STARCH LEVELS IN FALL-LIFTED HARDWOOD PLANTING STOCK AND THEIR RELATION TO FIELD SURVIVAL AND GROWTH

by

W.J. Rietveld, Robert D. Williams, and F. Danny McBride 1/

Abstract. Starch concentration of 1-0 black walnut, red oak, white ash, and yellow poplar seedlings was measured at nine fall lifting dates, during overwinter cold storage, and at spring planting. Peak starch concentrations in shoots were reached in. September and early October, followed by peat concentrations in roots in late October, followed by peat concentrations in roots in late October and November. Root starch concentration of all species declines during storage. Seedlings lifted in early fall had lower root starch concentration at lifting and at planting time than those lifted after the peak. Lifting time confounded with root starch concentration at time of planting was positively, but weakly, correlated with survival of red oak and yellow poplar seedlings. It was also positively correlated with first-year height and diameter increment of red oak and white ash seedlings. Seedlings lifted before complete leaf fall had lower first-year survival and growth, but second-year growth of all species was good regardless of lifting date. There were no meaningful correlations with survival or growth of black walnut seedlings. The small effect of lifting date and starch concentration was attributed to unusually favorable testing conditions. Starch concentrations determined by a staining and visual estimation technique were acceptably close to concentrations determined by chemical analysis.

Additional keywords: *Juglans nigra, Quercus rubra, Fraxinus americana, Liriodendron tulipifera,* nursery practices, cold storage, planting stock quality.

Starch is the principal storage carbohydrate in hardwood seedlings and the taproot is the main repository (Farmer 1975). Improper overwinter storage or moisture stress or defoliation during the growing season affect seedling starch levels (Farmer 1978, Kramer and Kozlowski 1960, Parker and Patton 1975). Stored food reserves provide an energy source during the tenuous period after transplanting when seedlings must: (1) regenerate new roots, (2) burst buds and grow new foliage, and (3) endure environmental stress until they become

1 Principal Plant Physiologist, Principal Silviculturist, and Research Technician, North Central Forest Experiment Station. Rietveld and McBridge are located at Carbondale, IL, and Williams is located at Bedford, IN. We wish to thank Dr. Jim Wichman, nurseryman at the Vallonia Forest Nursery, IN, for his cooperation and Drs. Philip M. Wargo, Robert E. Farmer, and J.W. Van Sambeek for manuscript reviews.

established and self-sufficient. It is thus assumed that high root starch concentration is an important characteristic of seedling quality.

Starch concentration may provide a useful indicator of planting stock quality and of potential vigor in the field, if other factors that affect quality are not limiting. However, to evaluate the contribution of stored starch to seedling quality, we first need to identify: (1) typical starch levels in healthy nursery stock, (2) patterns of accumulation and depletion in the nursery and in cold storage, and (3) the relation between seedling starch content at planting time and field survival and growth.

METHODS

One-year-old (1-0) black walnut (*Juglans nigra* L.), northern red oak (*Quercus rubra* L.), white ash (*Fraxinus americana* L.), and yellow poplar (*Liriodendron tulipifera* L.) from the Vallonia Forest Nursery in IN (Lat. N 38°47, Long. W 86°05') were used in the study. Seedlings were randomly lifted by hand from beds of regular nursery stock at nine approximately weekly intervals between September 22 and December 1, 1975, and were immediately bundled and refrigerated at 3°C until planted on April 9, 1976. Because of partially frozen soil on December 1, seedlings lifted on that data probably received greater injury during lifting. Average height and caliper 10 cm above the root collar of the seedlings at time of lifting were: black walnut 50 cm x 5 mm, red oak 15 cm x 3 mm, white ash 39 cm x 4 mm, and yellow poplar 49 cm x 4 mm.

Ten sample seedlings of each species were taken for starch measurements at lifting time, at monthly intervals during storage, and at planting time. Spring-lifted (April 5) walnut and yellow poplar seedlings were also sampled from the same beds. Stock of the other two species were not available in the spring. Shoot and root starch concentrations were measured by two methods: (1) chemical analysis using the extraction method of Hassid and Neufeld (1964) and the colorimetric method of Siminovitch et al. (1953), and (2) visual starch estimation using a sectioning and staining technique developed by Wargo (1975).

