

ESTIMATING TWIG STARCH CONTENT IN SUGAR MAPLE (ACER SACCHARUM): EVALUATION OF THE VISUAL TECHNIQUE

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INTRODUCTION

Nonstructural carbohydrate reserves stored by plants are sometimes described in terms of personal banking. The reserves are added when production from photosynthesis exceeds the demand from immediate living and growth (similar to depositing money in the bank) and reserves are consumed when the reverse is true (like a withdrawal) (Chapin 1990). The analogy can be extended further by saying that soluble sugars (predominately sucrose) are like a checking account (more easily spent) whereas water-insoluble starch is like a savings account. The nonstructural carbohydrate balance of trees has thus been used as an indication of overall health of trees (Wargo 1972). It is also used to assess a trees ability to withstand stress. During the dormant season, the reserves are allocated to several essential processes (such as cold season acclimation, cellular respiration and defense) which allow trees to survive in cold climates. Total nonstructural carbohydrates (TNC), which includes total starch + total sugar, is used as a measure of

the reserve material available to plants. Starch is the dominant reserve carbohydrate in sugar maple (*Acer saccharum*) (Jones & Bradlee 1933). Starch is stored in vascular rays throughout the xylem (Gregory H. 1981). Starch can represent 50-80% of the total nonstructural carbohydrate reserves in sugar maple (Isselhardt, unpublished data). Trees with depleted starch reserves are more prone to die back and decline from various biotic and abiotic stressors (Gregory R. A. 1986).

Root starch has been used to estimate tree vigor and health with some success (Gregory 1986, Renaud & Mauffette 1991). Precise carbohydrate chemical analysis of wood tissue is a time consuming, expensive endeavor that requires significant investment in equipment and technical expertise. A relatively simple, low cost, visual technique was developed to give an estimation of the starch reserves in maple roots (Wargo P. 1975). This experiment used a simple iodine solution to stain root tissue from trees that had been defoliated by hand (as well as non-defoliated controls). Chemical carbohydrate analysis was used to quantify the starch concentrations in sugar maple root samples, and iodine stained root cross sections were sorted into four categories of starch concentration (depleted 0-1%, low 2-5%, medium 6-10% and high 12-25%) based on the intensity of staining. There was sufficient agreement with the chemically determined starch values to suggest the visual technique had promise and represented a technique that could be easily mastered by a wide range of professionals.

It has been noted that root sampling can be a laborious and inexact process (Perkins, personal communication). In a forested setting, determining the actual source of a given tree root can be challenging. Additionally, there is no clear delineation for where roots end and stems begin. Wong (2003) proposed the use of twigs in carbohydrate analysis. Twig sampling is easier, less damaging than root sampling and allows for collection of numerous samples and repeated measures on the same tree over successive years. In the present paper, a visual method of starch determination similar to that already used for roots has been undertaken for twigs. If successful, this method would simplify the process of assessing overall tree health and vigor in sugar maple trees.

METHODS

Study site

This experiment was performed at the University of Vermont, Proctor Maple Research Center, in Underhill Center, Vermont. The site was described in more detail in a previous study (Wilmot 1995). Generally the forest has low site quality. The terrain is hilly and at an approximate elevation of 1430', with a generally west-facing aspect and slopes of 10-15%. This site is an actively-managed sugarbush and sugar maple (*Acer saccharum*) is the dominant overstory species. Lesser amounts of red maple (*Acer rubrum*), white ash (*Fraxinus americanum*), American beech (*Fagus granifolia*) and yellow birch (*Betula alliganiensis*) are also present.

Sample collections

Twelve visually healthy sugar

maple trees were selected from a group of trees actively tapped for maple syrup production. Mean diameter of the study trees was 7.7". Twigs were collected just prior to bud-break (April 6, 2010), from the upper 1/3 of the crown using a shotgun. Three separate twigs from the same general area of the crown were collected from 12 trees. Samples were immediately placed in a cooler with ice and transported to the lab.

Laboratory Procedures

Following the sample collection, twigs were placed in an ultra-low freezer (-93 degrees F) until chemical and visual analysis could be performed. Starch was determined chemically (Wong 2003). A 3-4 cm section was removed from the base of each twig. The bark, phloem and cambium were removed with a razor blade. The pith was removed with the aid of a cordless drill. The remaining sample of xylem tissue was submerged in a vial containing 5 ml of 80% ethanol, placed in a boiling water bath for 15 minutes and then evacuated at 24" Hg in a cold vacuum oven.

Samples were homogenized in a Brinkman Instruments Polytron™ and transferred to a 50 ml centrifuge tube. The ethanol soluble sugar fraction of the xylem tissue was extracted twice with 5 ml of 80% ethanol. The wood pellet was analyzed for starch concentration by an enzymatic method. The enzyme hydrolyzes the starch to glucose, which is then quantified colorimetrically with a spectrophotometer using a glucose standard curve. The residual wood tissue pellet was dried to uniform moisture and weighed. Starch concentration was

calculated in terms of milligrams/gram dry weight of wood and then converted to percent starch.

Cross sections of whole twigs were stained with iodine for visual analysis of starch. The sections were made to a uniform thickness of 25 with a rotary microtome, placed on a glass slide, and saturated with a 15% iodine solution for five minutes, at which time the iodine was rinsed off with water and a cover slip placed on top. Mounted slides were observed and photographed at 10x magnification. Digital color images were captured for visual analysis.

