EVALUATION OF ELECTRICAL TECHNIQUES TO DETERMINE LIFTING TIME FOR HARDWOOD PLANTING STOCK

by

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Abstract. A convenient, fast, non-destructive method is needed for evaluating seedling fitness for lifting and storage. Measuring certain electrical charcteristics of plant tissue appears to have these attributes. However, our research with the oscilloscope technique, dormancy meter, and resistance to pulsed direct current has shown that all three methods in their present form have serious problems and limitations. None of the methods were substantiated by experimental verification of dormancy status of seedlings. Several factors were identified and quantified that substantially influence instrument readings. Excessive variability from these factors and from attempts to measure changes in seedling physiology over time greatly reduce the accuracy of predictions. We conclude that the three methods tested are not presently suitable for quantifying the dormancy status or predicting the physiological fitness of nursery stock for lifting.

Additional keywords: Dormancy, oscilloscope technique, MEDC dormancy meter, Shigometer, pulsed direct current, root growth potential, *Juglans nigra, Liriodendron tulipifera, Liquidambar styraciflua, Quercus rubra, Q. alba, Fraxinus pennsylvania,* F. *americana, Pinus taeda,* P. *strobus, Platanus occidentalis.*

Cold-storing hardwood seedlings between lifting and planting is a convenient and widely used practice. Date of lifting is an important factor affecting the success of overwinter storage because it determines the status of bud dormancy that exists at the time seedlings enter storage, and is strongly related to seedling root growth potential (RGP) after chilling (Ritchie and Dunlap 1980). RGP, a measure of the ability of planting stock to rapidly grow new roots after transplanting, is considered a good indicator of potential survival and vigor. In previous research, we found that lifting black walnut *(Juglans nigra* L.) seedlings too early in the fall resulted in reduced root growth potential the following spring (Rietveld and Williams 1981). The physiological factors related to seedling readiness for lifting are not clearly understood, and we know little about how the relationship varies for

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different species. We have assumed that some degree of dormancy and/or cold hardiness must develop before seedlings can be lifted without adverse effects. Two important questions remain: (1) what degree of dormancy is sufficient, and (2) how can be conveniently detect the degree of dormancy?

A clear distinction should be made between cold hardiness and dormancy. Although both processes parallel the general trend of decreasing physiological activity in the fall, their mechanisms and significance are different. Temperature is the key environmental factor for synchronizing a plant's capacity to develop cold hardiness. After vegetative growth ceases in the summer, the maturation and cold acclimation of tissues begin in response to cold temperatures in autumn. Species differ in the timing and amount of acclimation that develops in response to warmer temperatures. Cold hardening results from certain changes in the properties of the protoplasm and cellular membranes that favor outward movement of water during freezing and reduces the likelihood of intercellular ice formation and other damaging stresses in the cellular environment (Steponkus 1978).

Dormancy, defined as the absence of growth (Samish 1954), is often assumed to be caused by cold weather, but this is only partially true. In many species, growth commonly ceases long before the temperature is low enough to check growth. Dormancy is a phenomenon associated with the buds and is the result of changes in photoperiod, soil and air temperature, and availability of water and nutrients (Perry 1971). The cessation of growth is caused by changes in concentrations and proportions of certain growth-regulating substances. Once the seedling enters dormancy, exposure to cold temperatures for a certain amount of time is necessary to overturn the balance of these growth-regulating substances so that growth will be renewed when favorable conditions return.

Although most research on the dormancy cycle in tree seedlings has been done on conifers, available evidence indicates that the general pattern is applicable to hardwood seedlings (Ritchie and Dunlap 1980). The cycle documented for Douglas-fir is divided into four district but continuous phases: (1) dormancy induction, (2) dormancy deepening, (3) true dormancy, and (4) post-dormancy (Cleary et al. 1978). When Douglas-fir seedlings are lifted before or after the period of true dormancy, buds are not physiologically responsive to chilling, storage is often unsuccessful, and RGP can be seriously reduced (Hermann et al. 1972). Although there is a lack of information relating seedling dormancy and cold hardiness to lifting and storing of hardwood species, recent evidence suggests that some hardwood species may be lifted and stored during the dormancy deepening phase without adverse effect (Rietveld and Williams 1981, 1982 in press).

The objective is to identify the earliest date that planting stock can be lifted in the fall and stored overwinter without adversely affecting seedling physiological quality at time of planting. Although experienced nurserymen have successfully used phenological events such as leaf fall or weather events such as the occurrence of one or more frosts as criteria to begin lifting, it is preferable to have a convenient and precise method that directly measures seedling readiness for lifting.

Measurement of electrical properties of plant tissue has been studied for many years to determine various physiological conditions in plants. Electrical techniques are convenient and non-destructive. However, the relationship between electrical properties of plant tissues and dormancy is implicit and not clearly defined. In this paper we summarize 5 years of research with three electrical techniques to detect dormancy in tree seedlings: (1) the oscilloscope technique, (2) the Missoula Equipment Development Centre (MEDC) dormancy meter, and (3) resistance to pulsed direct current (RPDC). This paper is a progress report on research to develop an electrical technique to determine degree of dormancy in tree seedlings.

OSCILLOSCOPE TECHNIQUE

Background

Wanek (1971) tried to assess the degree of dormancy by observing changes in the shape of an oscilloscope trace when a square-wave voltage - a mathematically composed spectrum of frequencies - was applied to Douglas-fir needle. He believed differences in trace shapes indicated life, dormancy, or death in plant tissue. Zaerr (1972) confirmed that the changes in waveform were an effective early indicator of dead plant tissue, and reasoned that the resultant oscilloscope trace is an indicator of impedance (alternating current resistance) over a wide range of frequencies. Ferguson, Ryker, and Ballard (1975) developed what is presently known as the oscilloscope technique for determining seedling dormancy (Fig. 1). The method involves passing a square-wave voltage through plant tissue by means 'of a four-needle electrode probe. The resultant waveforms displayed on an oscilloscope screen characterize dead, dormant, and active tissue (Fig. 1). When the square-wave is passed through dead tissue, a greater attenuation of high frequencies results in a rounded leading edge (sawtooth pattern) on the oscilloscope waveform. If a seedling is in an active state, greater attenuation of the midrange frequencies results in a peaked leading edge on the oscilloscope waveform. When a tree is dormant, the square-wave pattern is returned to the oscilloscope at a smaller amplitude but is unchanged in shape.

Experimental

Materials and methods. We tested the oscilloscope technique's ability to detect dormancy in connection with a lifting and storage study of black walnut seedlings. Because the detailed methods and results of that research are published elsewhere (Rietveld and Williams 1977), they will only be briefly summarized here. Seedlings were lifted at approximate 2-week intervals between October 6 and April 25. Twelve seedlings representing each lifting date were tested by the oscilloscope technique at room temperature before overwinter cold storage, and again after 4-week-long RGP tests in the spring. Individual seedlings were simply scored as active, dormant, or dead, based on waveform characteristics.

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[2] For excellent reviews of electrical impedance techniques in seedling physiology research, see Glerum (1980) and Tattar and Blanchard (1976).

Fig. 1. Equipment for the oscilloscope technique consists of square-wave generator, portable oscilloscope, and four-needle electrode probe. Bottom: oscilloscope waveforms indicating active-growing, dormant, and dead plant tissue. (Photo courtesy of Missoula Equipment Development Centre, USDA Forest Service, Missoula, MT.)

