Forest Genetics. 1959:25-50.
4. Little, S.; Sores, H.A. 1961 Results from the 1955 planting of hybrid pines and ordinary nursery stock. [Unpublished report from the New Lisbon (NJ) Research Center on file at the U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station, Broomall, PA.] 1962.6 p.

A Tissue Culture Solution to a Forestry Problem—The Propagation of a Tetraploid European Aspen

S. R. Wann, G. W. Wyckoff, and J. L. Wyckoff

Industrial research fellow, Union Camp Corporation, Princeton, NJ, and research fellow and research assistant, the Institute of Paper Chemistry, Forest Biology Division, Appleton, WI

A simple tissue culture method based on the production of multiple shoots from dormant buds is described. The application of the method to tetraploid European aspen (Populus tremula *L.*) *illustrates that in vitro propagation can be an attractive alternative when conventional methods prove unsuccessful.* Tree Planters' Notes 39(3):28-30; 1988.

Tissue culture has been proposed as a method for the large-scale clonal propagation of forest species. To date, *in vitro* propagation techniques have not been developed to the extent necessary for commercial forestry operations. Nevertheless, propagation of forestry species from tissue cultures may provide solutions to other forestry problems not directly related to mass propagation. The history of the propagation Ta-10, a tetraploid European aspen (*Populus tremula*) represents a case in point.

Hybridization of native, diploid quaking aspen (*P. tremuloides* Michx.) with tetraploid European aspen results in the formation of triploid aspen that are highly valued by the pulp and paper industry for their increased growth rate, specific gravity, and fiber length (2). Ta-10 originated in southern Sweden and was first crossed with native aspen at The Institute of Paper Chemistry (IPC) in 1958.

Since then, Ta-10 that has been grafted in the United States has been crossed with numerous *P. tremuloides* to the extent that up to 500,000 to 1,000,000 triploid hybrid seeds have been produced annually for the past 20 years. Although Ta-10 can be readily grafted onto diploid or triploid rootstock, attempts have been made at vegetative propagation. As a member of the section *Leuce*, Ta-10 is not easily propagated from hardwood cuttings but is amenable to propagation via root sprouts. Attempts to stimulate roots on Ta-10 by burial of grafts below the graft union were successful, but sprouting from those roots in the particular clone was minimal.

Therefore, tissue culture techniques became an attractive option to circumvent these difficulties in vegetatively propagating Ta-10. The following describes a general procedure that has been successfully used to propagate not only Ta-10 by tissue culture, but also other hardwood species as well.

Materials and Methods

Dormant lateral buds of Ta-10 were collected in January and February from the IPC arboretum near Greenville, WI. Buds were rinsed under cold water for 30 minutes and treated for 15 minutes with a 10% (v/v) solution of commercial bleach (Hilex). After three rinses with sterile water, the bud scales and outer leaves were aseptically removed, and the apical meristems with several layers of intact leaf primordia were again treated with 1% bleach for 5 minutes. Following three rinses with sterile water, the explants were placed on woody plant medium (WPM) (3) containing 0.05 mg/liter naphthaleneacetic acid (NAA) and 1.0 mg/liter benzyladenine. The medium was adjusted to pH 5.8 prior to autoclaving and solidified with 0.8% agar (Bacto, Difco). The cultures were incubated at 22 °C and 3000 lux (cool-white fluorescent; 16/24-hour photoperiod).

Every 2 to 3 days, the explants were transferred to renewed medium by sliding them to a different portion of the petri dish. Every 2 weeks, the explants were subcultured to dishes of fresh medium. After 6 to 8 weeks "bud break" occurred, and shoots formed and multiplied. After 4 months, stable "shoot cultures" (fig. 1) could be maintained on the above medium without NAA. These cultures provided a continuous source of shoots suitable for rooting.

Root formation was accomplished in vitro as previously described (5). Briefly, one-third-strength medium (macro and microelements) containing 0.1 mg/liter indole butyric acid was used. Alternatively, shoots were transferred directly from

Figure 1—*Shoot culture of tetraploid European aspen (× 3).*

tissue culture to a mist bed for simultaneous rooting and hardening. Shoots or rooted plants were transferred to a soilless mixture containing equal parts sand peat, and perlite and watered to saturation with 1 liter benomyl (Benlate). Once estlablished in soil, plants assumed growth rate and characteristics consistent with plants obtained from root sprouts (fig. 2).