For chemical analysis, shoots or roots of five seedlings representing each treatment were put into one sample, ovendried as quickly as possible, ground to pass a 20-mesh screen, and one sub-sample analyzed within 5 days. Starch concentration was expressed as percent of ovendry weight.

The remaining five seedlings were used for visual estimation of starch. One hundred micron thick cross-sections of shoot and root tissue were taken 2.5 cm above and below the root collar. These cross-sections were then stained with an iodine solution and one seedling cross-section with average color intensity was photographed through a microscope to represent each treatment. Data from the chemical analyses were then consulted to determine the range of starch concentrations. Finally, starch concentration was estimated from the photographs to the nearest 2.5 percent.

Starch measurements were repeated for black walnut the following year. Regular nursery stock was lifted on 11 approximately biweekly dates during fall 1976 and spring 1977 up to the time of flushing. Seedlings were bundled and refrigerated at 3°C until planting time (March 8, 1977). Root starch concentration was measured by chemical analysis of four individual seedlings at lifting and at planting time.

The field planting was established on a fertile well-drained bottomland site in southern Illinois. Each of the 36 treatment combinations (4 species x 9 lifting dates) was represented by 10 seedlings planted in each of S randomized complete blocks. Seedlings were root pruned to 20 cm at time of planting and were hand planted using KBC bars. Plantation weeds were controlled by mechanical tilling four times a year. The site and growing conditions were excellent for the establishment and growth of hardwoods. Precipitation during May-July was 21% above normal the first year and near normal the second year.

Seedling total height and diameter 10 cm above ground were measured at time of planting and at the end of the first two growing seasons. Differences in survival, height increment, and diameter increment among lifting dates were tested for significance (a = 0.05) by analysis of variance and Duncan's multiple range test.

RESULTS

Starch Concentration at Lifting

Leaf fall was complete by September 22 for black walnut and white ash and by October 6 for red oak and yellow poplar. The four species showed similar patterns of starch accumulation during early fall (Fig. 1). The taproot was the main repository for starch; concentration in roots was above five times greater than in shoots. Highest shoot starch concentrations of approximately 5 to 7 percent were reached in September and early October, followed by peak root starch concentrations of approximately 25 to 30 percent in late October and November. The earliest peaking of shoot starch indicated that after leaf fall, carbohydrates were still being translocated from shoot to root and were being converted to starch.

Although walnut, ash, and yellow poplar showed uniform distribution of starch within stained root cross-sections, newly-formed xylem and phloem in red oak were distinctly low in starch until October 13. This is probably due to late development of tissues resulting from late-season flushes, followed by starch deposition.

Starch Concentration During Storage and at Planting Time

Root starch concentration in fall-lifted seedlings declines constantly and substantially during storage (Fig. 1), to lows of 18.9, 18.4, 12.1, and 14.8 percent, respectively, for black walnut, red oak, white ash, and yellow poplar at planting time (April 9). Shoot starch concentration was constant at approximately 1 to 3 percent during storage. Starch concentrations measured in spring-lifted (April 5) black walnut