Results and discussion. By carefully adjusting the oscilloscope, we found that with practice we could obtain waveforms for black walnut seedlings resembling those reported by Ferguson et al. (1975) for different degrees of growth activity (Fig. 1). Waveforms for conspicuously actively growing, dormant, and dead seedlings were easy to interpret. However, waveforms for seedlings in transitional stages were more difficult to interpret. To be confident, the evaluator should wait until a consistent dormant waveform is obtained before beginning lifting operations.

We obtained a "dormant" waveform approximately October 20, about 2 weeks after leaf fall. However, RGP tests of the same seedlings after overwinter storage indicated that seedlings lifted and stored before November 1 had reduced RGP (Rietveld and Williams 1981). From that study we estimated that the earliest date to begin lifting black walnut seedlings is about one month after leaf fall. Thus, if we had judged seedling readiness by the oscilloscope technique, we would have begun lifting about 1-2 weeks too early.

The delicate instrument adjustments required are a major drawback of the oscilloscope technique, e.g. if the gain control is set improperly, the waveform on the oscilloscope may not be large or distinct enough to be accurately interpreted. Holbo et al. (1981) also pointed out that the oscilloscope itself influences the waveform characteristics.

Holbo et al. (1981) recently reported their attempts to experimentally substantiate the oscilloscope technique with an independent verification of the stage of dormancy in Douglas-fir seedlings. They found that waveform characteristics in late summer and fall were erratic and poorly correlated with time to bud break - a method of determining the dormancy status of seedlings by observing the number of days needed for seedlings to flush in a standarized environment. However, the correlation was food for seedlings coming out of dormancy and resuming growth in the spring. They concluded that the lack of consistency in the early season did not allow prediction of when true dormancy would be reached. Askren and Hermann (1979) tested the suitability of the oscilloscope technique for predicting seedling survival after planting. Waveform characteristics measured at time of lifting and at time of planting were not significantly related to survival after planting. They concluded that the oscilloscope technique apparently does not indicate vigor as such, and thus is poorly suited for predicting survival potential.

Evaluation

Although some individuals have developed much skill and confidence in the oscilloscope technique, we believe that general application is constrained by the following combination of factors: (1) instrument cost, (2) complexity of instrument adjustments, and (3) lack of experimental substantiation with the true dormancy status of seedlings.

The oscilloscope technique appears to be sensitive to the degree of growth activity in seedlings. Since we have defined dormancy as the absence of growth, we have assumed that decreasing growth activity means increasing dormancy. This may not be true. Additional research is needed to solve these problems. Based on these factors, we conclude that the oscilloscope technique, in its present form, is not a convenient and objective method suitable for general practice.

MEDC DORMANCY METER

Background

The MEDC dormancy meter was designed to be a simple, one-piece, inexpensive instrument that duplicated the function of an oscilloscope and square-wave generator. Instead of a square-wave, the dormancy meter introduces an electrical sinusoidal wave voltage that alternates between a high frequency of 3 KH_z and a low frequency of 500 KH_z (Andrews and Deland 1979). The compact instrument is equipped with an analog meter and indicator lights that indicate directly the status (dead, dormant, or active) of a seedling impaled on its 4-needle electrode probe. The null (center) point of the meter is zero; values to the right (positive) indicate an icnreasing degree of growth activity, and values to the left (negative) indicate a decreasing degree of growth activity. Although the prototype instrument is calibrated to oscilloscope data for ponderosa pine (*Pinus ponderosa* Laws.), it has been released for testing on various species by researchers around the country.

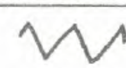
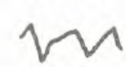
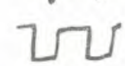
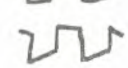
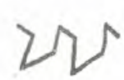
Experimental

We received one of the prototype instruments in 1978 and tested its ability to predict the dormancy status of seedlings of several hardwood and coniferous species at the Union Forest Nursery near Jonesboro, IL (lat. N 37°30', long. W. 89°20'). Three separate experiments were run to: (1) substantiate the MEDC dormancy meter with the oscilloscope technique, (2) verify the instrument-measured dormancy status of seedlings in the nursery with the experimentally determined dormancy status of the same seedlings, and (3) determine the influence of air temperature and seedling stem diameter and moisture content on readings. The methods and results of the three experiments will be presented separately.

Substantiation of the MEDC dormancy meter with the oscilloscope technique. Because the MEDC dormancy meter was developed to replace the oscillopscope and square-wave generator, the two methods should give similar indications of the status of individual seedlings. Instrument comparisons were made of dormant seedlings and of seedlings at different stages of growth resumption. This preliminary experiment gave us an early idea of how well the dormancy meter would work for different species.

Materials and methods. Approximately 150 seedlings of each of four species - sycamore (*Platanus occidentalis* L.), yellow poplar (*Liriodendron tulipifera* L.), sweetgum (*Liquidambar styraciflua* L.), and loblolly pine (*Pinus* taeda L.) - were tested with both instruments upon removal from storage in May (test 1). These seedlings were in post-dormancy, i.e. they were adequately chilled and ready to resume growth upon exposure to favorable temperatures. Comparisons on actively growing seedlings (test 2) were made by potting 1-0 planting stock of several species - yellow poplar, eastern white pine (P. *strobus* L.), white ash (*Fraxinus americana* L.), sycamore, northern red oak (*Quercus rubra* L.), white oak (Q. *alba* L.), and black walnut - and placing them in a greenhouse. Each species was divided into three groups and measured with both instruments after 1, 2 or 3 weeks in the greenhouse. Seedling growth activity ranged from bud swelling to rapid shoot elongation. All seedlings were probed just below the meristem or terminal bud.

Determinations of the status of individual seedlings by both instruments were converted to the following classes:

Class	Status	Dormancy meter reading	Oscilloscope waveform
0	Dead	-100 to - 45	
1	Transitional dead/dormant	- 44 to - 25	
2	Dormant	- 24 to + 3	
3	Transitional dormant/ slightly active	+ 4 to + 15	
4	Active	+ 16 to +100	

Oscilloscope waveforms were interpreted as described by Ferguson et al. (1975), and equivalent dormancy meter ranges were obtained from David Gasvoda, Missoula Equipment Development Center. Determination by the two instruments were tested for similarity by chi-square tests of independence.

Results and discussion. The growth status indicated by the two instruments was found to be similar for actively growing seedlings (test 2) of most species, but significantly different for dormant seedlings (test 1) (see Table A-1 in appendix for statistical data). In most cases, the two instruments agreed on the qualitative active or dormant status of seedlings; they disagreed mainly on the degree of activity or dormancy. These results suggest that: (1) dormancy meter scale readings indicative of dead, dormant, and active seedlings will be different for different seedlings, i.e. the calibration for ponderosa pine will not work for all species; and (2) like the oscilloscope technique, the dormancy meter gives inconsistent readings for dormant seedlings, but the two instruments are in fairly close agreement for active seedlings.

Verification of MEDC dormancy meter readings with the actual dormancy status of seedlings.