Results and Discussion

Tetraploid European aspen could be readily propagated through tissue culture. Cultures were established that provided a year-round source of rootable shoots. Both bud break and root formation *in vitro* were nearly 100%. The procedure developed is not new but represents some of the best elements of several procedures for propagating aspen (1, 5) and sweetgum (4).

In the case of Ta-10, tissue culture provided a convenient means of vegetative propagation where several methods proved unsatisfactory. Situations similar to that encountered in Ta-10 can also arise in *Leuce* poplars in which clones have been maintained by grafting but the ortet has been lost. In most hardwoods the establishment of shoot cultures from lateral meristems is relatively simple and straightforward. For species that are difficult to root, the tissue

Figure 2—*Tissue culture-derived tetraploid European aspen 10 weeks after transfer to potting medium.*

culture systems can be considered as providing material at the requisite physiological stage (i.e., rejuvenated) for root formation. Aside from grafting, tissue culture in difficult-to-root species may provide an attractive method of vegetative propagation.

Literature Cited

1. Ahuja, M.R. Short note: a commercially feasible micropropagation method for aspen. Silvae Genetica 33:174-176; 1984.
2. Einspahr, D.W. Production and utilization of triploid hybrid aspen. Iowa State Journal of Research 58:401-409; 1984.
3. Lloyd, G.; McCown, B.H. Commercially feasible micropropagation of mountain laurel (*Kalmia latifola*) by use of shoot tip culture. Combined Proceedings of the International Plant Propagators Society 30:421-427; 1980.
4. Sutter, E.G.; Barker, P.B. *In vitro* propagation of mature *Liquidambar styraciflua*. Plant Cell Tissue Organ Culture 5:13-21; 1985.
5. Wann, S.R.; Einspahr, D.W. Reliable plantlet formation from seedling explants of *Populus tremuloides* (Michx.) Silvae Genetica 35:19-24; 1985.

Effects of Soil Media on the Growth and Survival of Micropropagated Black Cherry

Kimala L. Dills and Richard R. Braham

Honors student and assistant professor, North Carolina State University, Department of Forestry, Raleigh, NC

Micropropagated plantlets of black cherry (Prunus serotina Ehrh.) were transplanted in the greenhouse from an agar medium to plastic tubes containing one *of* three different soil mixtures *of* peat, perlite, vermiculite, and sand at ratios *of* 1:1:0:1, 2:1:2:0, or 3:0:2:1. Each mixture had 10 tubes, each with one plantlet, and each of three clones was represented in each soil mixture. There was no significant difference among soil mixtures for plantlet root or shoot growth, although the 1:1:0:1 mixture was preferable because it provided the greatest number *of* plantable plantlets. Tree Planters' Notes 39(3) :31-34; 1988.

Although tissue culture is commonly employed for eastern cottonwood (*Populus deltoides* Barts. ex Marsh.), to date no suitable technique exists for black cherry *(Prunus serotina* Ehrh.). Such techniques are needed, because black cherry offers landowners an alternative to planting black walnut *Juglans nigra* L.) for high-value lumber. Being able to tissue-culture black cherry would allow not only mass production of genetically improved planting stock but also production of individual plants for seed orchards.

This experiment tested the effect of soil media on the survival and growth of black cherry plantlets, originally micropropagated on agar. The objective was to improve the initial survival rate of 60% attained by Tricoli *et al.* (5), who used a soil mixture of peat, perlite, and sand in a 1:1:1 (vol) ratio.

Methods

Thirty plantlets, micropropagated on agar according to techniques developed by Tricoli *et al.* (5) and represented by three clones (B20, M018, and B9), were first obtained from Dr. Charles Maynard, forest geneticist at State University of New York at Syracuse.

