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Estimating Starch Content in Roots of Deciduous Trees—A Visual Technique

by Philip M. Wargo

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ABSTRACT

A visual technique for determining starch content in roots of forest trees, based on iodine-staining of starch granules, was compared with a chemical method. Although the chemical method was more precise, roots could be sorted with the visual method into groups that are probably biologically important. The visual technique is simple and can be adapted for use in the field.

KNOWLEDGE OF TREE vigor is important in judging resistance of trees to disease and insect attack or predicting the ability of trees to tolerate stresses such as defoliation, drought, and air pollution *(Kozlowski 1969)*. Often tree vigor is determined after the fact; for example, trees that survive a pest attack or an adverse environmental condition are considered vigorous. Even vigor classes used to predict growth potential are based on the absence of disease and injury and the healthy appearance of the crown *(Trimble 1960, 1969)*. Vigor determination based on radial growth indicates how vigorous the tree has been, but does not necessarily show how vigorous it is now.

Starch content of the roots of deciduous trees may be a good index of tree vigor *(Wargo et al. 1972)*. Photosynthetic capacity is an indicator of the well-being of the tree; and starch, the major reserve carbohydrate in most deciduous trees, reflects photosynthetic capacity of the plant *(Kramer and Kozlowski 1960)*. Starch is stored in high concentrations in the roots *(Jones and Bradlee 1933, Murneek 1942, Wenger 1953, Wargo et al. 1972)*; it is sensitive to stresses such as defoliation *(Staley 1965, Parker and Houston 1971, Wargo et el. 1972)*, drought *(Parker 1970)*, and air pollution *(Miller et al. 1968)*; and changes can be observed within the same growing season when the stress is imposed *(Wargo et al. 1972)*.

If starch content of roots is a reliable index of tree vigor, its determination could be a valuable aid in decision-making for the practicing land manager. For this purpose an accurate and easy method of determining root starch is necessary. Several chemical extractions and analysis procedures are available for starch determination, but these require extensive training and laboratory equipment and cannot be adapted readily for use in the field.