Materials and methods. Techniques are not available for rapidly determining the stage of seedling dormancy. The only reliable method for quantitatively determining the actual dormancy status of seedlings is observation of the number of days required for seedlings to flush in a standarized forcing environment. Our assumption was that seedlings in the dormancy deepening phase would not resume growth under favorable conditions, but once the true dormancy phase was reached seedlings would resume growth slowly under favorable conditions. As the seedlings received more chilling, they would resume growth more rapidly.

We related dormancy meter readings to the experimentally determined dormancy status of five species: yellow poplar, sweetgum, northern red oak, green ash (F. pennsylvanica Marsh.), and loblolly pine. These species were chosen because they apparently enter dormancy at different times. Yellow poplar, sweetgum, and loblolly pine enter dormancy gradually and do not reach dormancy until late in the season. Their dormancy status is difficult to diagnose, and they are more likely to be lifted and stored too early.

From October to December 1978, 25 scattered 1-0 bedrun seedlings of each species of average root-collar diameter + 1 mm) and average height (+ 1 cm) were tagged in the nursery beds. At weekly intervals, the growth status of each seedling was measured with the dormancy meter by impaling the seedling just below the meristem or terminal bud on four 0.4-cm-long stainless steel needle electrodes spaced 0.5 cm apart and encased in a plastic handle. When the instrument stabilized, the meter reading was read and recorded. Then the same seedlings were hand lifted, packed in moist peat in sealed plastic bags, and potted within 2 hours of lifting. The 25 seedlings representing each species and lifting date were randomly divided between two 5-gallon pots of washed sand (i.e. 12-13 seedlings per pot) and placed in a standarized greenhouse environment consisting of continuous fluroescent and incandescent light, minimum air temperature of 30°C, and root temperature of constant 24°C. The root temperature was kept by pumping water from a reservoir maintained at that temperature through coiled plastic tubing in each pot. Seedlings were inspected for bud burst (defined as when the expanding bud doubled in length and began unfolding) twice weekly and the date of bud burst was recorded for each seedling.

Results and discussion. Dormancy meter readings showed a gradual and variable pattern in the five species during the dormancy induction and dormancy deepening periods in the fall of 1978 (Fig. 2). We planned to continue measurements into the winter months, but found that the instrument would not function at temperatures lower than 5-8°C. According to dormancy meter readings, green ash, red oak, and yellow poplar seedlings were less dormant in December than they were in October.

The time required for bud burst in a forcing environment, based on 80 percent of the seedlings that burst buds during a 20-week period, reflected a similar pattern of gradual dormancy induction for all species except green ash and red oak (Fig. 3). Linear regressions of days to bud burst on dormancy meter readings showed an almost complete lack of relationship (Fig. 4, see appendix Table A-2 for regression data). This is attributed to: (1) high variability and (2) the narrow range of meter readings. Dormancy meter readings of red oak, sweetgum, and yellow poplar seedlings were significantly correlated with air temperature fluctuations before each measurement (see appendix Table A-3 for correlation data).

Factors influencing MEDC dormancy meter readings. Our evaluation of the dormancy meter included tests of the separate effects of seedling internal temperature, stem diameter, and moisture content on meter readings.

Materials and methods. Twenty-five dormant black walnut, white ash, and yellow poplar seedlings, lifted in mid-November and cold stored, were used in the tests. Each seedling was numbered and six 2.0-cm stem segments were marked on each seedling below the terminal bud. Stem diameter at the center of each segment was measured and recorded. Seedlings of each species were placed in random order in a separate flat bundle with roots wrapped in moist burlap and plastic and tops exposed. The bundles were then placed in a small growth chamber set at approximately 2°C and allowed to equilibrate over night. A cardboard door with a plastic window and arm holes allowed us to reach into the growth chamber to probe seedlings without modifying the environment. The instrument was kept outside the growth chamber at room temperature. In one continuous 16-hour period, the 25 seedlings of each species were probed at the initial temperature, then repeatedly at five approximately 5°C intervals. Each measurement was taken on a different stem segment, which was assigned at random. Two hours were allowed for temperature equilibration between measurements. A needle thermister was used to determine that the internal temperature of the seedlings had adjusted to the air temperature before measurements were made. At the end of the test, the six measured segments were cut from each seedling and their moisture content determined. Dormancy meter readings taken at six temperatures were subjected to multivariate analysis of variance (a = 0.05) using stem diameter and moisture content as covariables.

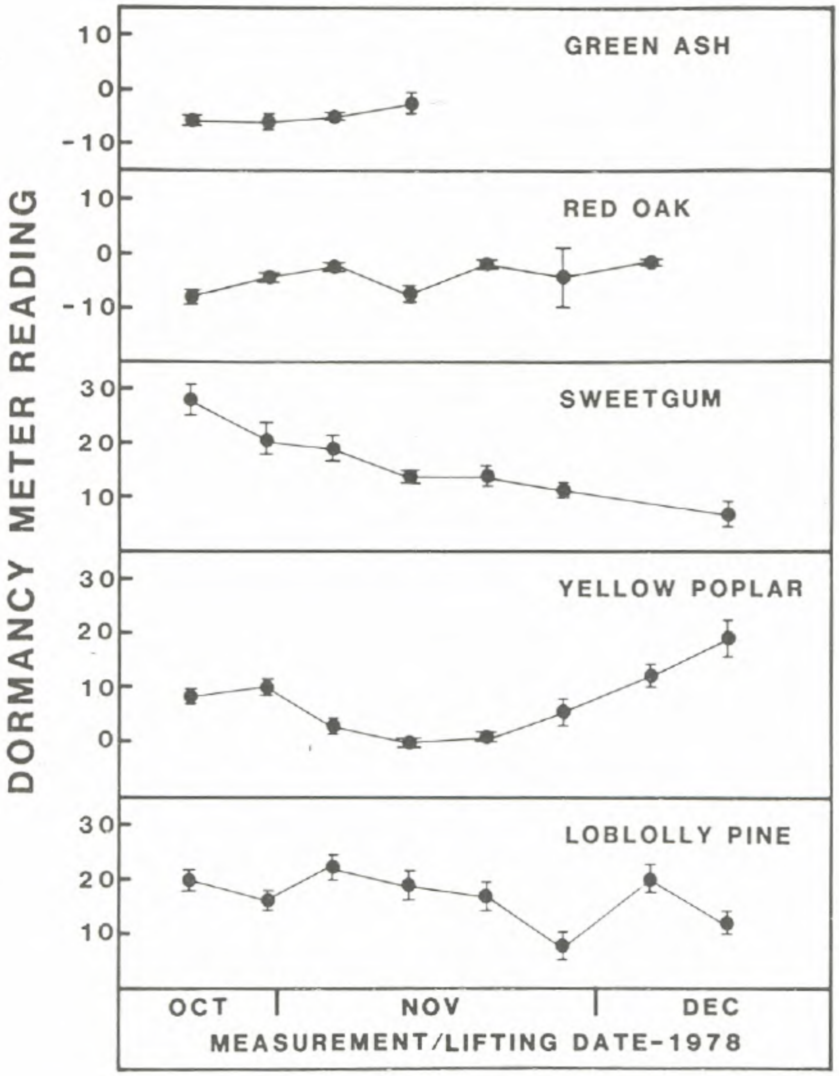


Fig. 2. Patterns of MEDC dormancy meter readings of five species at the Union Forest Nursery, IL, in fall 1978. Readings are not adjusted for temperature effects. Each value is the mean of 25 seedlings + 1 standard error.