RESULTS AND DISCUSSION

Chemical analysis

The range of mean starch values observed in sugar maple twigs collected from at the end of the dormant season was much lower than previously found in roots (Wargo 1975). Trees that were artificially defoliated in that experiment had root starch concentrations ranging from 0-30% on a dry weight basis. The twig samples in this study had a range in starch concentration from 1.1-8.9%. Wargo (1975) collected three sub-samples for each of four trees and found little visual variation in root starch concentration. In contrast, the twig samples collected for this study showed a substantial amount of tree variability (Table 1).

The high variability within tree starch concentrations represents an impediment to using twig starch as a reliable measure of sugar maple non-structural carbohydrate reserves. It is possible that the variation in observed starch concentration was a result of sampling error or subtle non-

Table 1. Sugar maple twig starch concentration (% dry weight basis) and standard error for 12 sugar maple trees.

Tree #	Sub-samples	Starch Concentration (%dry wt.)	SE
1	3	3.5	0.68
2	3	6.4	0.37
3	3	3.8	0.78
4	3	1.7	0.32
5	3	5.7	0.33
6	3	3.3	0.14
7	3	4.3	0.24
8	3	2.2	0.40
9	3	5.7	1.40
10	3	6.4	1.00
11	3	6.1	0.99
12	3	4.1	0.54

visual differences in spring-time twig phenology.

Visual analysis

Wargo (1975) determined that stained root cross-sections could be placed in four general categories of starch concentration (depleted 0-1%, low 2-5%, medium 6-10% and high 12-25%). A similar set of twig starch concentration categories could not be easily made with stained twig sections. The pattern of increased staining intensity with starch concentration (Figure 1) was not as observed in twig sections. A pattern of increasing stain intensity with increased starch content can be seen in the first column of the root sections. The pattern is less clear but still present in the second column of the root sections. No similar pattern can be seen in the twig sections, despite a comparable, 10 fold increase in the starch content.

Visual analysis was undertaken in hopes of finding some identifiable

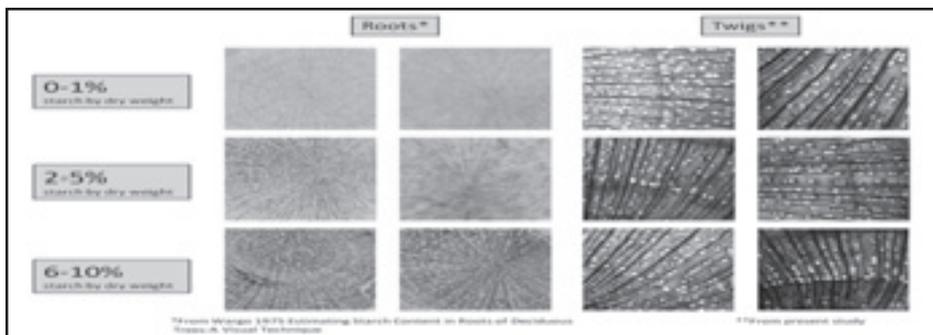


Figure 1. Visual comparison of iodine stained root and twig cross sections at three ranges of starch concentration (0-1%, 2-5% and 6-10%). Root section images are reprinted from (Wargo P. , 1975). Root sections are 7X and twig sections are 10X magnification.

source for the within tree variability in twig starch concentration. Figure 2 shows iodine stained cross sections from two sugar maple twigs used in the present study. The two twigs represent the lowest and highest starch concentrations recorded with chemical analysis in the present study. The twig on the right shows a fairly uniform distribution of xylem anatomical constituents (large vessel elements, darkly stained vascular rays and fibers). The twig section on the left has areas that are similar to the image on the right but it also includes a much greater collection of stained starch grains near the center of the twig. It appears that some starch is being stored in the area surrounding the pith as well as the radially oriented vascular rays. If not all the pith was removed in the sample preparation stage of the experiment (as was the case in the example in figure 2), the retained starch would have added to the overall twig starch concentration values and thus to the variability of twig starch concentration within trees. This would account for the highly variable starch concentration values but not for the failure to produce a discernable pattern of staining.

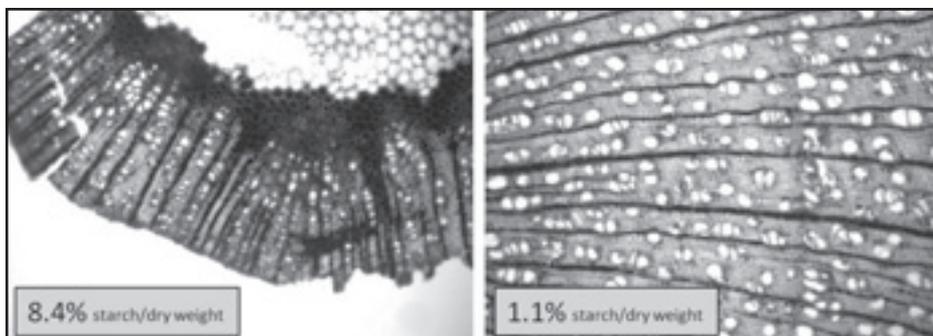


Figure 2 Cross-sections of twigs of sugar maple stained with iodine solution, showing maximum (image left) and minimum starch concentration (image right). Darkly stained vascular rays are present in both cross sections. The twig on the left appears to have starch storing amyloplasts around the pith lining as well.

Another possible explanation for the observed variation in twig starch concentrations is the timing of sample collection. Warm spring days in late March 2010 greatly accelerated the phenology of spring twig development. It is possible that subtle differences in twig development, concentration of vegetative vs. flower buds, or micro-site could have affected the starch concentrations. It appears that spring twig starch concentrations may be inherently too variable to be useful in assessing overall tree starch reserves. Perhaps sampling earlier in the year (January) would avoid the confounding factors of spring growth and warm temperatures. Additional work will be necessary to further investigate these sources of variability.

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