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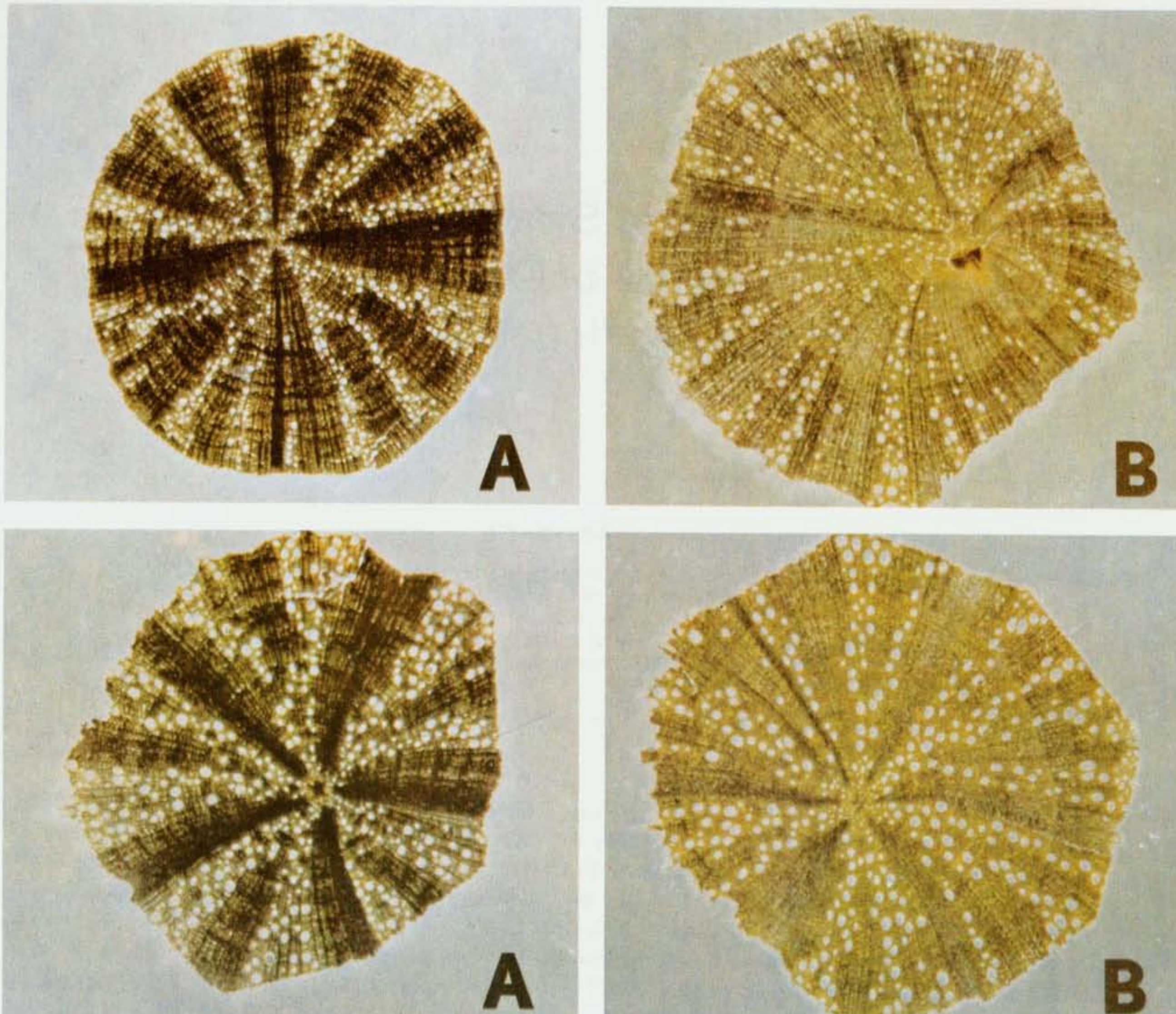
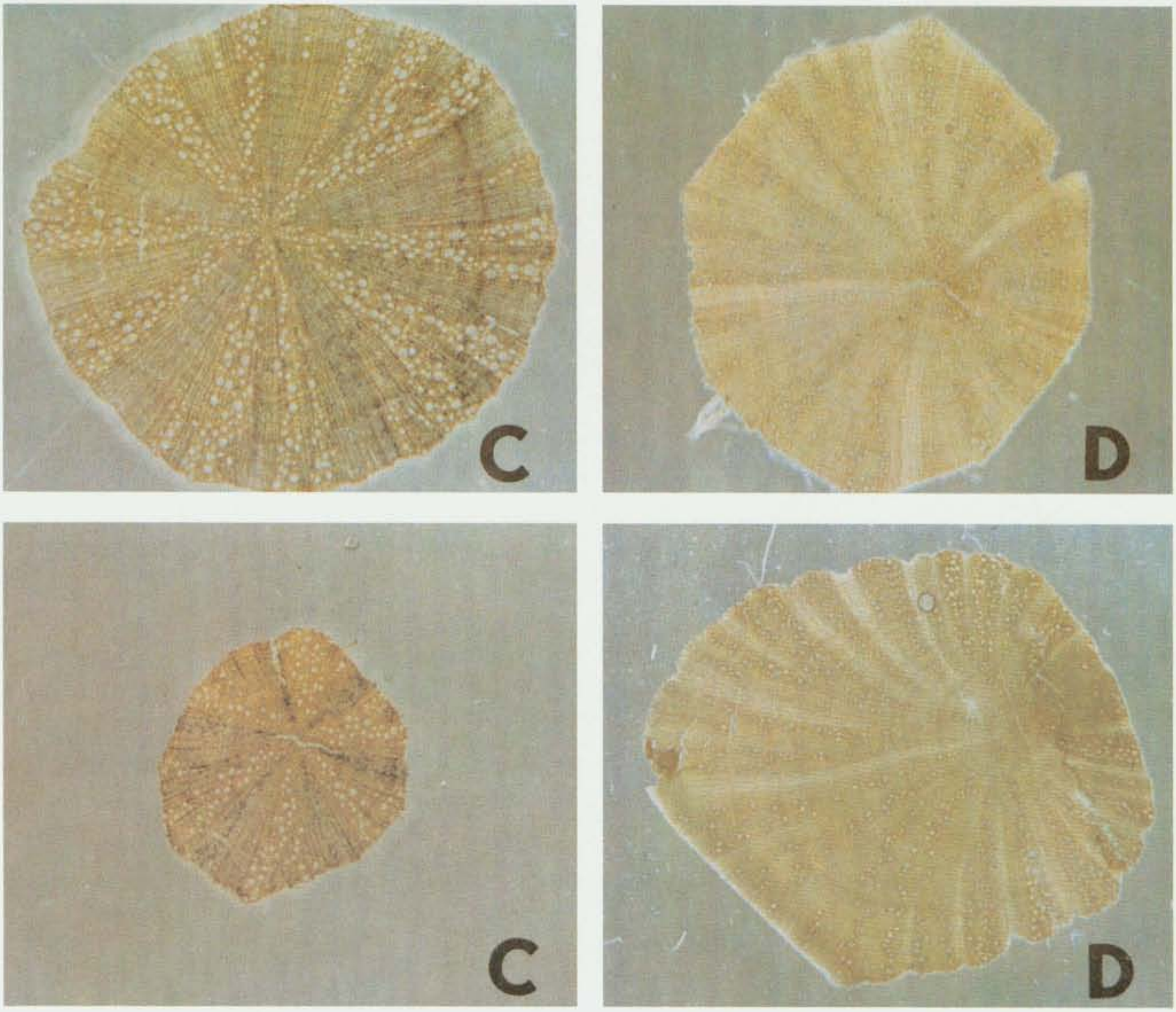