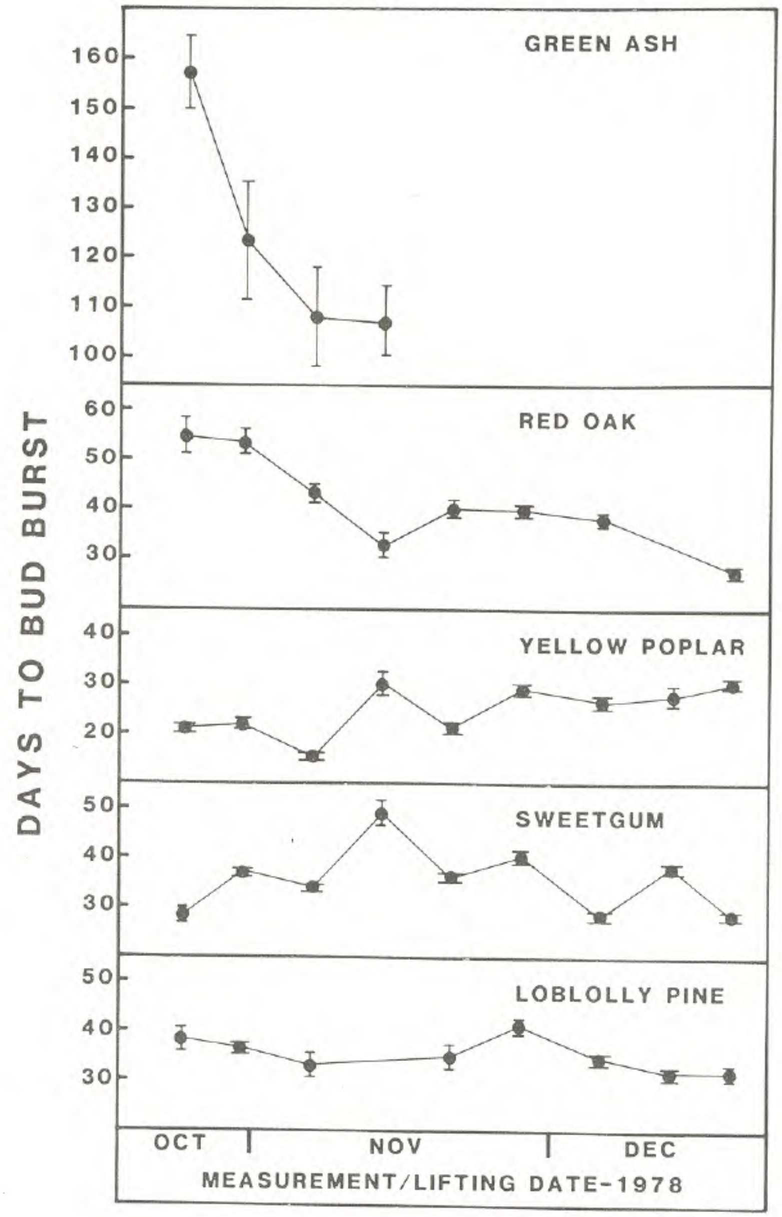


Fig. 3. Days to bud burst, determined in a standard forcing environment, of seedlings of five species lifted immediately after their dormancy status was measured using the MEDC dormancy meter. Values are the means + 1 standard error of 80 percent of seedlings that burst buds during a 20-week forcing period.

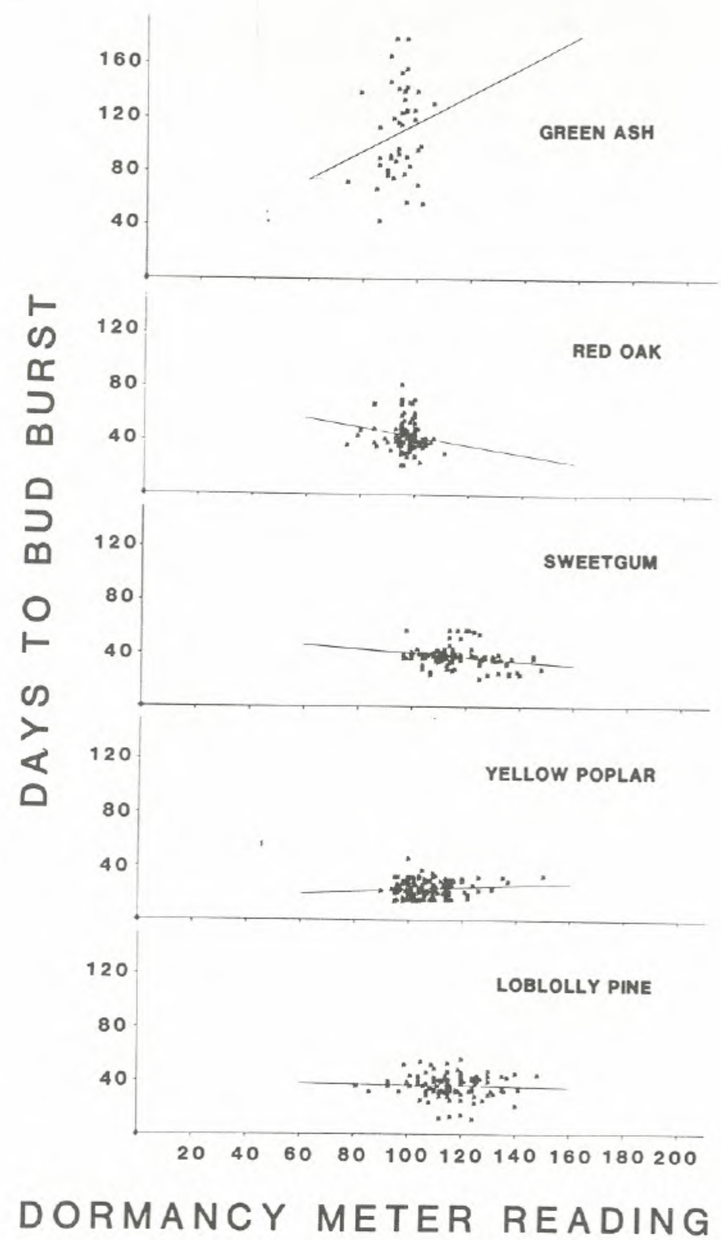


Fig. 4. Linear regressions of days to bud burst on MEDC dormancy meter readings for five species. Dormancy meter readings were converted to a linear 0-200 range with 100 equivalent to the null point on the meter. Seed Table A-2 in appendix for regression data.

Results and discussion. Dormancy meter readings of all three species were significantly affected by temperature (Fig. 5), although the actual differences were small (approximately 5 units over a 24°C range). The relation between dormancy meter readings and temperature was significantly different among the three species. These findings do not invalidate the technique, but they do imply that it will be necessary to apply a different temperature correction for each species measured. Dormancy meter readings in figure 2 were not adjusted for temperature effects because a temperature correction was available for only one (yellow poplar) of the five species. Seedling stem diameter and moisture content had no significant effect on dormancy meter readings.