Three soil mixtures of four ingredients with ten plantlets per mixture were tested in a splitplot design. The first mixture was that used for black cherry tissue culture by Tricoli et al. (5): peat, perlite, vermiculite, and sand in a 1:1:0:1 volume ratio. The second soil mixture was that used for loblolly pine (*Pinus taeda* L.) tissue culture by Amerson *et al*. (1): peat, perlite, vermiculite, and sand in a 2:1:2:0 volume ratio. The third mixture, selected to explore the effects of additional peat, was peat, perlite, vermiculite, and sand in a 3:0:2:1 volume ratio. Because the amount of peat used in the soil mixture affected soil reaction, lime was added to standardize soil reaction to pH 5.5 (about 1.5 g lime/liter of peat), according to techniques developed by Amerson *et al*. (1).

By this procedure, soil reaction was similar for all treatments.

A 3-mm screen was used to sift the soil mixtures, to concentrate the vermiculite and perlite in the top 2.5 cm of soil, thus providing a more favorable environment for developing roots. The soil mixtures were next transferred to plastic tubes, measuring 3.8 cm by 20.3 cm, and perforated on the bottom for drainage. One plantlet was then transplanted into each tube with each clone represented in each soil mixture.

Photoperiod, moisture regime, fertilizer type, and application rate were similar for all mixtures using the following procedures developed by Amerson et al. (1). Plantlets received 16 hours of light each day. Two irrigation and fertilization regimes were used for all treatments during the experiment, depending upon plantlet development. Immediately after transplanting, the plantlets were placed under a Mist-a-Matic® irrigation system.

The soil was fertilized 3 to 5 times per week until saturation using 1.2 ml of 15-30-15 fertilizer dissolved in 4.2 liters of water. Fifty cubic centimeters of captan dissolved in 4.2 liters of water was applied once a week in a like fashion. After shoot elonga-

tion began, the plantlets were removed from the irrigation system and irrigated with tap-water acidified with HCl to pH 5.5 and with a solution consisting of 39.5 ml of 20-19-8 fertilizer dissolved in 134 liters of tap-water, acidified with 35 ml of 1*N* HCl. These solutions were applied at 2-day intervals to the point of soil saturation.

Root and shoot lengths were measured to the nearest 0.5 mm when the plantlets were transplanted from the agar to the soil mixtures on May 14, 1985. Shoot lengths were measured from the shoot tip to the soil level. The measurements were repeated on August 2, 1985, after they had grown in the soil mixtures for 11 weeks. The relative value of each soil mixture was evaluated using a mean analysis based on least-square differences. In order to reduce variation, data for dead plantlets were deleted from analyses, emphasizing differences between mixtures.

Table 1—*Average root and shoot length of surviving plantlets by clone and soil mixture*

			May 14, 1985		August 2, 1985	
	Clone no.	No. of plantlets	Root length (mm)	Shoot length (mm)	Root length (mm)	Shoot length (mm)
Peat/perlite/vermiculite/sand (1:1:0:1)						
	B20	4	1.5	14.0	171.0	17.0
	M018	2	32.5	27.5	174.0	27.5
	B9	2	1.0	24.5	135.0	24.5
Peat/perlite/vermiculite/sand (2:1:2:0)						
	B20	3	0.0	19.0	146.7	18.0
	M018	2	17.0	30.0	104.0	32.0
	B9	4	9.2	15.7	154.2	15.7
Peat/perlite/vermiculite/sand (3:0:2:1)						
	B20	2	0.0	10.0	100.0	9.5
	M018	2	20.0	26.0	171.5	19.0
	B9	2	10.5	24.5	209.0	17.0

Results

Prior to experimentation, the plantlets showed considerable variation in root length among the three clones. In addition, many roots were broken during shipment from Syracuse. Clone B20 had the shortest roots and clone M018 the longest roots (table 1). Less variation occurred among clones in shoot length.

At the end of the experiment, root growth was greater than shoot growth. Even plantlets that initially lacked visible roots developed fibrous root systems during the experiment. Root growth of the 1:1:0:1 mixture averaged 148.3 mm, growth of the 2:1:2:0 mixture averaged 126.2 mm, and growth of the 3:0:2:1 mixture averaged 150.0 mm, but these differences were not statistically different.

