A NEW, MORE EFFICIENT METHOD TO EVALUATE ROOT GROWTH POTENTIAL OF PLANTING STOCK USING A ROOT AREA INDEX

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ABSTRACT

Root growth potential (RGP), the ability of seedlings to promptly and abundantly initiate and elongate new roots after transplanting, is an important and useful attribute of planting stock performance. However, it is generally laborious, tedious, and subjective to measure. A method was developed that employs aeroponic culture of seedlings in a root mist chamber (RMC) and measurement of root growth by changes in root area index (RAI) with a TV camera-based microprocessor area measurement system. The area meter scans each horizontal TV line and sums the segments that are traversed by roots. A high resolution camera was used for accurate area measurement of roots. The method consists of:(1) premeasuring RAI of individual seedlings, (2) growing seedlings in the RMC for ca. 2 weeks (depending on species), (3) staining new roots to make them visible to the camera, and (4) remeasuring RAI of individual seedlings.

An experiment was conducted to compare xylem water potential (XWP) of seedlings grown in the RMC with that of seedlings grown in pots of medium and seedlings grown in hydroponic culture. XWP, measured with a pressure chamber, of seedlings grown in the RMC was similar to that in potted seedlings, and increased (became less negative) when new roots were initiated. Seedlings in the RMC initiated new roots 1 week sooner than potted seedlings. XWP in hydroponically grown seedlings steadily decreased and very few new roots were present after 20 days.

A second experiment determined the relationship between root growth quantified by difference in RAI and that quantified by direct measurement of new root number and length. A range in RGP was accomplished by placing groups of 10 jack pine 2-0 seedlings in a forced-air oven (40°C, 30% RH) for 0, 10, 20, 30, and 40 min, then growing them in the RMC for 17 days. Root growth of individual seedlings was evaluated by the RAI method and by counting and visually estimating length of all new roots >0.5 cm. Linear regression of individual seedling data revealed r^2 values of 0.88 and 0.90 for predicting number of new roots and length of new roots, respectively, from difference in RAI. Eleven seedlings/person/hr were completed using the visual estimation method compared to 32 seedlings/person/hr using the RAI method.

This research documents the accuracy and productivity of the RAI method. Observer subjectivity is nearly eliminated.

EQUIPMENT

The test is conducted in a domestic chest freezer that can attain temperatures of -25 to - 30°C. A thermometer with remote readout is used so that the temperature in the chest can be monitored without opening the lid. A 50 CFM fan is used to circulate the air within the freezer. The fan should be less than 15 watts to avoid unnecessary heat load within the chest.

A cold room or refrigerator at +1°C is ideal for slowly thawing the seedlings. If neither is available, a styrofoam cooler will do. A greenhouse is best for allowing injury symptoms to develop, but a warm well-lighted room will suffice.

CONDUCTING THE FREEZING TEST

The plants to be tested are potted with roots in moist growing medium. Styrofoam containers work well because they help insulate the roots. Number of seedlings per container is not important as long as their crowns are well spaced for adequate air circulation.

The objective is to choose target temperatures that bracket the killing temperature. A wrong estimate may leave the killing temperature off the scale of the test, in which case a second test must be run. We recommend at least three target temperatures, each represented by 10 plants. Before a test begins randomly, divide the plants among the target temperatures and give them color coded labels to facilitate their removal.

Set the containerized plants in the bottom of the freezer and pour vermiculite around the containers so the pots are covered with 5 cm of vermiculite. It is important that the root systems get no colder than -2°C during the tests (0°C if the roots are actively growing).

To run the test, turn the freezer and fan on and set the freezer control at its lowest setting. The rate of cooling down to 0°C is not important, but below that level it should not exceed 2°-6°C/hr. At 0 to -10°C cooling may be too fast, but can be reduced by inserting a wedge (such as a pencil) under the chest lid to hold it open slightly. When the first target temperature is reached, open the freezer and quickly remove the designated plants and place them in a cold room or in a styrofoam cooler containing ice. Turn the fan off before opening to reduce loss of cool air. Then repeat this procedure with the remaining target temperatures.

The rate of thawing can be much faster than the rate of freezing, up to 20°C/hr. An overnight thawing period is sufficient. Then move the potted plants to a favorable growing environment and keep them well watered.

