DETECTION OF DORMANCY IN BLACK WALNUT SEEDLINGS WITH THE SHIGOMETER AND AN OSCILLOSCOPE TECHNIQUE

W.J. Rietveld and Robert D. Williams
Research Plant Physiologist and Principal Silviculturist
North Central Forest Experiment Station
USDA Forest Service
Carbondale, Illinois and Bedford, Indiana

ABSTRACT

The oscilloscope technique will work for black walnut seedlings, but the instruments must be carefully calibrated and considerable experience is needed. There are many variables in this technique which contribute to instrumental and personal errors. The Shigometer is simple, relatively inexpensive, and easy to operate. Shigometer pulse resistance readings are strongly correlated with degree of physiological activity in seedlings and seasonal periodicity of activity.

It is generally accepted that bare-root nursery stock, such as that of black walnut (*Juglans* nigra L.), should be lifted when fully dormant for best overwinter storage and transplanting success. Disturbance of the plant's physiological processes is minimized.

A device to detect seedling dormancy would eliminate depending on less certain signs of dormancy such as the development of terminal buds and shedding of leaves in autumn and give an extra margin of confidence.

Electrical resistance measurements have been studied for many years to detect various physiological conditions in plants, such as cold and drought stress (Cordukes, Wilner, and Rothfield 1966), suppression and drought (Snow 1942), injury (De Plater and Greenham 1959), death of tissues (Zaerr 1972), and vitality (Glerum 1970). Seasonal trends in electrical resistance reflect properties and changes in the physiological state of plant cells and tissues (Polozhentsev and Zolotov 1970). This suggests the possible usefulness of resistance measurements in detecting the onset of dormancy in seedlings prior to lifting.

Following the earlier work by Wanek (1971) and Zaerr (1972), Ferguson, Ryker and Ballard (1975) developed a portable oscilloscope technique for detecting dormancy in nursery stock. The method involves passing a square wave signal through plant tissue by means of a four-needle probe, and evaluating the shape of the transmitted signal on an oscilloscope screen. Different wave traces characterize active, dormant, and dead tissue. The technique was developed using conifer nursery stock primarily, although several deciduous shrubs were tested and found to follow a similar pattern.

The Shigometer was originally developed to detect discolored and decayed wood in utility poles and living trees (Skutt et al. 1972, Tattar et al. 1972, Shigo and Shigo 1974). It is based on the principle that resistance to a pulsed current decreases as concentrations of cations increase in wood. Recently the Shigometer has been used to measure the degree of vigor in intact trees (Smith _et_ al. 1976, Wargo and Skutt 1975).

In the present study, measurements by the oscilloscope and Shigometer techniques were superimposed on a study of lifting date and storage of black walnut seedlings. Rather than measure intact seedlings in a nursery bed, all measurements were made soon after lifting, or after specified periods of storage. Then the root regenerating potential (RRP, the ability of a seedling to regenerate an extensive root system rapidly after transplanting) (Stone and Schubert 1958) was determined for the same seedlings. This approach had the advantage of "measuring" the degree of dormancy of a group of seedlings with the instruments, then determining the corresponding growth responses of the same seedlings in a 4-week-long RRP test. Our preliminary results show that the oscilloscope technique is applicable to black walnut seedlings and Shigometer readings are strongly correlated with RRP and seasonal periodicity of physiological activity.

METHODS

Black walnut seedlings (1-0) were lifted at 2-week intervals, when the ground was not frozen, at Indiana's Vallonia Forest Nursery near Brownstown. Eleven liftings were made between October 6, 1976 and April 25, 1977. The seedlings were shipped to Carbondale, Illinois where oscilloscope and Shigometer measurements were made the following day. Measurements were made 3 cm above the root collar. For each lifting, seedlings were potted immediately (no storage) or bundled for storage, 12 per treatment, and stored in a cold room at 20° C for 4, 8, and 12 weeks. Oscilloscope and Shigometer readings were taken at the end of each storage period, giving a total of 44 treatment combinations (11 lifting dates x 4 storage periods).

In a second phase of the study, groups of seedlings from each lifting date were stored for extended periods until they were planted in the field on December 6, March 10, and May 12. This test represented the usual practice of lifting in the fall and storing until planting time. Oscilloscope and Shigometer measurements were taken and an RRP test was made on 12 seedlings representing each lifting date/storage combination (confounded) at the time of planting.