Figure 1.—Cross-sections of roots of red oak stained with I_2KI solution, showing several levels of starch content: A—high (15 to 30%), B—med-



ium (7 to 12%), C—low (3 to 6%), D—depleted (0 to 1%). Magnification 7x.

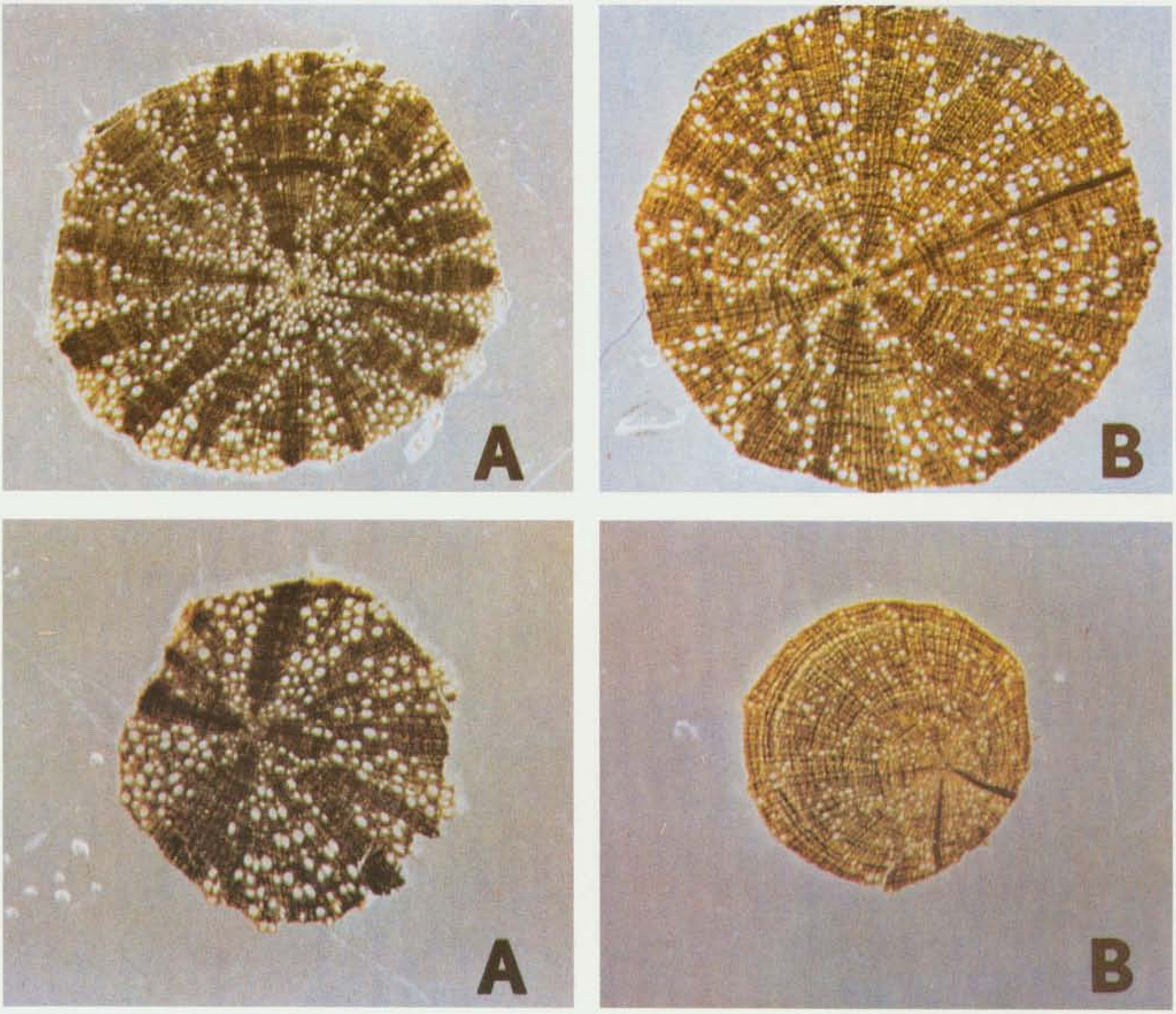
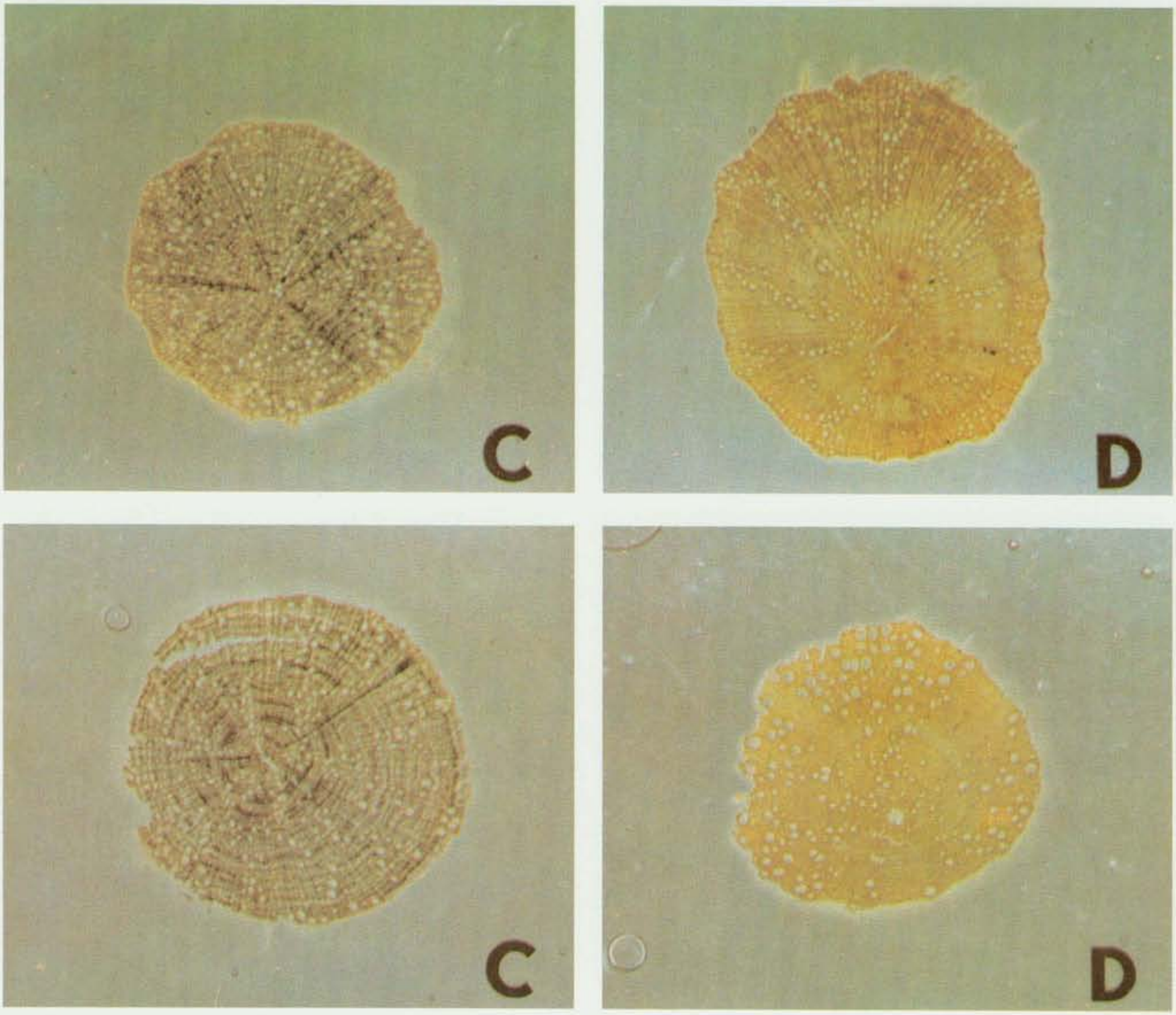