Shoot growth was very slow, with an overall average increase of 2.3 mm; shoot length of some plantlets actually decreased. Growth of the 1:1:0:1 and 2:1:2:0 mixtures both averaged 1.0 mm; growth of the 3:0:2:1 mixture averaged - 4.7 mm, but none of these differences were statistically significant either. All plantlets developed some reddening of the new leaves between the leaf margin and midrib as early as 1 week after transplanting. Reddening continued throughout the experiment. Red mottling and tissue necrosis of leaf tips and margins developed on older leaves.

Forty-three percent of the plantlets did not develop adequate root and shoot systems by the end of the experiment to allow field planting. Plantlets were judged to be field-plantable when the roots were at least 10 cm long. The variation in root and shoot length between plantlets of the same clone after 11 weeks was dramatic (fig. 1). Although the survival rate in all three treatments equaled or exceeded that obtained by Tricoli et al. (S), the proportion of plantlets suitable for field planting was much lower: 40% for 1:1:0:1, 30% for 2:1 :2:0, and

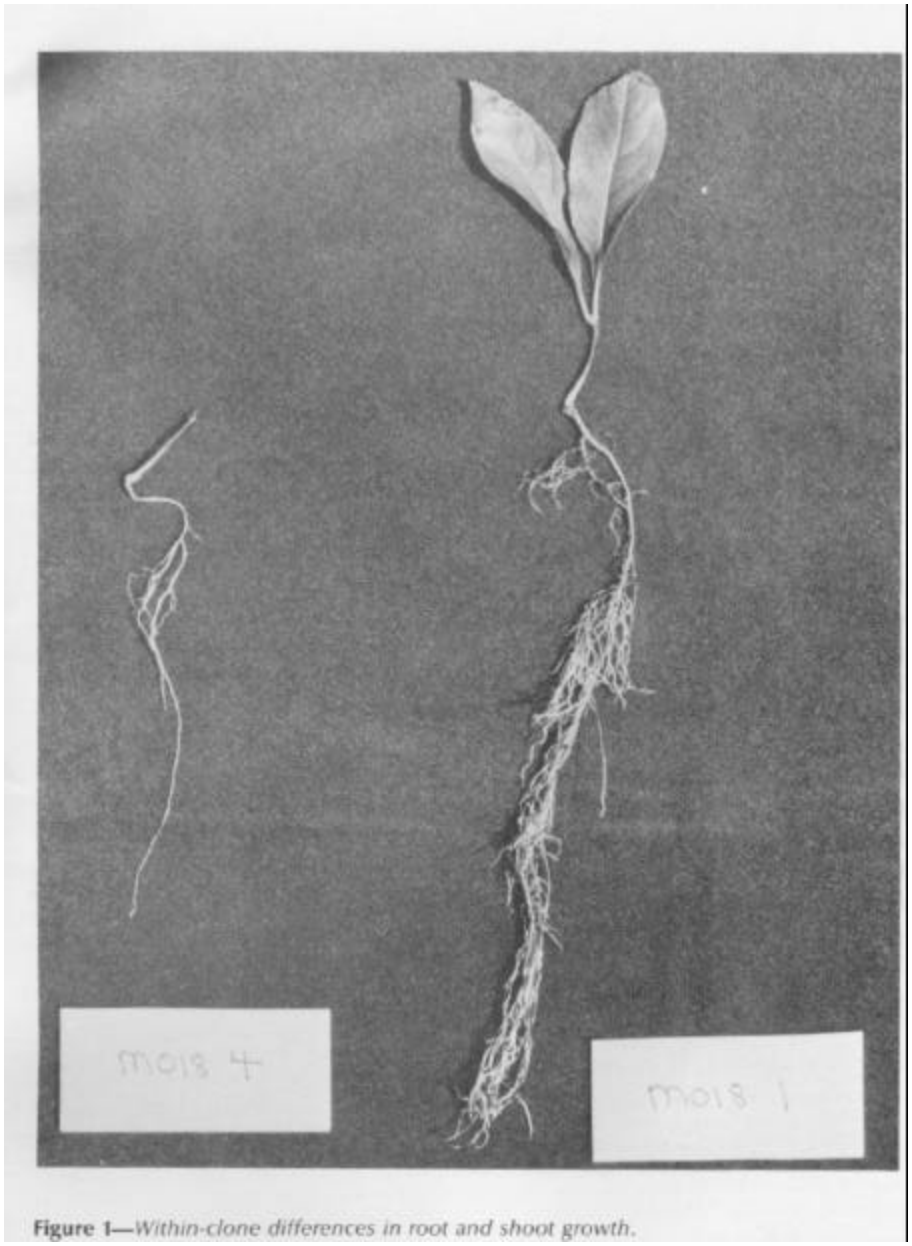

Figure 1—*Within-clone differences in root and shoot growth.*

30% for 3:0:2:1 (table 2). The soil mixture containing equal parts of peat, perlite, and sand (1:1:0:1) gave the largest number of field-plantable plantlets.

Table 2—*Survival rate and plantability of black cherry plantlets grown for 3 weeks in three different soil mixtures after micropropagation*

Peat/perlite/vermiculite/sand (vol)	% Survival	% Plantable
1:1:0:1	80	40
2:1:2:0	90	30
3:0:2:1	60	30

Discussion

The large differences in plantlet condition prior to experimentation may be partly due to the rough treatment during shipment from Syracuse. The plantlets exhibited different amounts of root development and elongation, possibly because some of the plantlets may have not received enough root-inducing hormones during the micropropagation stages conducted at Syracuse. Better methods that promote uniformity in size of shoots and roots need to be developed for the micropropagation stages.

There were no significant differences in root growth among soil mixtures. Nevertheless, the 1:1:0:1 mixture provided the largest number of field-plantable plantlets, and thus it is the preferred mixture. However, we consider these results to be preliminary because of the experimental limitations provided by the initial plantlet condition and