We used a dual trace A-C oscilloscope (fig. 1A) and a homemade square wave generator that was calibrated to introduce a signal of 1000 hertz and 2 volts. The Shigometer (fig. 1B) is manufactured by Northeast Electronics, Concord, N.H.1/ Voltage of the transmitted signal of each seedling was recorded and one photograph of the signal trace was taken for each treatment. The 2-needle probe handle for the Shigometer (model number 2-E) and the 4-needle probe handle for the oscilloscope (model number 62-E) are available from the Delmhorst Instrument Co., Boontown, N.J. (fig. 2).I/ Both probe handles have sturdy, replaceable stainless steel needles available in different lengths.

1/ The use of trade, firm, or corporation names in this paper does not constitute endorsement by the Forest Service or the U.S. Department of Agriculture.

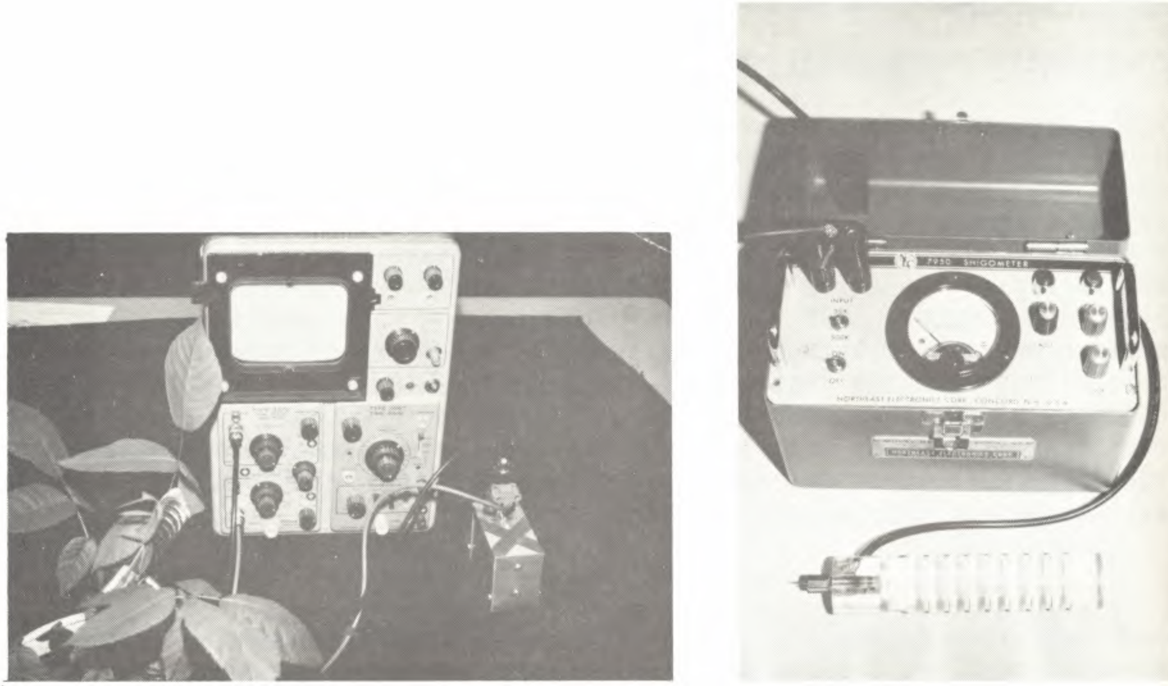

Figure 1.--The oscilloscope (A) and Shigometer (B) apparatus used in the experiment.

