Plant Physiol. (1985) 78, 427–429
0032-0889/85/78/0427/03/$01.00/0

Short Communication

Stable-Carbon Isotopic Composition of Maple Sap and Foliage[1]

Received for publication December 7, 1984 and in revised form March 7, 1985

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ABSTRACT

The $^{13}C/^{12}C$ ratios of *Acer grandidentatum* sap sugar collected during the dormant period are compared to those of buds, leaves, and wood developed over the following growing season. As the primary carbon source for cellulose manufacture at initiation of annual growth in deciduous trees, sap sucrose would be expected to have an isotopic composition similar to first-formed cellulose. Although constancy in concentration and $^{13}C/^{12}C$ ratios of the maple sap sugar suggests any gains or losses (*e.g.* to maintenance metabolism) do not appreciably alter composition, the $^{13}C/^{12}C$ ratios of cellulose of the enlarging buds in the spring are quite distinct from those of the sap sugar, seemingly precluding a simple direct biochemical pathway of sap sucrose→glucose→cellulose in favor of a more complex pathway with greater likelihood of isotopic fractionation. The $^{13}C/^{12}C$ ratios of the leaves and in the growth ring were initially similar to the sap sugar but decreased steadily over the growing season.

Seasonal variations of stable-carbon isotopic ratios ($^{13}C/^{12}C$) in whole tissue of maple leaves were first reported about a decade ago (10). Recently, Leavitt and Long (7) established a link between the timing and trend of seasonal variations in leaves and growth rings of a juniper tree. Some of this seasonal isotopic change may owe to the initial growth flush deriving primarily from carbohydrates manufactured during the previous season, followed by increasing reliance on photosynthates of the current season as the leaves mature. The previous year's sugars may carry an isotopic composition reflecting the environmental conditions at their formation, perhaps distinct from those of the current growing season. Unfortunately, with juniper, photosynthetically active leaves are retained from several previous seasons (1, 11) so that the carbohydrates utilized at the initiation of growth may originate from the stored carbohydrates or from the actively photosynthesizing older leaves. In the case of deciduous trees, however, carbohydrates stored from the previous season's growth should be the dominant, if not only, carbon contribution at the onset of growth.

We sampled sap and leaves from an *Acer grandidentatum* (bigtooth maple) individual to determine if this situation might be demonstrated isotopically. This species is deciduous, and it is considered (9) a variety of the Eastern sugar maple (*A. saccharum*) so that abundant sap flow during the growing season, dominated by sucrose (Leaf [6] reported 95% of solids from *A. saccharum* sap are sucrose), would provide ample material for analysis.

MATERIALS AND METHODS

The selected *A. grandidentatum* individual grows at an elevation of 7840 ft (2390 m) in the Santa Catalina Mts. in southeastern Arizona. We first put out taps on two of the three large trunks of this tree (~40 cm diameter) during the winter 1983 and collected the sap which accumulated in large plastic containers approximately every 2 weeks from February 18 to May 8, 1983. The samples were kept refrigerated and generally frozen within 48 h. Along with the sap, buds (unbroken) were collected in early May. Leaves were collected on June 12 and again near the end of the growing season on September 25. An increment core was also taken from one trunk during the September collection. The leaf and wood samples were first ground to 40 mesh, and oils and resins were extracted with toluene/ethanol in a Soxhlet extraction apparatus. Cellulose was then isolated from these resin-extracted leaves and wood with an acidified sodium chlorate solution in a method modified after Green (4). All samples were combusted to CO_2 at 800°C in a recirculating microcombustion system. The $^{13}C/^{12}C$ ratios of cellulose and whole tissue of the buds, leaves, and growth ring, and of the sap sugars were determined mass spectrometrically from analysis of the CO_2. The $^{13}C/^{12}C$ ratios are expressed as standard $\delta^{13}C$ values in permil units with respect to the PDB standard (PeeDee belemnite carbonate) first defined by Craig (2).

RESULTS AND DISCUSSION

The sugar concentrations are shown in Figure 1A. These represent the mass ratio of the crystalline precipitate remaining after hotplate heating (70°C) and vacuum drying (30°C), to the original mass of the sap which was evaporated. Replicate analysis of five different sap samples gave a mean concentration difference of 0.10% and SD of 0.13%. Overall, there is no distinct trend, and the range of these concentrations is not unlike the day to day variability found by Taylor (12) in a study of Vermont *A. saccharum*. The sugar $\delta^{13}C$ results in Figure 1B show a tight range of only −24.3 to −23.7‰. Given the precision of about 0.1‰ for preparation and analysis, however, a clear $\delta^{13}C$ trend does not emerge. Furthermore, no systematic differences appear among the different taps collected during the same time period. Although microbial action might have occurred on the sap sitting out in the collection containers, the sap collected April 14 which had accumulated over 2.5 weeks had virtually the identical sugar isotopic composition (−24.08‰) as an instantaneous sample collected in 0.5 h on the same day (−24.14).