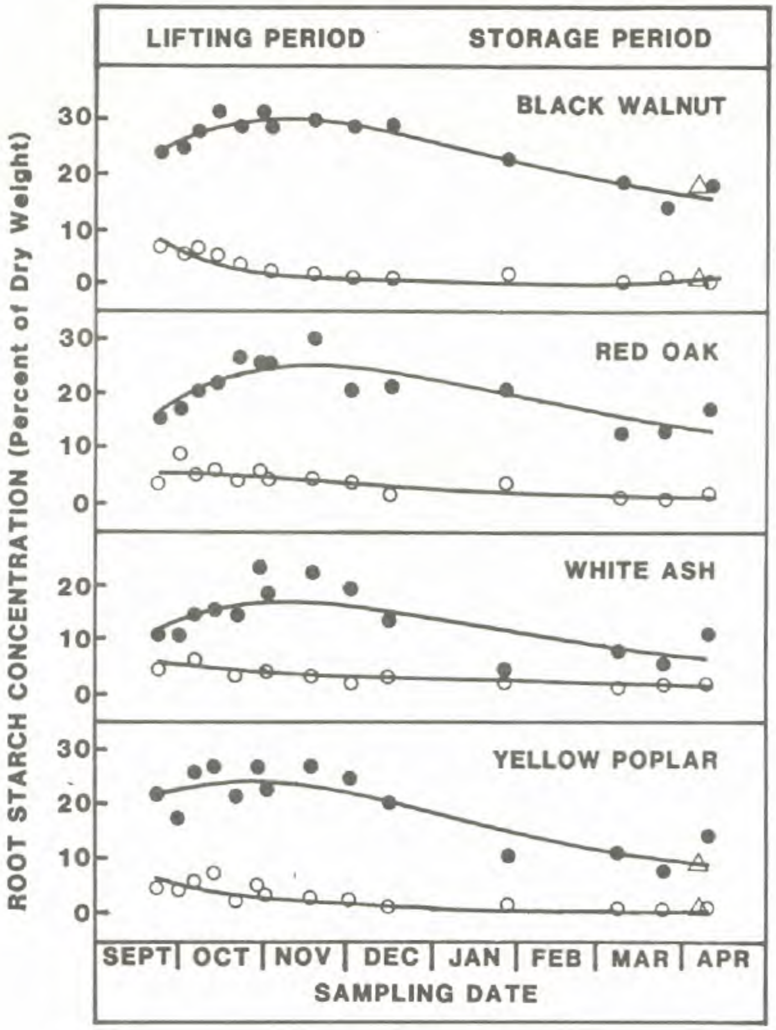

Fig. 1. Shoot (o) and root (•) starch concentrations in 1-0 seedlings of four hardwood species at time of lifting, during storage, and at time of planting. Starch concentrations in spring-lifted seedlings (Δ) are plotted for black walnut and yellow poplar only. Each lifting date is represented by a composite sample of five seedlings; each storage period is represented by 45 seedlings. Data for regression curves are given in Table A-1 in the appendix.

and yellow poplar seedlings were very similar to those in cold-stored seedlings (Fig. 1), indicating that a similar pattern of starch depletion had occurred in seedlings overwintered in the nursery beds.

Root starch in black walnut seedlings lifted and stored in six dates in fall 1976 did not decline during storage to planting time, averaging 31.6 percent starch at time of lifting and 31.4 percent after