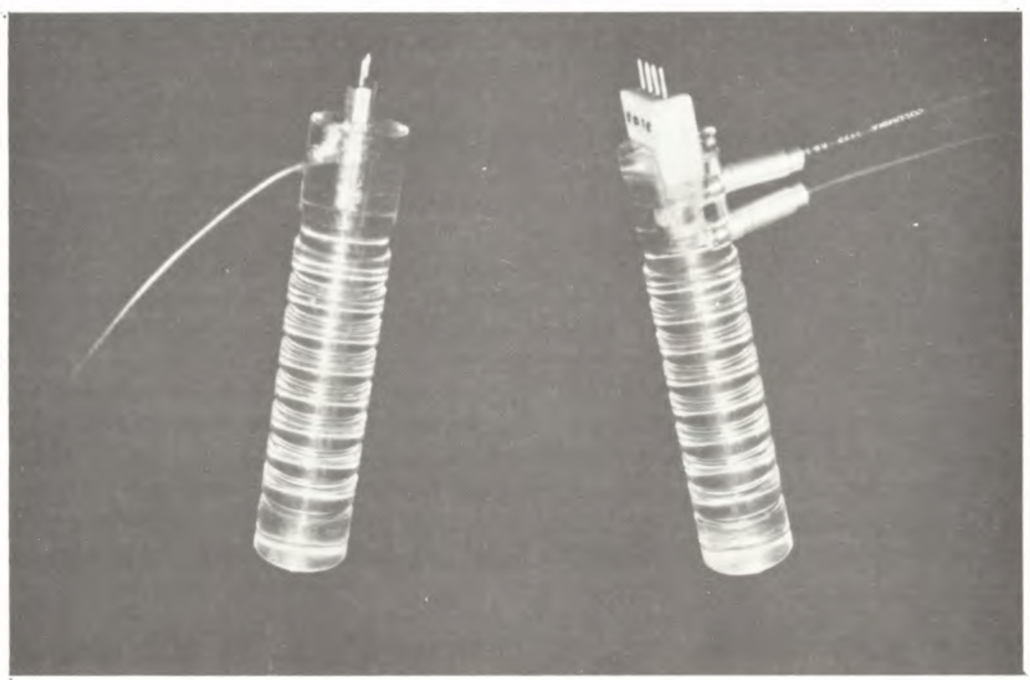

Figure 2.--Electrode handles used with the Shigometer (left) and oscilloscope. Both have sturdy, replaceable needles.

Following the oscilloscope and Shigometer measurements of freshly-lifted or stored seedlings, the same 12 seedlings were potted and grown in a greenhouse for 4 weeks. Air temperature in the greenhouse varied seasonally (minimum 16° C), a photoperiod of 16 hours was maintained by supplemental lighting, and soil temperature was maintained at 24 C by pumping water from a reservoir maintained at that temperature through plastic tubing coiled in each pot. At the end of the 4-week root regeneration test, the seedlings were removed from the pots and oscilloscope and Shigometer readings were taken. Also, total shoot growth, total length of new primary roots (those >1 mm in diameter were summed), and ovendry weight of new roots were determined for each seedlings. These measurements represented the growth reponses corresponding to the lifting date and storage period treatments, and were compared with the respective oscilloscope and Shigometer measurements.

RESULTS AND DISCUSSION

OSCILLOSCOPE TECHNIQUE

By carefully adjusting the oscilloscope and after some experience, we found we could obtain oscilloscope traces for black walnut seedlings resembling those reported by Ferguson, Ryker, and Ballard (1975) for different degrees of growth activity (fig. 3). For seedlings just beginning to grow, the active trace (fig. 38) was weak and required considerable amplification or it would go unnoticed. Increasing voltage or frequency did not improve the trace. As growth activity increased, a more distinctive active signal trace developed (fig. 4).

Depth of penetration of the probes was found to be very important. For an actively growing seedling, a dormant trace can be obtained if the needles are pressed lightly into the wood, but an active trace resulted when the needles were pressed entirely through the stem. It is important, as Ferguson et al. (1975) point out, that the needles pass completely through the stem so they protrude from the other side.

Peak voltage of the transmitted signal did not have any relationship with seedling physiological activity. Although peak voltage varied considerably for individual seedlings, according to caliper, the mean values were uniform through the entire experiment.

SHIGOMETER

Shigometer readings (pulse resistance) were highly correlated with growth responses in the RRP tests and with the seasonal cycle of physiological activity (fig. 1, table 1). Correlation coefficients for days from October 1 versus shoot elongation, ovendry weight of new roots, and Shigometer readings are +0.90, +0.82, and -0.84, respectively. The correlations between Shigometer readings and the growth parameters (correlations 4-6 and 8-10) are equally strong. Thus, high resistance readings are associated with dormancy, while low resistance readings are associated with readiness to grow.