by the soil nutrition discussed below. The decrease in shoot length in some clones may be due to slight variations in the site of measurements. Because the soil level varied slightly within a container due to splashing during irrigation, a more accurate method would be to measure from the shoot tip to the lowest node.

The reddening and necrosis observed on leaves may be due to soil nutrient deficiencies or toxicities caused by soil reaction. At low soil reaction, many nutrients are fixed and not available for plant uptake; in addition, low soil reaction may result in aluminum and manganese toxicities (3). The symptoms observed resemble those associated with phosphorus or possibly nitrogen deficiency (2). The specific nutrient requirements for black cherry and interactions between soil reaction, nutrient absorption, and aluminum and manganese toxicity need to be determined in future research.

Since shoot growth was slow, a technique to stimulate shoot growth is needed. Spraying the bud and foliage with gibberellic acid, which promotes cell elongation and division (4), at time of transplanting, might increase shoot growth.

Conclusions

Two preliminary conclusions may be drawn from this experiment: a) of the three soil mixtures tested, the mixture containing equal parts of peat, perlite, and sand gave the most field-plantable plantlets, and thus is preferable; b) more research on the interactions of shoot elongation, soil reaction, and nutrient availability is needed to successfully grow black cherry tissue culture plantlets in the greenhouse.

Acknowledgments

The authors thank John Frampton, Charles Maynard, Steve McKeand, and K.O. Summerville.

Literature cited

1. Amerson, H.V.; Frampton, L.J., Jr.; McKeand, S.E.; Mott, R.L.; Weir, R.J. Loblolly pine tissue culture: laboratory, greenhouse, and field studies. In: Henke, R.R.; Hughes, K.W.; Constantin, M.J.; Hallaender, A., eds. Tissue culture in forestry and agriculture. New York: Plenum; 1985. 390 p.
2. Davidescu, D.; Davidescu, V. Evaluation of fertility by plant and soil analysis. Kent, England: Abacus Press; 1982. 162 p.
3. Hanan, J.J.; Holley, W.D.; Goldsberry, K.L. Greenhouse management. Berlin: Springer-Verlag; 1978. 289 p.
4. Kramer, P.J.; Kozlowski, T.T. Physiology of trees. New York: McGrawHill Book Co.; 1960. 642 p.
5. Tricoli, D.M.; Maynard, C.A.; Drew, A.P. Tissue culture of propagation of mature trees of *Prunus serotina* Ehrh. I. Establishment, multiplication, and rooting in vitro. Forest Science 31(1):201-208; 1985.