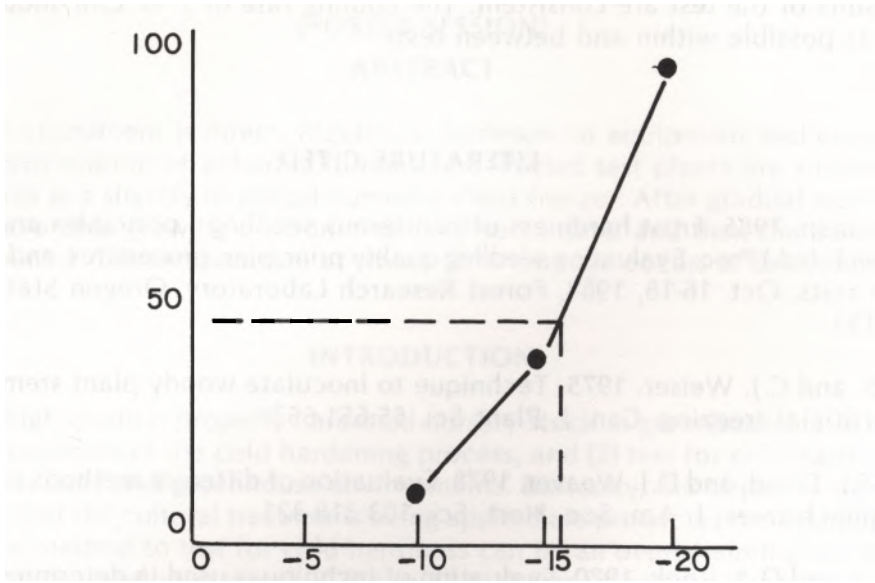
Freezing damage to leaves (or needles), buds, and cambium can be assessed separately and combined into a damage scale, but we prefer to measure only cambial damage. Damage symptoms to cambial tissue required 7 to 14 days to develop, depending on species and environment. To assess damage, use a knife or razor blade and scrape the entire length of the stem down to the cambium. The color of healthy tissue is fresh green, but damaged tissue changes over time from fresh green to drab olive green to brown.

The symptoms of injury will vary from one species to another, and the rate of symptom development will depend on (1) the hardiness of the plants, and (2) conditions in the greenhouse or room where the plants are incubated. With plants in early stages of cold acclimation, symptoms will be readily visible after 7 days. Hardy plants take longer. It is usually desirable to estimate damage as soon as possible, but the longer the incubation period, the less the chance of error.

Measure the length (± 0.5 cm) of the damaged stem down from the shoot tip, then measure the total stem length, excluding the 5 cm covered with vermiculite. Calculate the percent damage for each plant as: (damaged length/total length) X 100. Determine the mean percent damage for the test plants representing each target temperature. Next, plot percent damage versus temperature, as shown in figure 1. From the graph determine the temperature at which 50% damage would occur; this is the lethal temperature for 50% damage, or LT_{50} (the LT 50). Or, you may wish to determine the temperature at which no damage occurs, or 20%, 50%, or 100% damage—depending on your preferences and the purpose of the nursery stock.

TEMPERATURES (°C)

Figure 1. *Percent cambial damage plotted for three freezing test temperatures* (-10, -15,-20°C), *as determined with the whole plant assessment method. The temperature for* 50% *damage (LT_{50}), in this case about* -16C, *is determined from the graph.*



DISCUSSION

A domestic chest freezer has enough cooling capacity for testing during the hardening period to determine if plants have attained sufficient cold hardiness to withstand the natural environment. If you desire additional cooling capacity, replace the refrigerant to extend the lower limit to about -30. Or place a pan of liquid nitrogen in the freezer. Determining the maximum cold tolerance of plant materials requires more sophisticated (and expensive) equipment capable of reaching -70°C.

Our method assumes that the youngest parts of the seedling are the most vulnerable to freezing injury, so that the severity of injury increases as more of the stem is damaged, from the top down. If the species you are working with shows a different pattern of freezing injury, devise an alternative damage rating scheme.

The amount of damage that occurs in a freezing cycle depends on the low temperature attained, on the rates of freezing and thawing, and on the duration of the low-temperature period. Some authors recommend that each target temperature be maintained for aspecified time period (1 to 3 hours) before removing the plants (Glerum 1985). We prefer to remove plants immediately once the target temperature is reached. A test that requires holding the plants at specified minimum temperatures requires more time and equipment than one in which plants are removed immediately upon reaching a benchmark temperature. Although the results of the two methods may be somewhat different, they are both usable for predicting field reaction to freezing temperatures. The important issue here is to choose a technique, then apply it uniformly and consistently.

If maintaining each target temperature is desirable, you can install a microswitch temperature controller (or for more money, a cam-operated or electronic temperature controller) on the outside of the freezer wired to a light bulb mounted in front of the fan. Set the freezer control at its lowest setting. Then the temperature controller and light bulb, working against the freezer's cooling system, will maintain any desired temperature.

In some freezing tests, the plants are inoculated with ice to minimize the damaging effects of supercooling. Plant tissue supercools if the temperature of the cell solution falls below the freezing

point without the formation of ice. When ice does form in supercooled tissues, crystals form very rapidly and are more damaging to tissue than ice that forms slowly in association with little or no supercooling. Supercooling is of more concern when target temperatures fall in a range of 0-to-5°C. Wrapping the seeding stems with a 1- to 2-cm wide strip of damp cheesecloth inoculates plants with ice and circumvents supercooling during freezing tests (McKenzie and Weiser 1975). Because our method does not include seeding with ice crystals, supercooling and increased damage may occur. But results of the test are consistent. The cooling rate of 2°-6°C/hr should be maintained as uniformly as possible within and between tests.

LITERATURE CITED

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