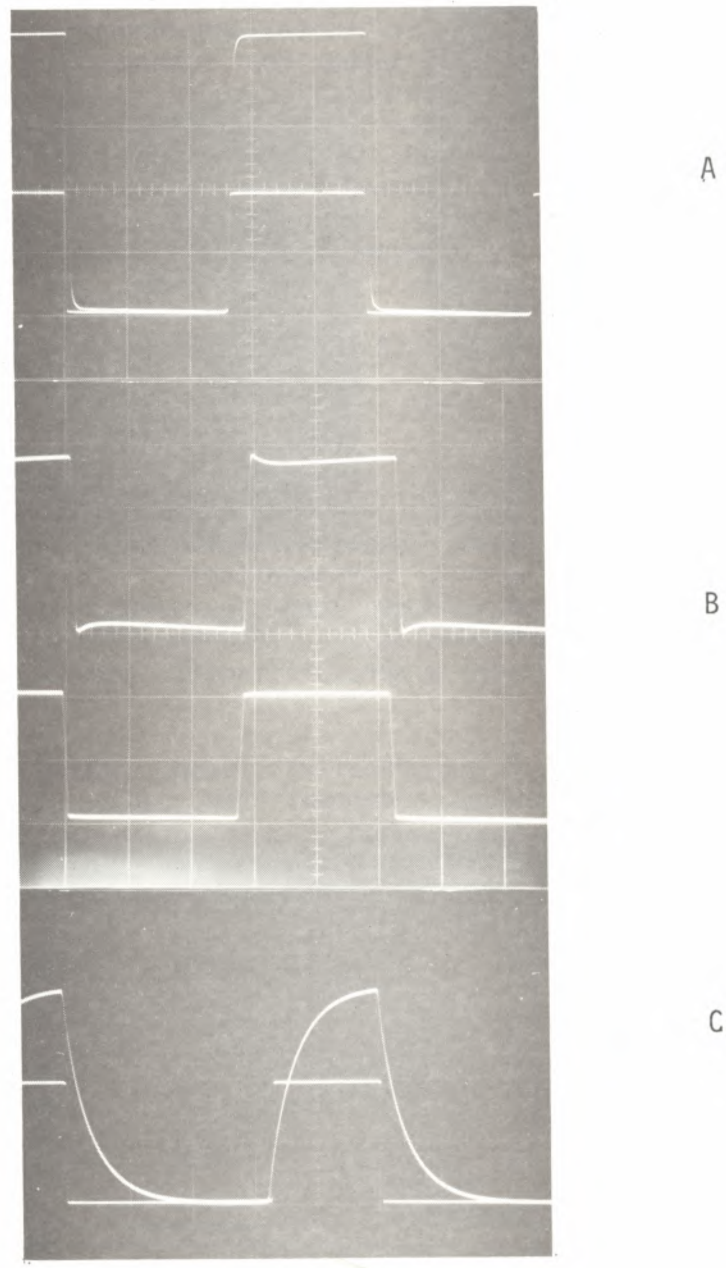

Figure 3.--Oscilloscope wave traces for (A) dormant, (B) actively growing, and (C) dead black walnut seedlings. In each photo the upper trace is the amplified output signal (0.1 volt/Div.) and the lower is the input square wave signal (2 volts/Div.).

Table 1.--Simple correlation coefficients of several paired variables, including relationships, of Shigometer readings and corresponding growth responses

Correlation number	Variable X	Variable Y	Storage treatment included (months)	Correlation coefficient
1	Days from Oct. 1 1/	Shoot elongation	0	+0.90
2	Days from Oct. 1 1/	Ovendry wt. of new roots	0	+0.82
3	Shoot elongation	Ovendry wt. of new roots	0	+0.95
4	Shigometer reading	Shoot elongation	0	-0.86
5	Shigometer reading	Length of new primary roots	0	-0.94
6	Shigometer reading	Ovendry wt. of new roots	0	-0.89
7	Days from Oct. 1 1/	Shigometer reading	0,4,8,12	-0.84
8	Shigometer reading	Shoot elongation	0,4,8,12	-0.82
9	Shigometer reading	Length of new primary roots	0,4,8,12	-0.77
10	Shigometer reading	Ovendry wt. of new roots	0,4,8,12	-0.77

1/ Days from October 1 to the beginning of the RRP test was arbitrarily chosen to determine the relationship of shoot elongation, ovendry weight of new roots, and Shigometer readings with time.