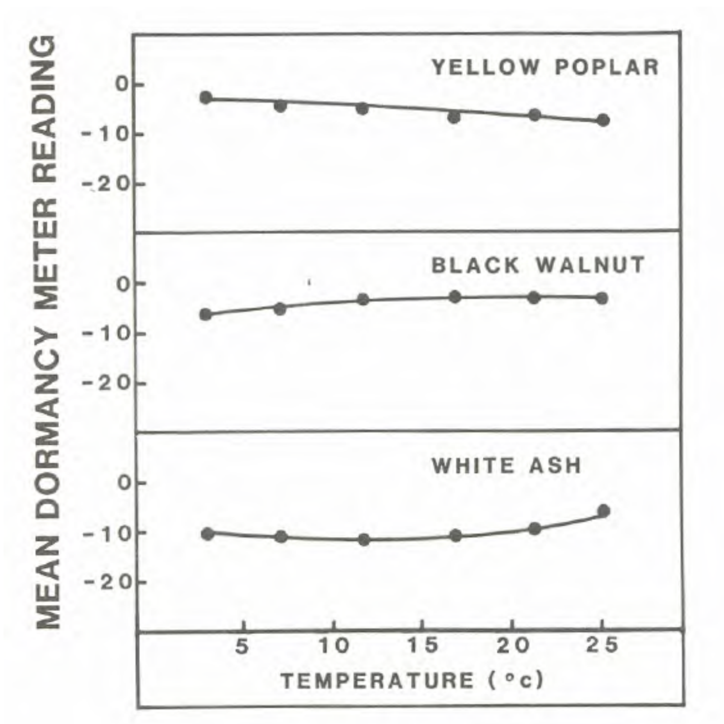


Fig. 5. Effect of temperature on MEDC dormancy meter readings of yellow poplar, black walnut, and white ash seedlings. Twenty-five seedlings were repeatedly probed at six temperatures in a controlled-environment chamber. Maximum standard error of the means were: yellow poplar + 1.46, black walnut + 0.88, and white ash + 1.55. The data were best-fitted with curvilinear functions; see Table A-4 in appendix for regression data.

Evaluation

Although dormancy meter readings appeared to reflect the degree of physiological activity in seedlings, they were only weakly related to the experimentally determined dormancy status of the same seedlings. Barnett (1978) also reported that dormancy meter readings of nursery and containerized loblolly pine seedlings varied considerably with variations in soil fertility and moisture, as well as in probe location and the presence of surface moisture. Results of the present studies suggest that future refinements of the instrument should account for temperature effects and individual species differences.

Based on the weak relationship with seedling dormancy and high variability, we conclude that the dormancy meter, in its present form, has no advantage over present empirical methods of determining when seedlings are ready for lifting.

RESISTANCE TO PULSED DIRECT CURRENT (RPDC)

Background

Electrical resistance varies as the concentration and mobility of cations in plant tissue vary (Glerum 1980). Low resistance means a high ionic concentration and high level of metabolic activity (growth); high resistance-means low ionic concentration and low metabolic activity (dormancy?). Some investigators have suggested that it is better to measure resistance to pulsed direct current rather than impedance to alternating current for three reasons: pulsed direct current minimizes electrode polarization, the pathway is almost entirely through the cell walls, and it least disrupts the process being measured (Dixon et al. 1978, Fensom 1966). However, Glerum (1980) contends that these claims have not been substantiated for the frequencies used.

RPDC has been applied to detect discolored and decayed wood in utility poles and living trees (Skutt et al. 1972, Shigo and Shigo 1974), stress due to defoliation (Wargo and Skutt 1975), presymptom presence of disease (Blanchard and Carter 1980), growth rates associated with fertilization and release (Smith et al. 1976), tree vigor as a guide to thinning (Shortle et al. 1979) wound closure (Shortle et al. 1977), and water potential of trees (Dixon et al. 1978). Seasonal changes in RPDC have been identified in inner bark of several tree species (Davis et al. 1979). Resistance was lowest in the summer when metabolic processes were most active and highest in the winter when metabolic processes were least active.

We previously reported that the pattern of increasing RPDC appears to coincide with the onset of dormancy in seedings of several hardwood species (Rietveld and Williams 1979). From these observations, we suggested that monitoring electrical resistance may provide a measure of the physiological fitness of nursery stock for lifting. In this paper we report the results of our continuing research on the method and its suitability for indicating seedling dormancy.

Verification of RPDC with actual dormancy status of seedlings.

Materials and methods. The verification experiment previously described for the MEDC dormancy meter - which involved nursery measurements with the instrument, followed by lifting the seedlings and determining days to bud burst in a greenhouse - also included measurements for RPDC. The same species and seedlings were sequentially measured with both the dormancy meter and a portable DC ohmmeter (Northeast Electronics Corp., Shigometer[R] Model 7950). The Shigometer[R] delivers a pulsed current of 0.5 pA for 0.5 ms with intervals of 10 ms between pulses (Skutt et al. 1972). Current was introduced into plant tissue through two 0.5-cm-long uninsulated stainless steel needle electrodes spaced 1.2 cm apart enclosed in a plastic handle (Delmhorst Instrument Co. Model 2-D). The electrodes were inserted into the seedling stem just below the meristem or terminal bud.

Results and discussion. Patterns of RPDC adjusted for air temperature (Fig. 6) were similar to those previously reported (Rietveld and Williams 1979). The pattern of increasing resistance during the fall appeared to coincide with the cessation of growth. The time required for bud burst in a favorable environment, based on 80 percent of the seedlings that burst buds, reflected a similar pattern of gradual dormancy induction for all species except green ash (Fig. 3). However, regressions of days to bud burst on RPDC of individual seedlings revealed that RPDC was a poor indicator of dormancy status for the species tested (Fig. 7, see Table A-5 in the appendix for regression data). The poor relation was the result of high variability attributable to: (1) interfering factors (reported in the following section), and (2) apparent variation in physiological activity that is not related to dormancy.

Although mean resistance values were adjusted for ambient temperature differences among treatments (Fig. 7), the data still reflected significant temperature effects. Temperature-adjusted RPDC was negatively correlated with air temperature fluctuations before each measurement in all species (see Table A-3 in appendix for correlation data. This implies that the temperature correction based on air temperature does not completely remove temperature effects from RPDC measurements.

Factors influencing RPDC.

Materials and methods. To define the effects of temperature and other variables on RPDC, additional experiments and analysis were preformed to account for variation in RPDC measurements. The effects of seedling internal temperature, stem diameter, and moisture content on RPDC in black walnut seedlings were determined in an experiment identical to that performed for the MEDC dormancy meter. Therefore, methods and procedures will not be repeated here.

Results and discussion. Tissue moisture content had no significant effect on RPDC. This finding agrees with Glerum's (1980) statement that moisture contents above the fiber saturation point (25-30%) have little effect on resistivity.