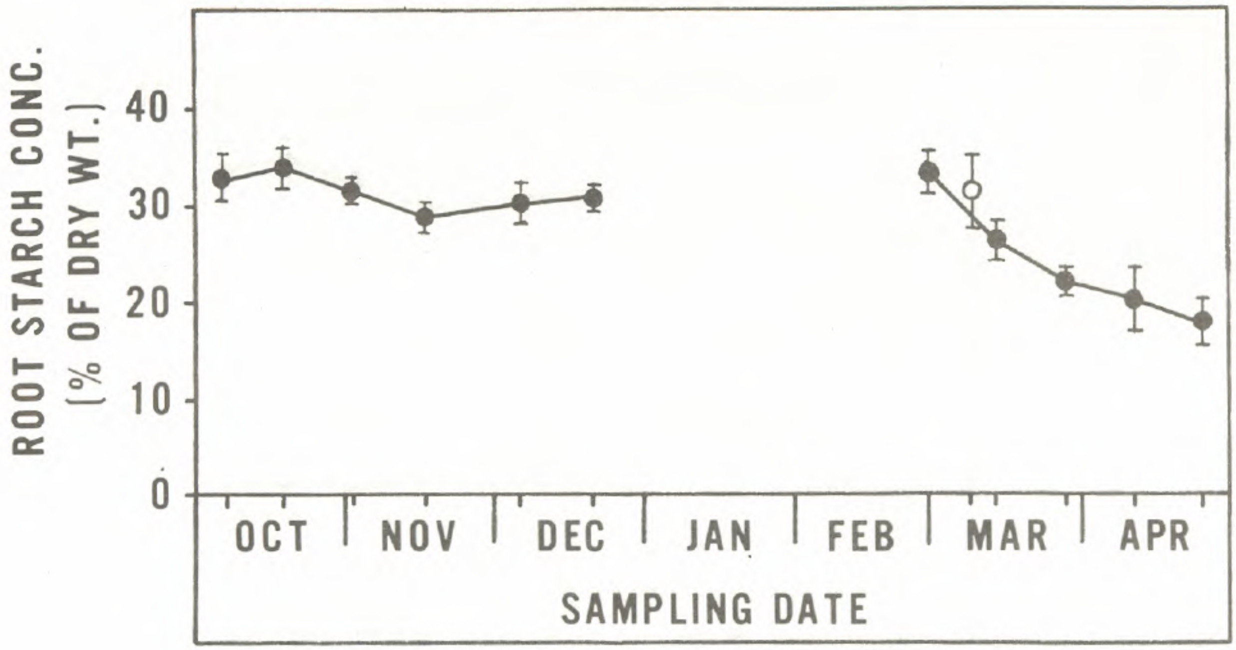

Fig. 2. Root starch concentration (percent of dry weight) in black walnut seedlings at time of fall or spring lifting (o) and after storage of fall-lifted seedlings to planting on March 8, 1977 (o). Each point is the mean of four seedlings + 1 SE.

Table 1. Root starch concentrations of four hardwood species by chemical analysis at lifting and at planting time. (In percent of dry weight.)

Lifting date	Black walnut		Red oak		White ash		Yellow poplar	
	Lift	Plant	Lift	Plant	Lift	Plant	Lift	Plant
Sept. 22	24.1	18.1	15.9	3.0	11.4	4.6	22.3	4.7
Sept. 30	25.3	14.6	17.6	7.6	10.8	6.3	17.6	11.2
Oct. 6	28.4	22.6	20.7	9.2	15.0	5.7	26.0	14.0
Oct. 13	31.8	26.3	21.9	14.8	15.7	9.1	27.3	14.8
Oct. 21	31.3	25.8	26.9	12.1	14.8	8.8	21.9	19.9
Oct. 29	31.8	24.4	26.0	19.5	23.4	8.0	26.9	10.2
Nov. 1	28.8	22.6	25.7	18.1	18.8	8.4	22.9	11.8
Nov. 17	30.0	23.0	30.5	16.2	22.8	9.3	27.3	13.2
Dec. 1	29.4	22.8	21.3	18.4	19.8	12.1	24.9	14.8

storage to planting time on March 8 (Fig. 2). Root starch concentration in spring-lifted seedlings was 33.5 percent on February 28, similar to that of stored seedlings; it then declined rapidly before and during flushing.

Time of lifting affected root starch concentration at planting time (Table 1). Root starch was lower in black walnut and yellow poplar seedlings lifted before October 6, and in red oak and white ash seedlings lifted before October 13. The patterns are mostly explained by (1) incomplete leaf fall and (2) higher stem starch concentration at lifting (Fig. 1), which imply that carbohydrate translocation from leaves to stem to taproot was still occurring. Leaf fall was already complete in black walnut and white ash by September 22 but still underway in red oak and yellow poplar until October 6. Seedlings lifted in early fall also had lower root starch concentration at planting time, compared to those lifted and stored after October 13 (Table 1).

Method of Starch Determination

Root starch concentrations determined by visual estimation were acceptably close to concentrations determined by chemical analysis. Coefficients of determination (r^2) were: black walnut 0.60, red oak 0.74, white ash 0.56, and yellow poplar 0.78. For regression data, see Table A-2 in the appendix. Higher r^2 values would be expected if starch concentration in individual seedlings was measured by both methods. Although starch concentrations above 30 percent were difficult to distinguish by visual analysis, the method is suitably definitive to identify differences that are biologically important. Thus, the visual technique offers a simple method for evaluating the adequacy of root starch reserves in hardwood planting stock.

