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Image-analyzing computer in plant science: more and larger vascular rays in sugar maples of high sap and sugar yield'

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The total area and number of xylem rays and vessels from tangential and cross sections of twigs of 12 sugar maples (Acer saccharum Marsh.) were determined by the use of an image-analyzing computer. A nested analysis of variance indicated that xylem rays of trees of high sap and sugar yield are more numerous and larger than the rays of other sugar maples. The total area and number of xylem vessels were about the same in all 12 trees.

Introduction

Some sugar maples consistently yield sweeter (7, 11, 16) and more abundant (7) sap than others, a fact of considerable economic significance for producers of maple syrup. Although rigid proof is lacking, these data suggest that high-quality maple trees may be genetically determined (17, 18). One phenotypic basis for this phenomenon may be the presence in their xylem of many large carbohydrate-storing rays; microscopic observations of wood sections were consistent with this hypothesis (Figs. 2, 3). Maples with large crowns, hence with increased potential for photosynthesis, are high-quality trees for both sugar and sap yield (12). In addition, environmental factors may influence growth release. Gregory and Romberger have shown a strong positive correlation between radial growth rate and number of ray cells per unit tangential area in the xylem of some conifers (3). Gregory has found this correlation to be true also in sugar maples (2). However, the factor or factors that stimulate ray initials and regulate the quantitative relationship between diameter ring size and vascular ray development are unknown. In a natural stand of maples, tree-vigor parameters are not correlated with sap concentration (F. M. Laing and T. T. Siccama, unpublished data).' Furthermore, the complex factors, including temperature changes, which determine sap sugar concentration (14) also influence sap volume yield (6, 7).

Methods and Materials

Twelve maples of approximately the same diameter were randomly selected from the top and bottom groups of 29 mature maple trees which were ranked according to the correlation coefficient of their sap sugar concentration averages and sap volume yields for 20 years (7). Data of two following seasons were added to the original 18-year study to come up with this 20-year total.

A preliminary analysis of variance of area size and number of xylem rays and vessels in wood sections of twigs of different size indicated that the random choice of twigs of 8, 12, and 18 mm in diameter from each tree was adequate. Twigs were excised in October when starch content in ray cells is high (8), and they were fixed in formalin – acetic acid – alcohol (FAA, 5:5:90). To measure xylem rays, 10- to 15-p.m-thick tangential sections were stained with iodine–potassium iodide and mounted in Aquamount. To measure vessel elements, 10- to 15-p.m-thick transverse sections were stained with safranin; adequate contrast was obtained without counterstaining.

The following numbers of fields were each magnified 100 x and analyzed with an image-analyzing computer (IAC): 10 at random from each of six transverse and six tangential sections per twig, a total of 120 per twig and 360 per tree. Initially developed for metallurgic research, the IAC, a Quantimet 720, has been used for measuring biological systems (1, 4, 9, 10), including leaf areas (13). Images scanned by the Quantimet 720 are transmitted to a video monitor and to a detector programmed to select features according to their optical density. A digitized feature is recognized as a whole by the IAC specific memory and counted as one. This involves conversion of an optical image, monitored on a TV screen, into an electrical image and its analysis with pattern-recognition computer modules. The IAC modules were programmed to read the number of rays or vessels of seven size classes (between 45 and 10 µm in diameter), their total number, and their area.

The data obtained on the IAC output device were in turn analyzed by another computer with an analysis of variance for nested or hierarchical classification (15) in which each random sample was composed of subsamples, which in turn were subsampled. This repeated sampling and subsampling led to the use of a nested design in which twig-within-tree mean square was used to test for differences among trees of the same quality, i.e., high or low sap and sugar yield, and tree-within-quality mean square for differences between high- and low-quality trees (Table 1).

Results

Results of the nested analysis of variance for the vascular rays are summarized in Table 1. Significant differences (P = 0.01) were found between high- and low-quality maples in the number of large

'Vermont Agricultural Experiment Station Journal Article No. 378.
'Deceased.
'F. M. Laing and T. T. Siccama. Young sugar maples. 1. Consistency in sap sugar concentration.