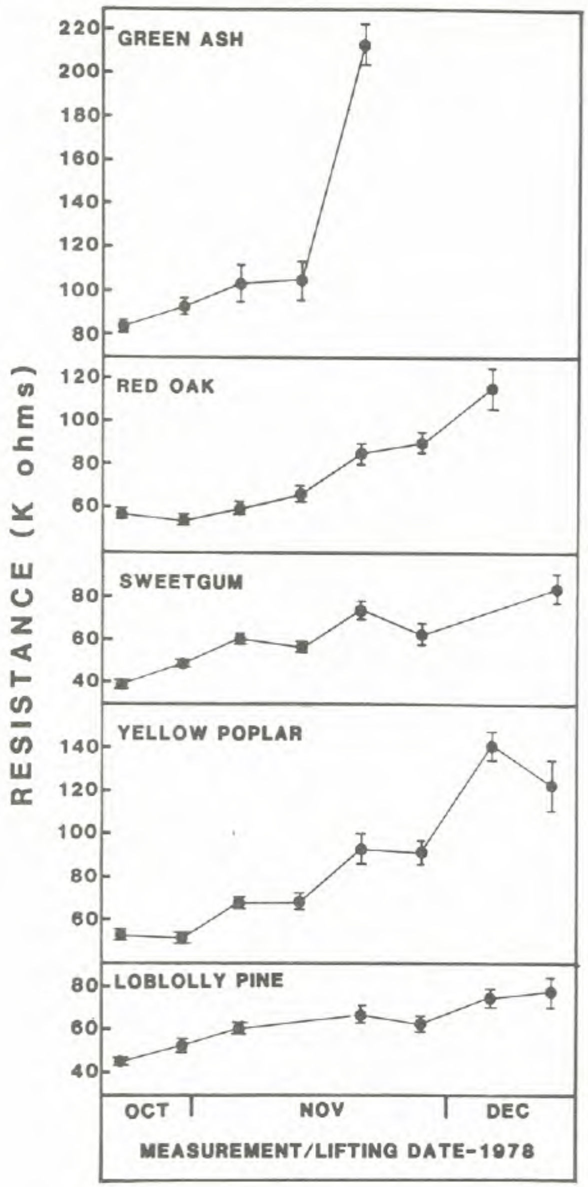


Fig. 6. Patterns of RPDC of five species at the Union State Nursery, IL, in fall 1978. Readings were taken just below the terminal bud. Each point in the mean resistance of 25 seedlings (+ 1 SE) adjusted to a standard temperature of 200C.

Stem diameter contributed significantly to RPDC readings; seedlings with small stem diameters usually had higher resistance than seedlings with large stem diameters.

Temperature was clearly the most important variable affecting RPDC measurements. As temperature decreased, RPDC increased. We found

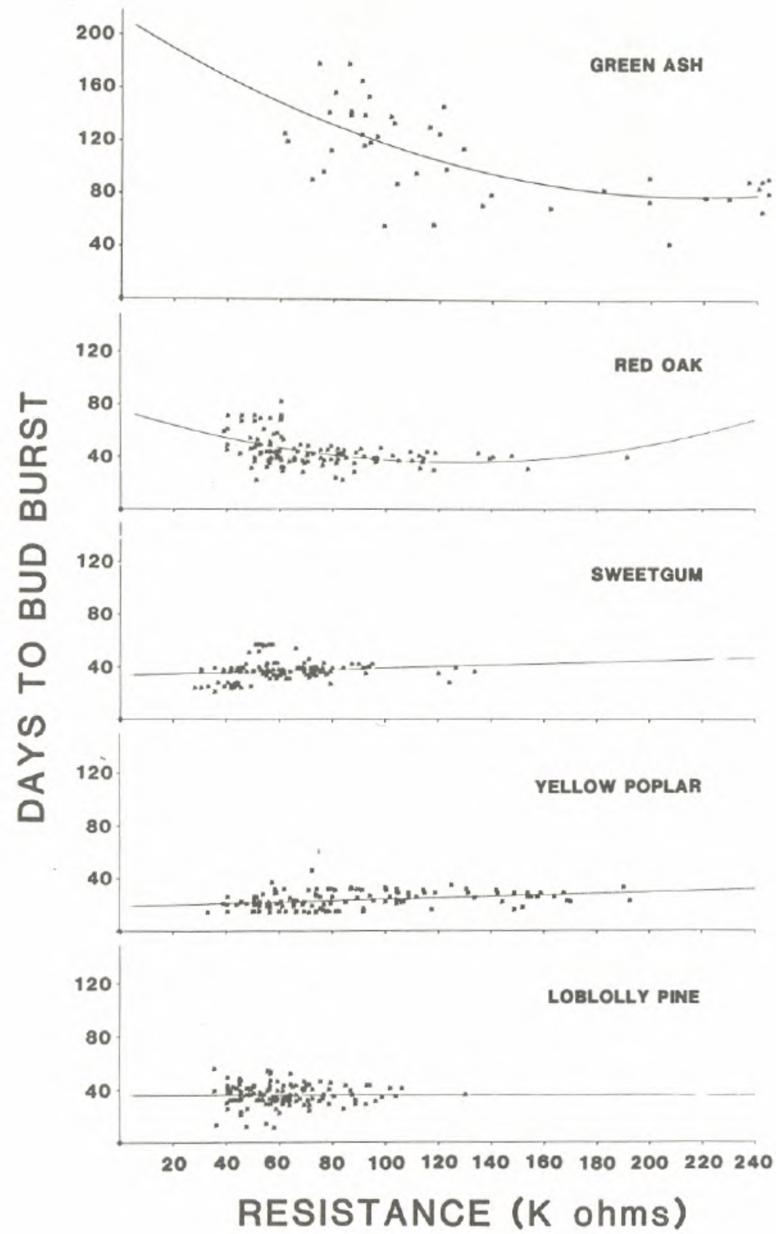


Fig. 7. Regressions of days to bud burst on RPDC for five species. See Table A-5 in the appendix for regression data.

that measuring and correcting for seedling internal temperature was more accurate than correcting for ambient temperature, as was done in our 1977 nursery measurements (Rietveld and Williams 1979) and 1978 nursery measurements (Fig. 6). In the nursery, seedling internal temperature was found to differ as much as 5°C from ambient temperature.

Using a stepwise least squares multiple regression procedure, we developed an equation that accounts for much of the physical effects of seedling internal temperature and stem diameter on RPDC measurements of black walnut seedlings. The best regression model was determined according to the following criteria: (1) a decrease in mean square error (MSE) or the subset regression index [C(P)], and (2) an increase in r^2. The best equation obtained was:

$$R = 211.1313 - 5.6142\ (T) + 0.083791\ (T^2) = 20.127762\ (D) + 1.218648\ (D^2) - 29.891948\ (D/T)\quad (r^2 = 0.88).$$

where:

R = resistance to pulsed direct current (RPDC)
T = internal seedling temperature in degrees Centigrade
D D = stem diameter millimeters

Relation of RPDC at lifting time to root growth potential after overwinter cold storage. By measuring electrical resistance of seedlings in the nursery bed before lifting, then determining RGP in the spring, we hoped to accomplish the following objectives: (1) experimentally determine the "best" date to begin fall lifting, and (2) determine if the pattern of seedling RPDC can be applied to predict when the seedlings were ready for lifting. Seedlings must be fully chilled before RGP can be measured (Rietveld and Williams 1978).