Relation of Lifting Time/Starch Concentration at Planting Time to Field Survival and Growth

Seedling starch concentration at planting time varied according to when seedlings were lifted, i.e., seedlings lifted early, just before or just after leaf fall, had lower starch concentration at planting time. Therefore, differences in field survival and growth were primarily due to the different lifting times (Table 2). Seedlings lifted from partially frozen soil on December 1 grew less the first year than those lifted on preceding dates, especially black walnut and yellow poplar.

Black walnut. First- and second-year seedling survival was high, 80 to 100 percent, and differences showed no relation to lifting time. Both height and diameter growth were generally slow during the first year, a condition referred to as "transplant shock". Second-year growth was generally good. Height increment varied significantly among the lifting dates, but there was no meaningful pattern, especially during the second year.

Red oak. First- and second-year survival were lower for seedlings lifted before mid-October. The same pattern generally holds for first-year growth of seedlings lifted before October, although significant differences existed only in diameter increment. Seeding growth was generally sluggish during the first two years after transplanting.

White ash. Seedling survival remained high through the second year, 86 to 100 percent, and differences showed no relation to lifting time. First-year height and diameter increment were generally higher for seedlings lifted from early October on. Seedling growth was generally good the first season after transplanting, and excellent regardless of lifting date during the second year.

Table 2. First- and second-year survival, height increment, and diameter increment of four hardwood species lifted on nine dates in fall, 1975 and stored until planted on April 9, 1976.

Species	Lifting date	Survival (%) First year	Survival (%) Second year	Height increment (cm) First year	Height increment (cm) Second year	Diameter increment (mm) First year	Diameter increment (mm) Second year
Black walnut	Sept. 22	94	90	− 2.0bc[1]	42.7a	3.0	12.5
	Sept. 30	98	96	1.4 abc	30.8abc	2.9	10.9
	Oct. 6	94	94	4.9abc	21.1c	2.8	11.9
	Oct. 13	98	98	6.1abc	35.5ab	3.5	12.0
	Oct. 21	92	86	− 4.6c	34.0bc	2.4	10.3
	Oct. 29	96	98	6.6ab	28.9bc	3.4	12.4
	Nov. 1	100	100	9.8a	31.5abc	4.3	11.8
	Nov. 17	94	94	6.6ab	22.2c	3.5	11.4
	Dec. 1	80	80	− 4.1bc	20.2c	2.7	10.6
Red oak	Sept. 22	52b	44b	5.3	14.2	0.8bc	2.8
	Sept. 30	2c	0c	− 0.9	–	− 0.2c	–
	Oct. 6	56b	40b	7.9	22.3	1.2ab	3.2
	Oct. 13	74ab	62ab	6.1	18.8	1.0bc	3.5
	Oct. 21	92a	74a	5.1	25.0	1.0bc	4.7
	Oct. 29	84a	68ab	4.1	16.5	0.9bc	2.6
	Nov. 1	84a	80a	13.2	26.4	1.9a	5.7
	Nov. 17	92a	84a	6.9	23.3	1.8a	4.3
	Dec. 1	82a	74a	6.1	19.9	1.4ab	3.6
White ash	Sept. 22	88	88	10.4c	58.6	3.2c	8.3
	Sept. 30	94	94	12.5bc	68.4	3.7bc	8.4
	Oct. 6	96	94	21.1a	62.1	5.1a	9.9
	Oct. 13	96	94	15.5abc	58.1	4.3ab	8.9
	Oct. 21	96	96	20.6a	65.4	4.5ab	8.3
	Oct. 29	100	100	17.5abc	65.4	4.7ab	9.6
	Nov. 1	100	98	22.5a	64.2	4.9ab	8.6
	Nov. 17	88	86	23.1a	63.7	5.1a	8.9
	Dec. 1	98	96	18.4ab	63.8	5.1a	8.6
Yellow poplar	Sept. 22	20b	16b	−10.7b	78.4	2.1b	13.2
	Sept. 30	34b	30b	4.3b	64.5	2.3b	11.5
	Oct. 6	80a	78a	4.2b	78.9	4.1b	12.3
	Oct. 13	78a	70a	− 8.9b	73.2	3.3b	11.2
	Oct. 21	66a	60a	−14.4b	77.9	2.6b	11.5
	Oct. 29	80a	72a	−12.5b	77.5	3.0b	11.9
	Nov. 1	68a	62a	−12.4b	66.8	3.5b	11.0
	Nov. 17	82a	80a	24.6a	75.0	6.8a	11.0
	Dec. 1	90a	82a	−12.3b	63.4	3.4b	11.3