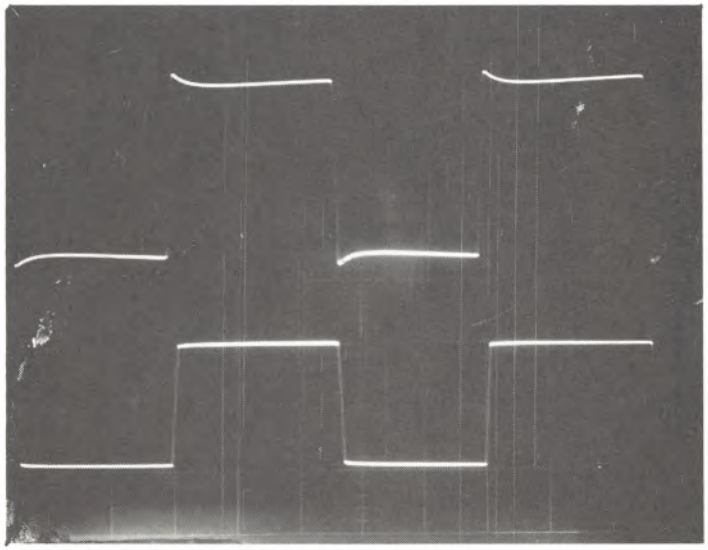

Figure 4.--Oscilloscope trace (top) of an actively-growing black walnut seedling taken at the conclusion of a root regeneration test. The lower trace is the input square wave signal (2 volts/Div.).

In this preliminary study, Shigometer readings greater than about 90 K Ohms (table 2 and fig. 5) appear to indicate dormancy.2/ Full dormancy was apparently reached by November 15. In fall-lifted seedlings, there was a tendency for pulse resistance to increase in cold storage, but in spring-lifted seedlings this trend was smaller and short lived. For nearly every combination of lifting date and storage period, pulse resistance at the end of the RRP test was lower than the reading from storage. This indicates that growth activity increased in all seedlings during the RRP test, although there were no exterior signs of growth in tests conducted in October-December. Visible growth (shoot elongation) first occurred when Shigometer readings at the end of the RRP test were less than about 50 K Ohms. Growth activity increased as the resistance readings decreased. At the storage temperature used (2° C) most of the seedlings sprouted in storage during late May-early June: Shigometer readings were less than 40 K Ohms.

Both freshly-lifted and stored walnut seedlings showed a strong dependence on fulfillment of the "chilling requirement" before any appreciable growth response was measured in the RRP tests (fig. 4). RRP tests conducted in October, November, and December showed that the seedlings could not be forced to grow in the greenhouse, even under a 16-hour photoperiod and 75 ° F temperature. A small growth response was measured in January and February. Then in early March, a sudden and strong growth response began--the seedlings were physiologically ready to resume growth. The pattern of growth responses shown by the RRP tests is clearly tied to the dormancy cycle as described by Samish

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2/ Please note that in this type of measurement, the comparisons among readings are more important than the absolute values. More research is needed before a specific interpretation can be ascribed to a particular resistance reading.