Materials and methods.' We measured RPDC of a random sample of 50 black walnut seedlings at weekly intervals between September 22 and December 15, 1980, at the Vallonia Forest Nursery, Indiana (lat. N 38°57', long. W 89°20'). At the same time, random samples of 24 seedlings were lifted and packaged in poly-lined bags for overwinter cold storage. In mid-April 1981, RGP was measured by potting the seedlings in sand and placing them in a favorable environment equivalent to that used in the forcing study described previously. After 4 weeks, the seedlings were unpotted and number, total length, and surface area of new roots longer than 1 cm were measured. Data was subjected to one-way analysis of variance and Duncan's New Multiple Range Test using a = 0.05.

Results and discussion. The pattern of mean RPDC (covariance adjusted for internal temperature and stem diameter effects) of black walnut seedlings during the fall was typical of that previously reported (Rietveld and Williams 1979), **i.e.** resistance increased as the season progressed (Fig. 8A).

RGP measured as mean number of new roots per seedling showed significant differences among the 13 lifting dates (Fig. 9B). Trends in number, length, and surface area were similar, so only lifting date effects on number of new roots are shown. Seedlings lifted before mid-October and on November 10 had significantly fewer new roots that those lifted later. The higher RGP response of seedlings lifted on December 8 was consistent within the sample, and coincided with a cold period. These results basically agreed with our previous finding that lifting and storing black walnut seedlings sooner than approximately 1 month after leaf fall results in lowered RGP after overwinter storage (Rietveld and Williams 1981).

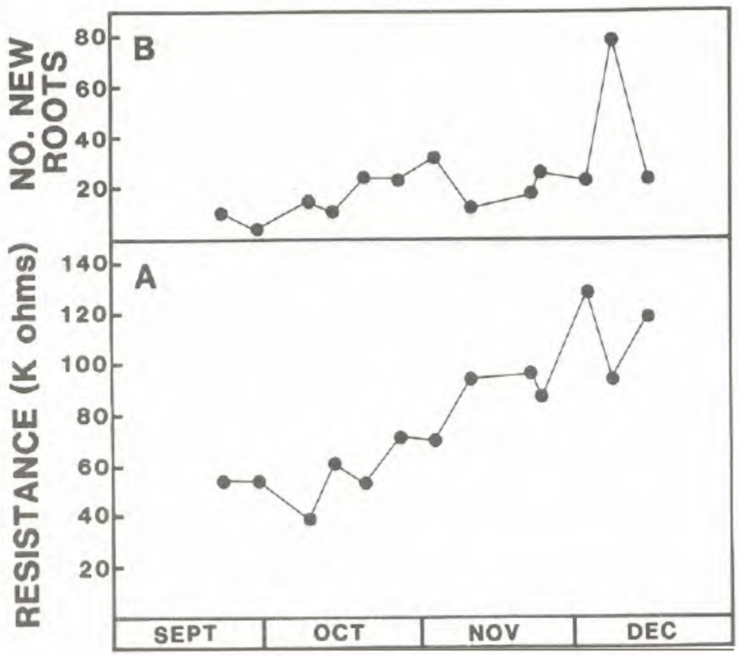


Fig. 8A. Mean resistance to pulsed direct current (RPDC), covariance adjusted for internal temperature and stem diameter effects, measured on 13 dates between September 22 and December 1980 at the Vallonia Forest Nursery, IN.

Fig. 8B. Root growth potential (RGP), expressed as mean number of new roots per seedling for seedlings lifted on the same dates and cold-stored overwinter.

Linear regression of RGP responses on mean RPDC at time of lifting revealed that RPDC is a poor indicator of the effects of lifting time on RGP (see Table A-S in the appendix for regression data).

Evaluation

Although a consistent pattern of increasing RPDC occurs in tree seedlings during the fall, which appears to coincide with the onset of dormancy, no such relation could be verified experimentally. RPDC was found to be a poor predictor of the actual dormancy status of individual seedlings of several species, and of the readiness of black walnut seedlings for lifting and overwinter storage.

Our results verified that lifting of black walnut seedlings may begin approximately 3-4 weeks after leaf fall without adverse effects on RGP. Thus, it appears that black walnut seedlings may be lifted and stored during the dormancy deepening phase, i.e. before they are fully dormant, without reducing seedling quality.

DISCUSSION

These results make it increasingly apparent that we are attempting to precisely quantify a physiological process (dormancy) that we only imprecisely understand in hardwood species. Additional research is needed to determine the relationship between: (1) environmental variables and seedling dormancy, (2) seedling dormancy and cold hardiness and seedling readiness for lifting and storage, and (3) electrical characteristics of plant tissue and seedling dormancy. The negative results from these experiments do not invalidate the electrical techniques tested. The electrical methods are only as good as our understanding of their use; the instruments measure different electrical characteristics of plant tissue, and it is up to us to determine what the readings mean. Indeed, the methods may give us some valuable clues about dormancy and cold hardiness and what they really are.

The experiments have shown that there are several serious problems to overcome if electrical diagnostic techniques are to be developed. Future refinements of the methods should account for temperature effects and individual species differences. An additional source of variance in the research on the dormancy meter and RPDC was our attempt to measure change in a physiological process over time. Each measurement was made under a different set of conditions, making it necessary to adjust readings for temperature and to restrict variation in stem diameter of seedlings sampled. We also found that it was necessary to adjust for internal tissue temperature. Thus, measuring electrical characteristics of plant tissue over time increased the complexity and variability of the methods, making it more difficult to predict the physiological fitness of planting stock for lifting. Apparently the decline in physiological activity in the fall cannot be assumed to mean that the seedlings are becoming increasingly dormant. Various factors, e.g. temperature, light, and water and nutrient availability, effect the level of growth activity in the fall. Variations in amount of these factors cause short-term changes in the level of physiological activity, and limiting conditions serve to induce seedlings into dormancy. But there appears to be a great deal of variation in physiological activity and in dormancy meter readings, directly or indirectly caused by environmental variables, that is not related to dormancy. Evidence supporting this came from the last experiment. Although RPDC readings at lifting time were not related to RGP after overwinter storage, multiple regressions (data will be presented in a subsequent paper) showed that combinations of day length plus soil and air temperature variables before lifting were strongly related to RGP after storage. Seedlings lifted during a cold period had higher RGP than those lifted during a warm period. We speculate that this means that, once leaf fall has occurred and the dormancy deepening phase has been reached, daily and weekly temperature fluctuations are more important for judging lifting time than dormancy. The seedlings respond to temperature fluctuations by increasing or decreasing their cold hardening. Once true dormancy has been reached, the seedlings no longer loose their cold hardening in response to warm temperatures.

The results also suggest that we can successfully lift hardwood seedlings during the dormancy deepening phase; i.e. we may not have to wait for full dormancy. The results also suggest that the physiological condition of seedlings is "fixed" at lifting, and effects their RGP after storage.

SUMMARY AND CONCLUSIONS

The physiological fitness of nursery stock at time of lifting is critical to its survival and growth after outplanting. There is a definite need for a convenient, fast, non—destructive method to evaluate dormancy. Measurement of electrical properties of plant tissue has these attributes. Patterns of electrical characteristics appear to parallel the pattern of decreasing physiological activity and presumed increasing dormancy in autumn. However, existing electrical techniques have not been substantiated by an independent verification of the dormancy status of seedlings.