Figure 2.—Cross-sections of roots of white oak stained with I_2KI solution, showing several levels of starch content: A—high (15 to 30%), B—med-



ium (7 to 12%), C—low (3 to 6%), D—depleted (0 to 1%).
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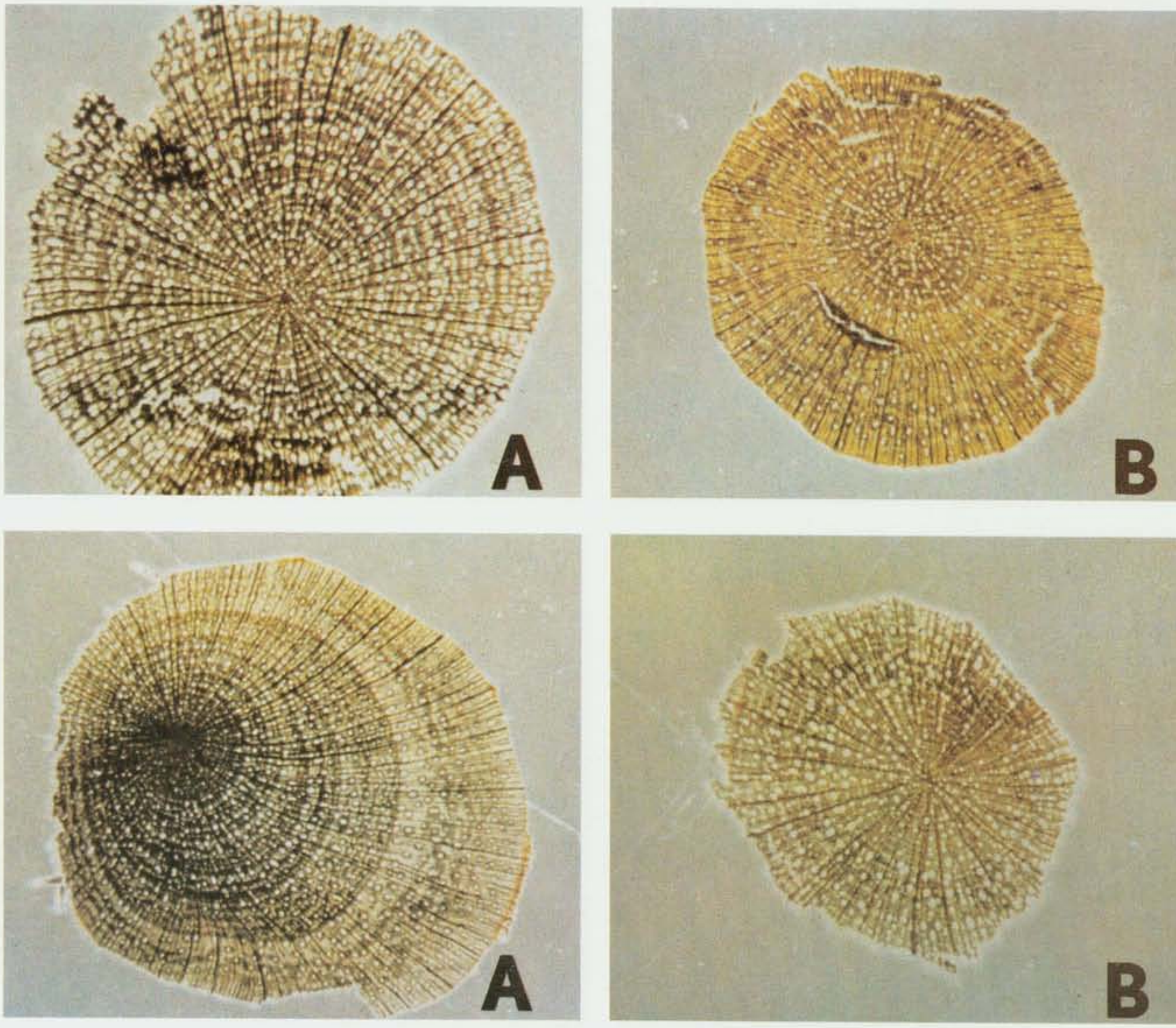