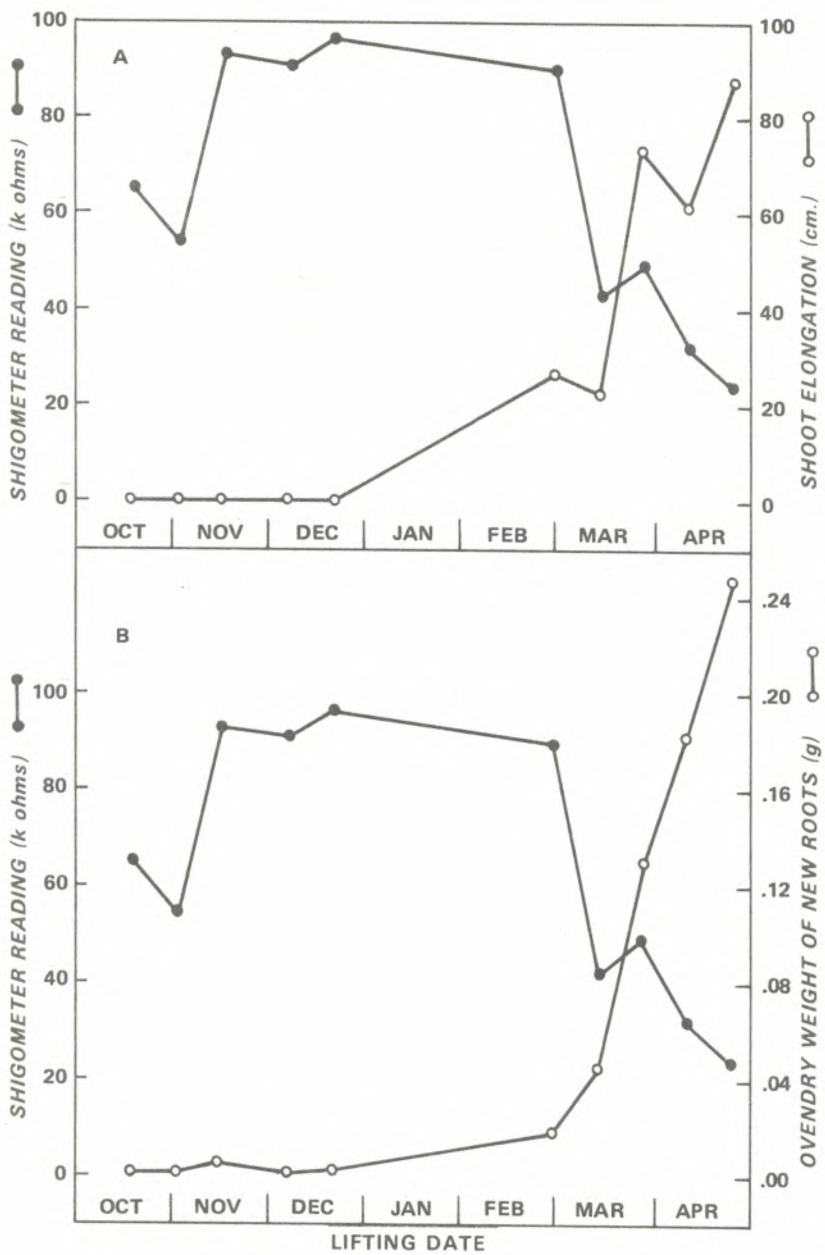

Figure 5.--Relationship between Shigometer readings (41--e) of freshly-lifted seedlings taken at the beginning of RRP tests, and the (A) shoot elongation (0__o) and (B) ovendry weight of new roots (o--o) measured at the end of 4-week tests. Each point is the mean of 12 seedlings. The simple correlation coefficients are -0.86 and -0.89, respectively.

Table 2.--Shigometer readings[1/] (pulse resistance in K Ohms), taken at the beginning and end of the 4-week root regeneration tests, of freshly-lifted and stored black walnut seedlings

	Weeks of storage and time of measurement							
	0		4		8		12	
Lifting date	Newly lifted	From green-house	From storage	From green-house	From storage	From green-house	From storage	From green-house
Oct. 6	--	--	--	84.8	--	75.6	93.8	61.9
Oct. 18	65.0	--	--	--	90.6	60.6	106.7	74.2
Nov. 1	54.0	--	93.9	74.4	87.1	60.6	89.4	40.2
Nov. 15	93.0	88.5	100.2	79.4	100.0	76.9	94.2	51.3
Dec. 6	90.6	82.3	91.9	69.8	112.5	52.9	87.5	38.5
Dec. 20	96.3	--	--	64.6	96.5	34.2	55.4	28.8
Feb. 28	90.0	40.6	60.4	--	34.6	40.6	57.9	42.1
Mar. 14	42.5	32.3	48.0	57.5	68.1	46.7	39.2	36.0
Mar. 28	49.4	40.2	52.7	43.8	53.8	41.7	41.7	--
Apr. 11	32.1	--	51.3	32.7	34.2	30.4	31.7	--
Apr. 25	24.0	39.6	51.3	40.8	39.6	--	--	--

1/ Values are the means of 12 seedlings measured 3 cm above the root collar.

(1954). The cycle consists of "preliminary rest", an early stage during which the dormant bud will no longer grow in response to favorable conditions, but can be "forced";3/ a period of "mid rest", when only the most drastic treatments will stimulate a weak growth response; and "after rest", a period having much in common with the first stage.