The principal findings of this research are as follows:

1. With practice, we found that the oscilloscope technique gives characteristic waveforms for conspicuously actively growing, dormant, or dead black walnut seedlings. However, waveforms for seedlings in transitional stages were often subtle and more difficult to interpret. The cost and delicate instrument adjustments required are a major drawback; if the instrument is improperly adjusted, the waveform chacteristics of a weak signal may go unnoticed. Other researchers have found that oscilloscope waveforms during late summer and fall are not correlated with the experimentally determined dormancy status of seedlings. We conclude that the oscilloscope technique, in its present form-, it not a convenient and objective method suitable for general practices.

2. We found that the MEDC dormancy meter and oscilloscope technique agree on the status of actively growing seedlings of several species, but disagree for dormant seedlings. Their disagreement was mainly in the degree of activity or dormancy. Dormancy meter readings of several species measured in the nursery were not significantly related to the experimentally determined dormancy status of the same seedlings. Readings were significantly influenced by temperature, but not by stem diameter and moisture content. The relation between temperature and dormancy meter readings varied with species. Although dormancy meter readings appear to reflect the degree of physiological activity in seedlings, they are only weakly related to dormancy. Apparently much of the variation in physiological activity is not related to dormancy. We conclude, based on the weak relation with dormancy and high variability when seedlings are measured over time, that the dormancy meter in its present form does not accurately predict when seedlings are ready for lifting.

3. Resistance to pulsed direct current (RPDC) increases as physiological activity decreases in late summer and fall, and seedlings presumably become more dormant. However, RPDC measured in the nursery over an 8—week period was poorly related to the experimentally determined dormancy status of the same seedlings. In a separate experiment, RPDC measured over a 12—week period in the nursery was poorly related to root growth potential of seedlings lifted on the same dates and stored overwinter. The lack of relationship with seedling dormancy and vigor, and excessive variation from several interfering factors, support our conclusion that RPDC is not a suitable method for determining the physiological fitness of seedlings for lifting.

4. At the present stage of development the oscilloscope technique, MEDC dormancy meter, and RPDC are not suitable for predicting the dormancy status or physiological fitness of nursery stock for lifting. This conclusion is based on the lack of experimental verification of instrument readings with the actual dormancy status of seedlings, and on excessive variability. Part of the problem lies in the fact that we are attempting to precisely quantify a physiological process that we imprecisely understand. Additional research is needed to elucidate the dormancy process in hardwood seedlings and its relation to lifting time and storage, as well as the relation between electrical characteristics of plant tissues and seedling dormancy. For the present, evaluations of readiness for lifting of hardwood seedlings can be based on the time after leaf fall and the occurrence of cold temperatures.

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APPENDIX

Table A-1. Chi-square tests of independence of seedling status indicated by the MEDC dormancy meter versus that indicated by the oscilloscope technique. The two instruments agreed on the status of actively growing seedlings of most species, but disagree for dormant seedlings.

Species	Number of seedlings	X^2	df	$P(X^2)$
		Dormant Seedlings		
Yellow poplar	90	11.073	4	0.026[1]
Loblolly pine	89	53.397	4	0.000[1]
Sweetgum	88	29.407	3	0.000[1]
Sycamore	90	65.576	4	0.000[1]
		Active Seedlings		
Yellow poplar	100	6.918	4	0.140
White pine	39	5.389	3	0.145
White ash	25	1.020	1	0.312
Sycamore	75	2.094	4	0.718
Red oak	15	14.333	2	0.001[1]
White oak	12	10.743	2	0.005[1]
Black walnut	25	1.022	2	0.600

[1] The two instruments give different readings at 5% level of significance.

Table A-2. Linear regression for days to bud burst on MEDC dormancy meter readings for five species.

$$\text{Bud burst} = b_0 + b_1 \text{ (dormancy meter reading)}$$

Species	Intercept b_0	Slope b_1	Number of observations n	Coefficient of determination r^2
Green ash	10.1971	1.0632	44	0.04
Red oak	76.3894	-0.3294	104	0.02
Yellow poplar	14.8775	0.0805	126	0.02
Sweetgum	55.4135	-0.1547	103	0.06
Loblolly pine	39.7358	-0.0306	112	0.00

Table A-3. Correlations of unadjusted MEDC dormancy meter readings and temperature-adjusted resistance to pulsed direct current (RPDC) with air temperatures prior to each measurement in fall 1978. Temperature data are for Anna, IL, 5 miles from the Union Forest Nursery.

Species	Average minimum temp. 3 days prior	Average maximum temp. 3 days prior	Average daily temp. 3 days prior	Previous night's minimum temp.	Previous day's maximum temp.
MEDC Dormancy Meter					
Green ash	+0.77	+0.78	+0.79	+0.29	+0.83
Red oak	-0.63	-0.83[1]	-0.78[1]	-0.20	-0.77[1]
Sweetgum	+0.75	+0.87[1]	+0.83[1]	+0.34	+0.83[1]
Yellow poplar	-0.67	-0.45	-0.54	-0.80[1]	-0.48
Loblolly pine	+0.35	+0.63	+0.53	+0.13	+0.39
MEDC					
Green ash	-0.67	-0.85	-0.81	-0.07	-0.93[1]
Red oak	-0.89[1]	-0.84[1]	-0.89[1]	-0.67[1]	-0.96[1]
Sweetgum	-0.76[1]	-0.84[1]	-0.82[1]	-0.43	-0.82[1]
Yellow poplar	-0.85[1]	-0.80[1]	-0.83[1]	-0.72[1]	-0.93[1]
Loblolly pine	-0.83[1]	-0.85[1]	-0.86[1]	-0.56	-0.87[1]

[1] Correlation coefficient differs significantly from zero at 5% level.

Table A-4. Regression equations for MEDC dormancy meter readings on temperature for 3 species, n = 25.

Dormancy meter reading = $b_0 + b_1$ (Temperature, $^{\circ}$C) + b_2 (Temperature^2)

Species	Intercept b_0	Slope b_1	Slope b_2	Coefficient determination r^2
Yellow poplar	12.342880	0.523224	0.003224	0.94
Black walnut	-7,327936	0.451447	-0.011904	0.95
White ash	-8.824928	-0.505634	0.023338	0.92

Table A-5. Regression equations for days to bud burst on RPDC (in K ohms) for five species.

Green ash, red oak: Bud burst = $b_0 + b_1$ (RPDC) + b_2 (RPDC^2)

Yellow poplar, sweetgum, loblolly pine: Bud burst = $b_0 + b_1$ (RPDC)

Species	Intercept b_0	Slope b_1	Slope b_2	Number of observations	Coefficients of determination r^2
Green ash	212.9754	-1.2312	0.002815	44	0.43
Red oak	75.2372	-0.6249	0.002479	110	0.19
Yellow poplar	18.8693	0.0539		126	0.10
Sweetgum	33.6057	0.0555		112	0.20
Loblolly pine	35.8396	0.0002		121	0.00

Table A-6. Linear regressions of root growth potential (RGP) - measured as mean number, length (cm), and surface area (cm^2) of new roots per seedling - on resistance to pulsed direct current (RPDC) in K ohms for black walnut seedlings; n = 13.

RGP = $b_0 + b_1$ (RPDC)

Species	Measure of RGP	Intercept b_0	Slope b_1	Coefficient of determination
Black Walnut	Number new roots	24.6462	0.1208	0.03
	Length new roots	59.3344	0.4159	0.04
	Surface area new roots	3.2551	0.0255	0.05