[1] Means within a column followed by the same letter are not significantly different (a = 0.05) according to Duncan's multiple range test. Data groups with no letters have no significant differences.

Yellow poplar. First- and second-year survival were distinctly lower for seedlings lifted before October 6. For the remaining lifting dates, survival averaged 72 percent at the end of the second year. Height and diameter increment varied among the lifting dates, but there was no meaningful pattern. Seedlings lifted on November 17 grew well,. but the poor performance of seedlings lifted December 1 clouded the existence of any trend with lifting date. Seedlings generally exhibited transplant shock during the first growing season and excellent growth regardless of lifting date during the second year.

Simple correlations were run between lifting time confounded with root starch concentration at time of planting and first- and second-year survival, height increment, and diameter increment. Significant correlation coefficients varied among the four species, but were consistently positive. Correlation coefficients are given in Table 3-A in the appendix. There were no significant correlations with growth of black walnut seedlings. Lifting time confounded with root starch concentration was significantly correlated with first- and second-year survival of red oak and yellow poplar seedlings, first-year diameter increment of red oak seedlings, and first-year height and diameter increment of white ash seedlings.

DISCUSSION

Although the pattern of starch accumulation in black walnut seedlings did not vary bvetween years, starch levels during storage were inconsistent. Root starch concentration declines approximately 11 percent during storage in 1975_1976, but remained constant in 1976-1977. Seedlings spring-lifted from the same beds had starch levels similar to stored seedlings in both years (Figs. 1 and 2). Because total carbohydrates and sugars were not measured, it was not possible to account for changes in the type and amount of carbohydrates in the seedlings. Some cold temperature hydrolysis of starch to sugars during the dormant period was expected (Kramer and Kozlowski 1960), but whether stored starch was converted to another carbohydrate or completely metabolized is unknown. The decline of starch in 1975-1976 was clearly apparent in comparing stained taproot cross-sections of all species taken at lifting and at five monthly intervals during storage. Starch concentration diminished uniformly from all root tissues. Root starch concentrations at flushing time in black walnut seedlings were similar for the two years, 18.9 percent on April 9, 1976 (Fig. 1), and 20.1 percent2/ on April 11, 1977. Thus it appears that the time and rate of starch conversion during the dormant period may vary depending on species and environmental conditions.

The actual starch concentrations reported here are lower than those reported for red oak and yellow poplar by Farmer (1975, 1978), due presumably to the low seedbed density in his experiments. Actual starch concentration can be expected to vary by species (Fig. 1), seedling (Farmer 1975), nursery, and year.

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2 Data from a supplemental experiment to evaluate long-term storage. Seedlings were lifted on December 20.

The results of our study suggest that carboyhdrate translocation to seedling roots should be nearly complete before seedlings are fall-lifted for overwinter storage. For highest root starch concentration at planting time, it appears that seedlings should not be lifted before root starch concentration peaks in the fall, or lower concentration at planting time may result (Table 1). Root starch concentration in the species sampled at Vallonia reached its highest level during a 3- to 4-week period after leaf fall.