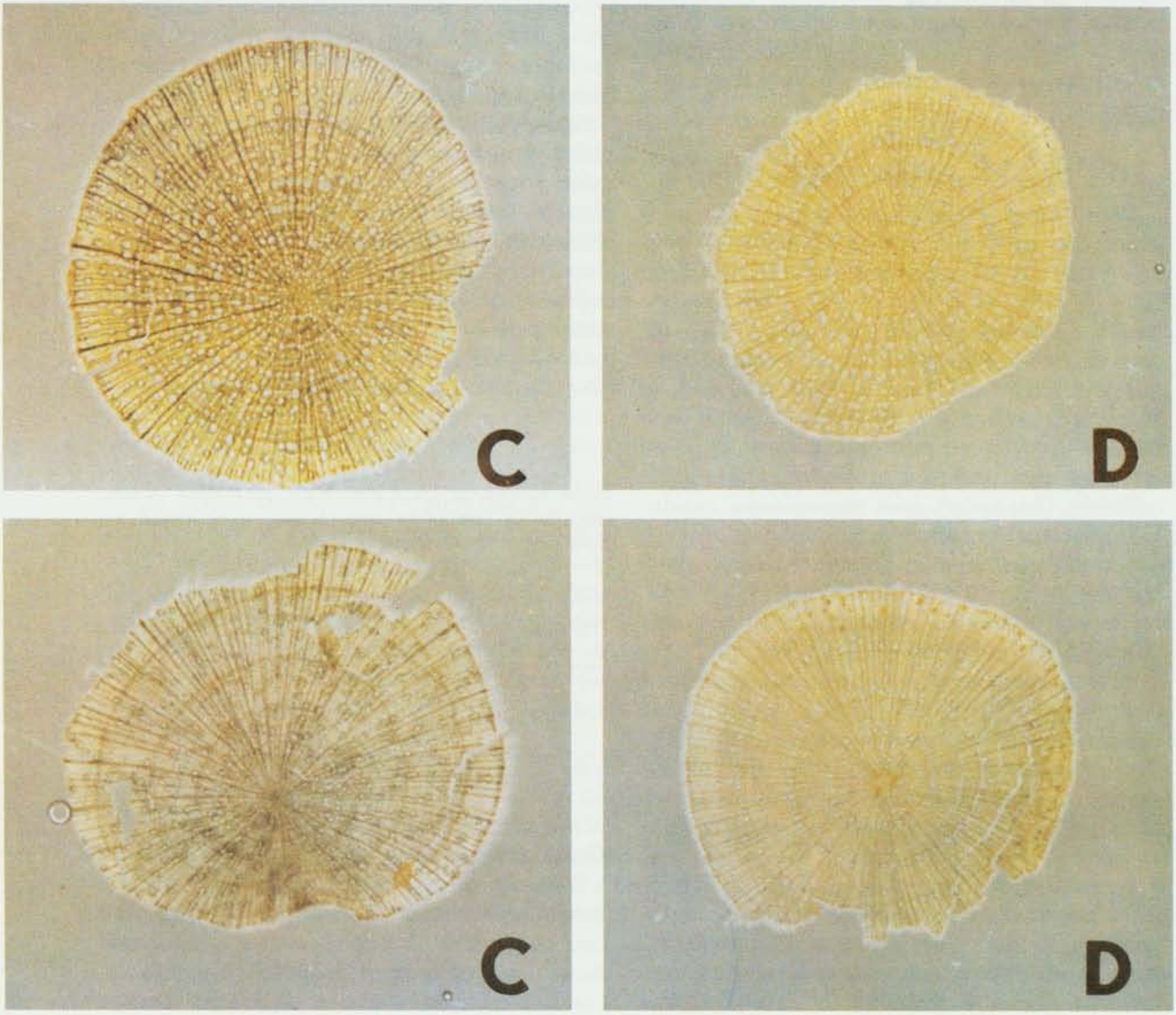


Figure 3.—Cross-sections of roots of sugar maple stained with I_2KI solution, showing several levels of starch content: A—high (12 to 25%), B—



medium (6 to 10%), C—low (2 to 5%), D—depleted (0 to 1%). Magnification 7x.