Figure 2 compares the $\delta^{13}C$ values of sap sugar to $\delta^{13}C$ values of whole tissue and cellulose of foliage of the following growing season (1983). These results were also statistically compared

[1] Supported under Subcontract No. 19X-22290C from Oak Ridge National Laboratory under Martin-Marietta Energy Systems, Inc., Contract DE-AC05-84OR21400 with the United States Department of Energy.

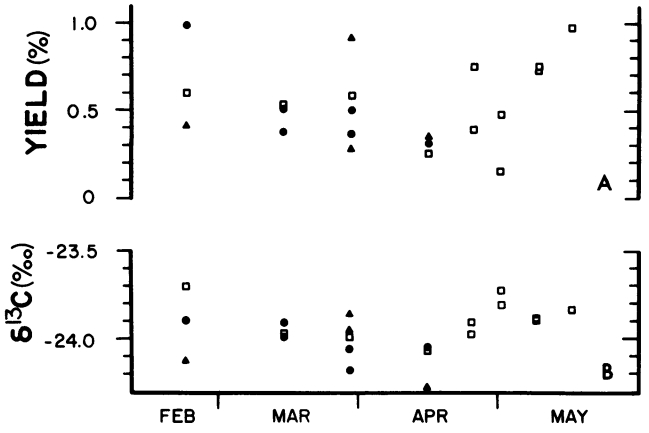

FIG. 1. The weight per cent yield (A) and the $\delta^{13}C$ values (B) of the precipitate (sucrose) obtained from maple sap collected during the winter-spring 1983 dormant period. The different symbols represent taps on different sides of two of the three large trunks of this tree. After April 14, only a single tap was maintained on one trunk. Duplicate symbols on several days represent small separate storage bottles into which large sap collections had been poured, so that the large yield differences but similar isotopic values suggest a density stratification effect in the large collection container.

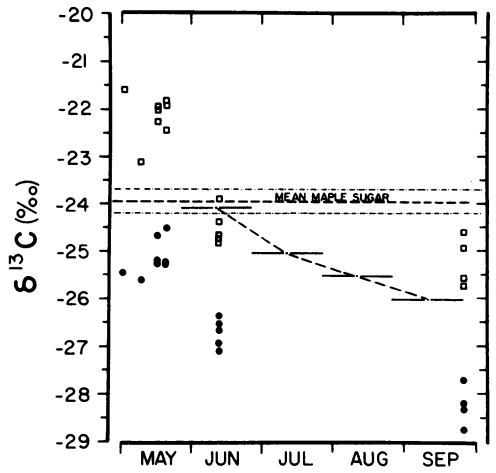

FIG. 2. The $\delta^{13}C$ values of cellulose (□) and whole tissue (●) of buds (May values) and leaves (collected on June 12 and Sept. 25, 1983). The dashed curve connects $\delta^{13}C$ values of cellulose from four equal subdivisions of the 1983 growth ring, which are positioned under assumptions that ring growth began when the buds broke and that the ring grew at a uniform rate. The mean sap sugar $\delta^{13}C$ (±2s) from the collections in Figure 1 has been plotted for comparison.

using *t* tests, with results included in the discussion where relevant, as the probability (P) of the observed difference occurring by chance (two-tailed test). The data in May represent analyses of the green tissue beneath the scales of the unbroken buds (sampled from same sides of trunk as tap locations, several buds pooled per side). By May 15, the buds had not broken, but had elongated to 2× to 3× their original size of May 1 and 8, indicating assimilation of carbon into new cellulose had begun. The isotopic composition of cellulose of the developing leaves within the buds is clearly much heavier than the sucrose ($P < 0.001$). If the source of carbon in this newly formed bud cellulose is glucose derived from the sucrose, it must involve a complex biochemical pathway resulting in fractionation. The simple enzymic breakdown of sucrose to glucose followed by incorporation of glucose into cellulose would probably not fractionate carbon due to the large masses of these compounds and their intermediaries. The June 12 leaves represent five branches on various sides of the three trunks and at heights ranging from 1 to 5 m with 3 to 20 leaves (average length, 4–6 cm) from each of these branches pooled prior to analysis. This pooling of buds and leaves from each branch was a useful precaution because we found a difference of ~1.5‰ between cellulose of two leaves from the same branch (−26.2 *versus* −24.6‰). Pooling as few as two buds and three leaves per branch with such potential variability may contribute to some of the scatter, but sufficient branches are represented to provide a clear picture of isotopic distinctions. The isotopic composition of whole tissue and cellulose of these leaves are distinctly lighter than the respective values from the bud tissue ($P < 0.001$ for both), and both are significantly lighter than the dormant-period sap sugars ($P < 0.05$ for leaf cellulose; $P < 0.001$ for whole tissue).