When seedlings were stored for extended periods prior to planting, Shigometer readings appeared to stabilize at an intermediate level (table 3). On March 10 and May 12 readings for fall-lifted stock were lower, while readings for spring-lifted stock were higher than readings taken at the time of lifting, listed in table 2. Compare the November 15 lifting date, May 12 planting date (60.0 K Ohms) in table 3 with 93.0 K Ohms in table 2. And compare April 25 (54.6 K Ohms) in table 3 with 24 K Ohms for April 25 in table 2. For the December 6 planting date (table 3), which involved rather short storage, resistance readings were quite high indicating full dormancy. The corresponding growth responses were negligible, which also indicate the seedlings were in the "mid rest" stage of dormancy (Samish 1954). By March 10, resistance readings averaged about 60 K Ohms, and the moderate growth response suggests that the seedlings were in the "after rest" stage of dormancy. On the May 12 planting date, resistance readings remained about 60 K Ohms, although there may be a trend toward lower resistance readings in the later lifting dates, especially for April 25 since seedlings had already flushed at the nursery when they were lifted. Growth response data again appear to follow the seasonal pattern, and are highest following the "after rest" stage of dormancy. Shoot and root growth responses for the October 5 and October 18 lifting dates were much lower than those for subsequent dates, indicating that some deterioration during storage had occurred. This suggests that nurserymen should wait for walnut seedlings to be fully dormant (about November 15 in 1976) before they are lifted.

The location to probe the seedlings is important. We found that fall resistance readings taken just below the terminal bud are higher than at the root collar. In the spring and after RRP tests the resistance reading at the terminal bud is lower than at the root collar. In other words, the terminal bud becomes dormant sooner in the fall and becomes active sooner in the spring. Seedlings taken from storage had about the same resistance reading at both locations. The terminal bud may be a better and more convenient location to take measurements than the root collar. Measurements reported in this paper were taken 3 cm above the root collar. Also, to decrease error due to penetration, we found that the probe needles should be pressed through the stem in order to obtain the lowest stable reading.

These preliminary results with the Shigometer indicate it has good potential as a sensitive, simple, and inexpensive instrument for the fieldman to use. It may also prove to be useful to obtain quantitative data for certain physiological processes. With an electronic technique, we may be able to detect many subtle changes that go on in plants which are not apparent by external signs.

3/ Subjection to cold or heat, wounding, treatment with anesthetics, etc.

Table 3.--Shigometer readings' and corresponding growth responses of black walnut seedlings stored for extended periods and planted on December 5, March 10, and May 12

Planting date	Lifting date	Length of storage	Shigometer reading from storage	4-week growth response: Terminal shoot elongation	4-week growth response: Length new primary roots	4-week growth response: Ovendry weight of new roots
		(Wks)	(K Ohms)	(cm)	(cm)	(g)
Dec. 6	Oct. 6	9	91.0	0.2	1.0	0.0031
	Oct. 18	7	83.3	0.1	1.1	0.0022
	Nov. 1	5	93.9	0.0	1.2	0.0009
	Nov. 15	3	103.1	0.0	0.4	0.0017
Mar. 10	Oct. 6	21	61.5	21.0	10.6	0.0267
	Oct. 18	19	62.3	22.6	6.9	0.0110
	Nov. 1	17	61.4	15.8	10.7	0.0207
	Nov. 15	15	53.3	17.1	19.0	0.0271
	Dec. 6	12	58.3	20.9	4.8	0.0182
	Dec. 20	10	65.2	20.2	11.6	0.0245
	Feb. 28	1	64.8	31.3	8.7	0.0216
May 12	Oct. 6	32	66.5	21.1	18.3	0.0715
	Oct. 18	30	68.1	22.8	1.9	0.0057
	Nov. 1	28	59.4	42.4	56.0	0.0154
	Nov. 15	26	60.0	55.4	132.8	0.3410
	Dec. 6	23	62.5	45.1	51.7	0.1752
	Dec. 20	21	55.8	50.3	78.1	0.2066
	Feb. 28	11	55.4	66.2	44.6	0.1607
	Mar. 14	9	57.9	63.4	32.8	0.1192
	Mar. 28	7	57.1	64.0	30.5	0.1234
	Apr. 11	5	67.7	83.7	83.9	0.2966
	Apr. 25	3	54.6	75.8	65.5	0.2314

1/ Values are the means of 12 seedlings measured 3 cm above the root collar.