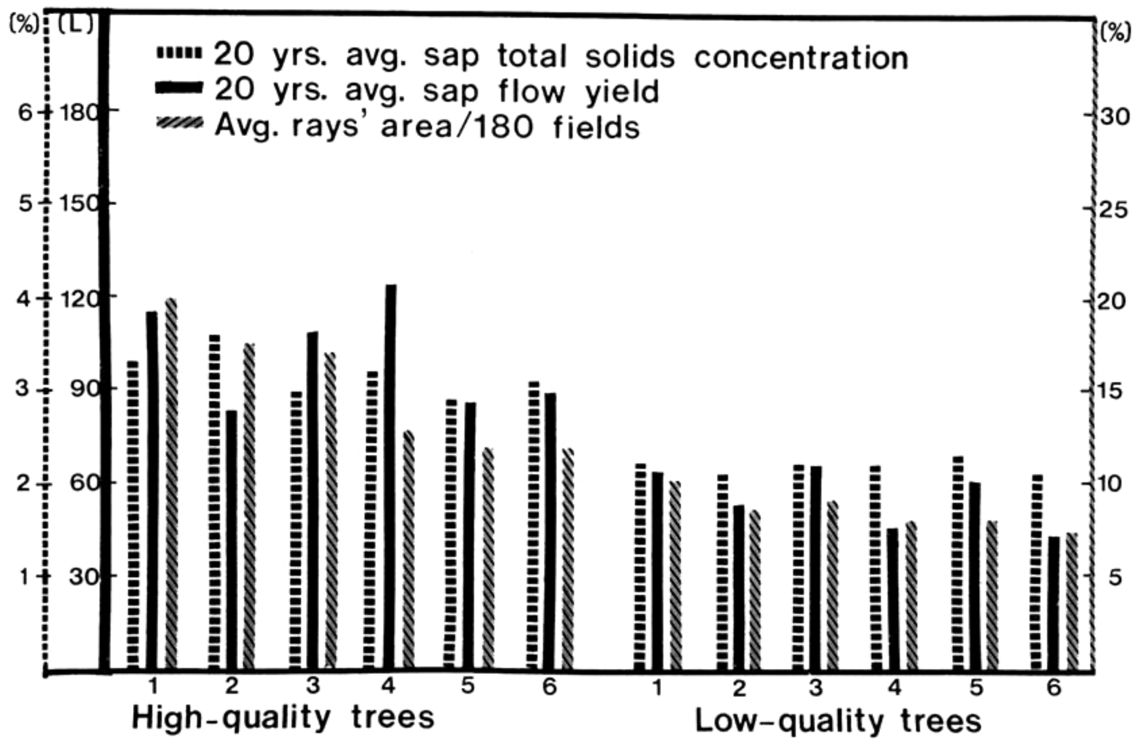

FIG. 1. Relationship of the percentage average tangential area of rays per 180 fields with the percentage average sap sugar (total solids) concentration and the average sap flow yield in litres (L) (20-season average for each tree). yrs. = years; avg. = average.

TABLE 1. Hierarchical (nested) analysis of variance

Source of variation	Degrees of freedom	Mean square	F	P*
		Total area of vascular rays, μm^{z}		
1 **2** **3** Total	2159			
Quality	1	6 673 231 089.44	19.343	0.001
Trees within quality	10	344 989 036.35	7.287	< 0.001
Twigs within tree	24	47 340 872.83	4.159	< 0.001
Sections within twig	180	11 383 086.52	2.072	< 0.001
Fields within section (error)	1944	5 493 452.24		
		Number of vascular rays > 40 μm in diameter		
Total	2159			
Quality	1	2 754.04	10.100	0.01
Trees within quality	10	272.69	9.029	< 0.001
Twigs within tree	24	30.20	6.551	< 0.001
Sections within twig	180	4.61	2.696	< 0.001
Fields within section (error)	1944	1.71		

*Probability of a greater F value.

vascular rays (40 μm in diameter) and in the size of their total tangential area (P = 0.001). The average number of large rays per field was 2.85 in high-quality maples and 0.59 in the others. The mean total area of rays per field was 7763 μm^2 for the high-quality trees and 4247 μm^2 for the others. The difference between these two means was highly significant (P = 0.001).

The number of large vessels per field was about the same in all trees. The difference was not significant (P = 0.44). The mean total tangential area of vessels per field was 4126 μm^2 for high-quality