Our study was made to evaluate a visual method for estimating levels of root starch in tree roots. A similar technique has been used to evaluate the effects of sheep grazing on root reserves in alfalfa *(Rather and Harrison 1939)*.

METHODS AND MATERIALS

Roots were collected before, during, and after the growing season, from red oak *(Quercus rubra* L.), black oak *(Q. velutina* Lam.), white oak *(Q. alba* L.), and sugar maple *(Acer saccharum* Marsh.). Trees of each species had been defoliated by hand during the growing season. This provided a range of starch levels for the test. Small sections of root were removed, placed in polyethylene bags, and kept in insulated bags with ordinary ice. Later, root pieces were cut in half; one-half was frozen and kept at —20 C until analyzed visually. The other half was debarked, dried, and ground in a Wiley mill to pass a 60-mesh screen and was analyzed chemically to determine starch content.

Starch was extracted chemically from the root tissue with perchloric acid according to the procedure of Hassid and Neufeld *(1964)*. Starch content, measured with the colorometric method described by Siminovitch et al. *(1953)*, was expressed as percent dry weight of tissue extracted.

Cross-sections of whole roots or portions of whole roots were stained with an iodine solution for the visual estimate of starch content. Root pieces were thawed rapidly, and cross-sections, approximately 100μ thick, were cut with a sliding microtome. The sections were kept moist at all times. At least two cross-sections were placed on a glass slide and flooded with I_2KI solution. The solution contained 15 g of KI and 3 g of crystalline I_2 in 1000 ml of distilled water. Excess solution was blotted, and fresh iodine solution was added. After 5 minutes the stain was blotted and the sections were rinsed twice with distilled water. Excess water was removed, glycerine was added, and a cover slip was placed over the sections. Staining results were recorded on Polaroid color film or 35-mm Ektachrome (Kodak Co.) color slides. Pictures were taken within 48 hours of staining because the stain faded after 72 hours.

RESULTS

Chemical Analysis

Chemically determined concentrations of starch ranged from 0 to 30% on a dry-weight basis. In general, the red oak group had the highest starch levels, followed closely by white oak and then sugar maple. The variation among subsamples of the same root tissue was small (table 1), and starch levels could be sorted into one of ten categories: 0%, 2±1%, 5±1%, 8±1%, 11±1%, 14±1%, 17±1%, 20±1%, 23±1%, and 25% and higher.

Table 1.—Variation of starch concentration in subsamples of the same root tissue, determined by chemical extraction and analysis.

[In percent dry weight]

Tree no.	Subsamples		
	1	2	3
1	7	7	7
2	0	0	0
3	3	3	3
4	19	20	20

Visual Analysis

With the visual technique, roots from all tree species could be separated easily into four categories of starch content: high, medium, low, and none or depleted (figs. 1 to 3). The low category could be separated further into very low and low within each species. In the oak groups, the corresponding chemically determined range of root starch in these categories was 15 to 30%, 7 to 12%, 3 to 6%, and 0 to 1%. For sugar maple the ranges were 12 to 25%, 6 to 10%, 2 to 5%, and 0 to 1%. Because of anatomical differences among species it was necessary to limit visual comparisons of starch content to within the same species or at least to related species such as red and black oak. Cross-sections 100 to 150 μ thick were suitable for staining and analysis, but sections 200 μ and thicker were not.

DISCUSSION

Chemical analysis of starch content permitted sorting of roots into more and narrower categories. With the visual technique, roots could be sorted easily into broader categories that are probably biologically important. Wargo et al. *(1972)* observed that defoliated trees had little or no starch in the roots, and that these levels reflected the adverse effects of stress on the trees. Unstressed trees, on the other hand, had high starch content in their roots. Drought also had a similar effect *(Parker 1970)*. Similar observations were made of alfalfa. Grazed alfalfa had lower reserves than ungrazed alfalfa, and starch content was important in winter survival of the plants *(Rather and Harrison 1939)*.