To learn more about starch levels and fluctuations in planting stock, it would be desirable to routinely measure root starch concentration at lifting time and again after storage. Because starch accumulation occurred gradually over a period of time, the maximum accumulation can be determined when two or more subsequent samplings show approximately the same concentration. We found that the visual estimation technique is fast and sufficiently accurate, although it requires some practice. Photographs of magnified cross-sections of known starch concentrations for each species would be helpful for standards. Such information can be used to refine nursery growing and storing method and to help establish grading standards for planting stock quality.

The low but significant positive correlation coefficients for red oak, white ash, and yellow poplar mean that there was a weak, but consistent, association between later lifting/higher root starch concentration at-planting time and better survival and/or growth in the field. The low correlation coefficients are attributed to the following factors: (1) the study lacked a wide range of seedling starch concentrations, especially seedlings with depleted starch reserves; (2) field test conditions were too favourable for survival and growth, i.e., more stressful conditions would have encouraged expression of treatment differences; and (3) the confounding of lifting dates with starch content effects added to the experimental error. Additional studies are needed to determine if a clear relationship exists between root starch concentration of planting stock and field survival and growth. Because environmental factors (planting sites and weather) vary and interact with seedling physical and physiological factors (injury, size, shoot/root weight ratio, chilling, root growth potential, starch concentration), starch concentration can be considered as only one of several indicators of planting stock quality, and then only to estimate potential vigor after planting.

On the basis of our study, it would appear that lifting and storing stock immediately after leaf fall has no detrimental effect on first-year field performance of transplanted seedlings. Height and diameter growth of all species was good the second year regardless of lifting date. However, these results may be misleading. Under the generally favorable growing conditions of the field planting, only the weakest seedlings would be expected to fail. Under more stressful testing conditions, lifting date effects are more pronounced, especially during the first year. In describing their method to test vigor of planting stock, Hermann and Lavender (1979) pointed out that the physiological quality of seedlings becomes apparent more quickly if the seedlings are stressed. Additional studies are needed under more stressful growing conditions so that lifting date guidelines can be developed which result in consistently good survival and growth under a variety of planting conditions.

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APPENDIX

Table A-1. Coefficients for regression equations and standard errors of estimation (SE_E) for the model $\log_{10}$ Starch = $b_0 + b_1 \log_{10}$ Days + b_2 Days, where Starch = root or shoot starch concentration and Days = days after September 1.

Species	Shoot				Root			
	b_0	b_1	b_2	SE_E	b_0	b_1	b_2	SE_E
1975-76 Study								
Black walnut	2.6868	-1.3420	0.0025	0.1438	0.7235	0.5498	-0.0036	0.0371
Red oak	0.5753	0.1992	-0.0038	0.1602	0.2672	0.7733	-0.0042	0.0634
White ash	1.0969	-0.2501	-0.0008	0.0889	0.0926	0.8137	-0.0053	0.1611
Yellow poplar	1.5480	-0.0011	-0.0011	0.1517	0.7893	0.4713	-0.0041	0.0978

Table A-2. Type II linear regressions of root starch concentration by chemical analysis on starch concentation by visual estimation: chemical analysis = a + b (visual estimation), for four species, n = 14.

Species	Intercept a	Slope b	Coefficient of determination r^2
Black walnut	10.90520	0.667052	0.60
Red oak	-11.86373	1.398039	0.74
White ash	4.39352	0.59359	0.56
Yellow poplar	5.04412	0.72994	0.78

Table A-3. Simple correlation coefficients for lifting date confounded with root starch concentration at planting versus first- and second-year survival, height increment, and diameter increment for four hardwood species.

Species	Survival		Height increment		Diameter increment	
	First year	Second year	First year	Second year	First year	Second year
Black walnut	+0.01	-0.10	-0.07	-0.06	-0.23	-0.11
Red oak	+0.56[1]	+0.57[1]	+0.14	+0.10	+0.37[1]	+0.16
White ash	+0.20	+0.10	+0.35[1]	+0.04	+0.47[1]	+0.01
Yellow poplar	+0.44[1]	+0.45[1]	+0.03	-0.01	+0.10	-0.15

1 Differs significantly from zero at the 0.05 probability level.