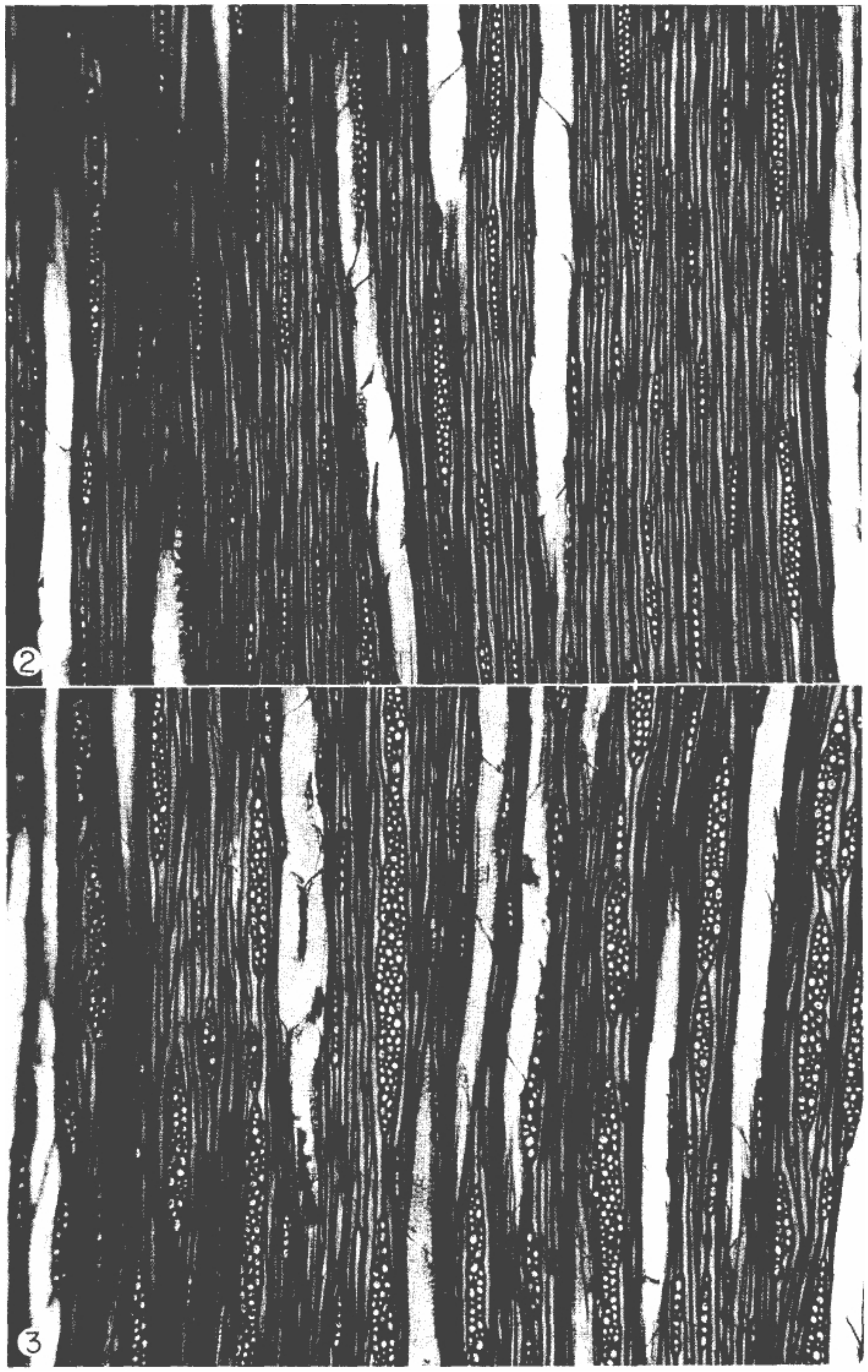

FIG. 2. Tangential section of twig from a low-quality sugar maple. FIG. 3. Tangential section of twig from a high-quality sugar maple.

maples and 3557 μm² for low-quality trees (P = 0.45). The analysis of variance indicated that the ratio between total vessel area and total ray area was different in the two qualities of trees (P = 0.008). The total area of rays was twice the total area of vessels in the high-quality maples, whereas in the low-quality trees, the total areas of rays and vessels were about the same.

Figure 1 shows the relationship of the average tangential area of rays per 180 fields, expressed as percentage of total field area, with the percentage average sap sugar (total solid) concentration and the average sap flow yield in litres as a mean of 20 sap seasons.

Discussion

Some methods traditionally used to measure wood anatomical features are time consuming. The image-analyzing computer (IAC) increases speed and accuracy in performing quantitative measurements of cell size and number in wood tissue and determines values through an appropriate choice of IAC modules. Its use has made it possible to rapidly assemble a number of observations which are statistically valid for determining a relationship among three phenotypic characteristics of maple trees: sap volume yield, sap sugar content, and size and total area of xylem rays and vessels.

The anatomical difference of vascular ray size and number among sugar maples of differing potential for final syrup production may indicate that differences of storage capacity exist among maples.

The field method of measuring sugar content to determine the long-range economic value of a sugarbush (5) is supported by these findings.

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