The visual technique is fairly simple and fast. It requires little equipment, and the procedure can be learned in a single day. The test can be done in a laboratory or, with some modification such as a hand microtome, in the field. The results can be read immediately or within 48 hours. The test is fairly accurate and repeatable, provided the staining solution is not more than 30 days old.

The visual technique offers a simple method for evaluating starch content in roots of deciduous trees. Such evaluation may be useful in determining tree vigor, in evaluating the effects of stress on trees, or in evaluating other methods and techniques of judging tree vigor.

LITERATURE CITED

Hassid, W.Z., and E. F. Neufeld.
1964. QUANTITATIVE DETERMINATION OF STARCH IN PLANT TISSUES. *In* METHODS IN CARBOHYDRATE CHEMISTRY, vol. IV. STARCH: 33-36. Roy L. Whistler, ed. Academic Press, New York.

Jones, C.H., and J. L. Bradlee.
1933. THE CARBOHYDRATE CONTENTS OF THE MAPLE TREE. Vt. Agric. Exp. Stn. Bull. 358. 147 p.

Kozlowski, T. T.
1969. TREE PHYSIOLOGY AND FOREST PESTS. J. For. 67:118-123.

Kramer, P.J., and T. T. Kozlowski.
1960. PHYSIOLOGY OF TREES. McGraw-Hill Book Co., Inc., New York. 642 p.

Miller, P.R., F.W. Cobb, Jr. and E. Zavarin.
1968. PHOTOCHEMICAL OXIDANT INJURY AND BARK BEETLE (COLEOPTERA SCOLYTIDAE) INFESTATION OF PONDEROSA PINE. III. EFFECT OF INJURY UPON OLEORESIN COMPOSITION, PHLOEM CARBOHYDRATES AND PHLOEM pH. Hilgardia 39 (6):135-140.

Murneek, A.E.
1942. QUANTITATIVE DISTRIBUTION OF NITROGEN AND CARBOHYDRATES IN APPLE TREES. Mo. Agric. Exp. Stn. Res. Bull. 348. 28 p.

Parker, J.
1970. EFFECTS OF DEFOLIATION AND DROUGHT ON ROOT FOOD RESERVES IN SUGAR MAPLE SEEDLINGS. USDA For. Serv. Res. Pap. NE-169. 8 p.

Parker, J., and D. R. Houston.
1971. EFFECTS OF REPEATED DEFOLIATION ON ROOT AND ROOT COLLAR EXTRACTIVES OF SUGAR MAPLE TREES. For. Sci. 17:91-95.

Rather, H.D., and C. M. Harrison.
1939. STARCH RESERVES IN ROOTS OF PASTURED ALFALFA WHEN GROWN ALONE AND IN MIXTURES. Mich. Agric. Exp. Stn. Quart. Bull. 21:281-291.

Siminovitch, D., C. M. Wilson, and D. R. Briggs.
1953. STUDIES ON THE BIOCHEMISTRY OF THE LIVING BARK OF THE BLACK LOCUST IN RELATION TO ITS FROST HARDINESS. V. SEASONAL TRANSFORMATION AND VARIATIONS IN THE CARBOHYDRATES: STARCH-SUCROSE INTERCONVERSIONS. Plant Physiol. 28:383-400.

Staley, J.M.
1965. DECLINE AND MORTALITY OF RED AND SCARLET OAKS. For. Sci. 11:2-17.

Trimble, G.R.
1960. RELATIVE DIAMETER GROWTH RATES OF FIVE UPLAND OAKS IN WEST VIRGINIA. J. For. 58:111-115.

Trimble, G.R.
1969. DIAMETER GROWTH OF INDIVIDUAL HARDWOOD TREES. THE EFFECT OF CERTAIN TREE AND ENVIORNMENTAL FACTORS ON THE GROWTH OF SEVERAL SPECIES. USDA For. Serv. Res. Pap. NE-145. 25 p.

Wargo, P.M., J. Parker, and D. R. Houston.
1972. STARCH CONTENT IN ROOTS OF DEFOLIATED SUGAR MAPLE. For. Sci. 18:203-204.

Wenger, K.F.
1953. THE SPROUTING OF SWEET GUM IN RELATION TO SEASON OF CUTTING AND CARBOHYDRATE CONTENT. Plant Physiol. 28:35-49.

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