Whole tissue and cellulose of leaves collected on September 25 show a substantial reduction in $\delta^{13}C$ values. For the whole tissue, this reduction from the June 12 values is statistically significant ($P < 0.001$), although for leaf cellulose the drop was not quite significant ($0.05 < P < 0.10$). These September 25 leaves were sampled from four branches on various sides of two trunks at heights ranging from 1.5 to 6 m. The branches were randomly chosen and not necessarily the same of those of June 12. Most branches had actually developed three pairs of leaves over the growing season, and in a few cases a fourth was in the early stages of development. Leaves from each branch were separated into pooled groups (2–13 leaves) of oldest pairs (average length, ~7 cm), second leaf pairs (~7 cm), third leaf pairs (~5 cm), and the fourth and youngest leaf pairs (~3 cm, and only on one branch), then analyzed separately. The various points for the September 25 leaves in Figure 2 represent the means of all leaf age-groups from each branch. The data revealed no relation of $\delta^{13}C$ and branch height for either the June 12 or September 25 leaves. Furthermore, although the September 25 leaves had first been analyzed in groups according to age on the branches (earliest pair, next oldest pair, etc.), the data do not evidence a trend of $\delta^{13}C$ with leaf age. A core from the base of one trunk, however, did show a clear seasonal trend of decreasing $\delta^{13}C$ in the 1983 growth ring (Fig. 2), with a similar slope to the whole leaf seasonal change but a steeper decline than in the leaf cellulose on average between June 12 and September 25. In light of the fact that this was a single core and that Leavitt and Long (8) have recently demonstrated that there may be typically about 1‰ variation within the same ring along different radii, there is a possibility that the stable-carbon isotopic composition of the earliest segment of the 1983 growth ring may not be quite as similar to the sap sugar as it appears from a single core.

The earliest leaf pairs of September 25 were actually equivalent in age to the leaf pairs sampled on June 12, yet these leaves had enlarged by 40% as of September 25 (average length 7 cm). These oldest leaf pairs of September 25, however, were 1 to 0.5‰ lighter than they were on June 12, supporting continued incorporation of carbon into the leaves over a substantial portion of the growing season. Whereas the cellulose isotopic composition of the earliest ring growth was similar to that of the June 12 leaves, by September 25 the composition of cellulose in all leaves was clearly heavier than that of cellulose in the final wood laid down in the ring. Thus, while the ring may faithfully record the continuum of isotopic changes during the growing season, each leaf apparently contains an integrated, rather than instantaneous, value representing some interval of the seasonal trend.

This study provides essentially a macro view of carbon cycling in a maple tree as evidenced in carbon isotopic compositions of sucrose and plant tissue; it provides virtually no information on micro carbon pathways, *i.e.* partitioning of carbon for assimilation *versus* respiration, distribution of glucose between cellulose

and other plant processes and constituents, etc. However, we may make some inferences from these results. First, the constancy of $\delta^{13}C$ of sap sugars throughout the dormant season suggests that if any sugars are utilized for maintenance metabolism or by microbial activity, or if any sugars are contributed from starches, the carbon isotopic composition of the sap sugars is affected little. Second, the buds (including those expanding) have an isotopic composition distinct from both the sap sugar and the leaves which had developed by June 12. If the carbon in the green tissue of the expanding buds originates from the sap sugar, significant isotopic fractionation occurs along the pathway to incorporation. There is the possibility of some isotopic bias because pure sucrose was not extracted and the small amount of remaining sap solids (≤5%) will include inorganic salts and a variety of organic compounds (5, 12). However, some of these organic compounds may be isotopically heavier and others lighter than the sucrose (Fig. 9-6 in Ref. 3) so that even if they were a net 2 to 3‰ heavier or lighter, this would only bias the sap measurements by a few tenths per mil, *i.e.* extremely unlikely to be the primary cause of the isotopic difference between sap and buds. Third, the cellulose of the first leaves and initial growth of rings is isotopically quite similar to the sap sugar, with a negative deviation from the sap sugar values as the growing season progresses. The starting point of the seasonal trend in the leaves and wood of maple thus seems determined by the isotopic composition of the dormant period sap sugar, accumulated at some interval during the previous season's growth. The deviation from this value may then reflect the photosynthetic contribution from the current season's growth whose carbon isotopic composition may be influenced by environmental variables such as sunlight and temperature. The $\delta^{13}C$ values of the leaves and wood toward the end of the growing season, however, may not be a good indicator of the values of sugars which will be stored over the following dormant period: a single sap sample collected on February 23, 1984, had a sugar $\delta^{13}C$ value (−24.23‰), within the scatter of values about the mean of sap sugar from the 1983 dormant period, and much heavier than the cellulose $\delta^{13}C$ of the late leaves and wood of the 1983 growing season. This last observation may imply that the seasonal isotopic changes in foliage do not simply result from direct environmental changes, but are a consequence of other processes such as shifts in reliance on different carbohydrate pools during the growing season. Alternatively, the seasonal isotopic changes in leaves may yet mirror environmental influences, but the last-leaf *versus* sap isotopic difference may indicate some late-season partitioning of photosynthates between the last leaves and the sugars to be stored in sap.

Acknowledgments—We give special thanks to L. Warneke for helping prepare and analyze plant and sap samples. C. W. Ferguson and T. Harlan helped identify the outer (1983) ring in the core. R. Jensen and F. Telewski provided helpful comments.

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