MANAGEMENT OBSERVATIONS

From this test of the oscilloscope technique and preliminary test of the Shigometer for detection of dormancy and measurement of vigor in black walnut seedlings, several inferences can be made. A few additional comments are included.

1. We found that the oscilloscope technique will work for black walnut seedlings, but the wave trace can be ambiguous. The active signal trace is often weak and could be miseed without amplification. There are many variables in this technique--instrument calibrations and adjustments, depth of probe penetration, and temperature--which contribute to errors.

2. The Shigometer is simple, inexpensive, and easy to operate. Shigometer readings are strongly correlated with the degree of physiological activity in the seedlings and with the seasonal periodicity of activity. More research is needed to relate the annual pattern of Shigometer readings to physiological activity in the nursery bed and cold storage, identify resistance values associated with specific degrees of physiological activity or growth events, and to place limits on the predictive accuracy of the Shigometer.

3. A new instrument, the dormancy meter, has been developed by the U.S. Forest Service's Equipment Development Center, Missoula, Montana, to determine physiological activity of nursery stock. Briefly, the engineers have found that an instrument that measures the ratio of voltage of 500 hertz and 10 kilohertz gives basically the same performance as the square wave generator and oscilloscope. This considerably simplifies the electronics required to determine the physiological activity of nursery stock, and allows the development of a simple, inexpensive instrument for the fieldman to use. We hope to test one of the prototypes on black walnut seedlings and compare its efficiency with the Shigometer.

LITERATURE CITED

Cordukes, W. E., J. Wilner, and V. T. Rothfield.
1966. The evaluation of cold and drought stress of turfgrass by electrolytic and ninhydrin methods. Can.J. Pl. Sci. 46:337-342.

DePlater, C. V. and C. G. Greenham.
1959. A wide range A.C. bridge for determining injury and death. Pl. Phys, 34:661-667.

Ferguson, Robert B., Russell A. Ryker, and Edward D. Ballard.
1975. Portable oscilloscope technique for detecting dormancy in nursery stock. USDA For. Serv. Gen. Tech. Rep. INT-26, 16 p.

Glerum, C.
1970. Vitality determinations of tree tissue with kilocycle and megacycle electrical impedance. For. Chron. 46:63-64.

Polozhentsev, P. A. and L. A. Zolotov.
1970. Dynamics of electrical resistance of bast tissues in pine trees as an indicator of changes in their physiological condition. Soviet Plant Physiol. 17:694-698.

Samish, R. M.
1954. Dormancy in woody plants. Annu. Rev. Plant Physiol. 5:183-204.

Shigo, Alex L. and Alex Shigo.
1974. Detection of discoloration and decay in living trees and utility poles. USDA For. Serv. Res. Pap. NE-294, 11 p.

Skutt, H. R., A. L. Shigo, and R. A. Lessard.
1972. Detection of discolored and decayed wood in living trees using a pulsed electrical current. Can. J. For. Res. 2:54-56.

Smith, Desmond E., Alex L. Shigo, L. O. Safford, and Robert Blanchard.
1976. Resistances to a pulsed electrical current reveal differences between nonreleased, released, and released-fertilized paper birch trees. For. Sci. 22:471-472.

Snow, A. C.
1942. Voltage gradient measurement in forest trees. J. For. 40:872-876.

Stone, E. C. and Gilbert H. Schubert.
1958. Seasonal periodicity in root regeneration of ponderosa pine transplants--a physiological condition. Proc. Soc. Amer. For. Annu. Mtg. 1958:154-155.

Tattar, T. A., A. L. Shigo, and T. Chase.
1972. Relationship between the degree of resistance to a pulsed electric current and wood in progressive stages of discoloration and decay in living trees. Can. J. For. Res. 2:236-243.

Wanek, J.
1971. Electronics now being used to "bug" Douglas-fir trees. The Forest Log 41(5):3. State of Oregon, Dept. of For.

Wargo, Philip M. and H. Richard Skutt.
1975. Resistance to pulsed electric current: an indicator of stress in forest trees. Can. J. For. Res. 5:557-561.

Zaerr, J. B.
1972. Early detection of dead plant tissue. Can. J. For. Res. 2:105-110.