

# Stachyose: An Early Product of Photosynthesis in Squash Leaves<sup>1</sup>

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## ABSTRACT

It was hypothesized that stachyose is translocated by squash because stachyose is supplied to the phloem loading system by the photosynthetic system. To test this hypothesis, <sup>14</sup>C<sub>2</sub> was supplied to squash leaves. The nonphosphorylated sugars containing <sup>14</sup>C were studied. A large proportion of <sup>14</sup>C appeared in stachyose very early in the time sequence, tending to confirm the hypothesis.

Sucrose is the sugar most commonly transported by plants (see summary by Crafts and Crisp [2]). It is also the first non-phosphorylated sugar produced by photosynthesis by a number of plants (3, 4, 8, 11). This correlation suggests that production by the chloroplast rather than selectivity of the phloem loading system determines which nonreducing sugar is transported.

Squash plants transport <sup>14</sup>C-stachyose when <sup>14</sup>CO<sub>2</sub> is supplied to leaves (6, 10). Recent work in this laboratory (7) demonstrated that squash plants will transport <sup>14</sup>C-sucrose if it is supplied to the leaves, thus indicating that the phloem loading system of squash is not specific for stachyose. Earlier work has demonstrated that the galactose moieties of stachyose become labeled more rapidly than the glucose and fructose moieties when <sup>14</sup>CO<sub>2</sub> is supplied to squash leaves (6). This suggests that stachyose is a product of squash photosynthesis. To further verify this point, we have done a time course study of the early nonphosphorylated products of squash photosynthesis. This study demonstrates that stachyose becomes labeled very early, indicating that it is a likely product of photosynthesis.

## MATERIALS AND METHODS

**Experimental Procedure.** Plants of *Curcubita pepo* L. var. *melo* were grown and prepared for experimentation using the procedures previously described (7).

The labeling apparatus is shown in Figure 1. This device was constructed from Plexiglas, except for the glass leaf cup. Two ml of lactic acid containing thymol blue indicator were placed in the leaf cup (A) along with a magnetic stirring bar. Anhydrous lanolin paste was spread around the lip of the leaf cup to ensure a tight seal between the cup and the leaf. The petiole was then inserted into the slot at (D) and the blade was placed over the hole at (C). The position of leaf placement is indicated by the circle on the leaf (Fig. 1). The top plate was then se-

cured tightly to the lower plate by four bolts, without crushing leaf veins. With the magnetic stirrer operating, a solution of Na<sub>2</sub><sup>14</sup>CO<sub>3</sub> was injected into the leaf cup through the side arm (B) which was covered with a serum vial stopper. The amounts of <sup>14</sup>C were varied, depending on the duration of the experiment.

The labeling period was terminated by cutting the leaf disc which was directly over the leaf cup, with a 2.1 cm diameter cork borer (F) and immediately plunging the tissue into a previously prepared Dry Ice bath containing 200 ml of crushed Dry Ice, 100 ml of 88% formic acid, and 100 ml of cold absolute methanol. The reported labeling time of each experiment was the elapsed time from the injection of the carbonate solution until the leaf disc was plunged into the Dry Ice bath. At the end of a 10-min freezing period, the tissue was transferred to a closed grinding cylinder containing 30 ml of the 1:1 (v/v) solution of 88% formic acid and absolute methanol. The tissue was pulverized for 5 min at approximately 1600 rpm in a Model 1677 Sorvall Omni-mixer. This procedure stops enzymatic activity, including phosphatase activity (1). Each sample was centrifuged at 5 C for 20 min at 27,000g. The supernatant was transferred to a 250-ml filtering flask and 40 ml of distilled water were added to prevent a highly concentrated solution of formic acid from forming while the sample was being dried in a VirTis lyophilizer. Each dried sample was rinsed from the flask alternately with 95% ethanol and water until a 5-ml mixture of 60% ethanol was obtained. Samples were stored at approximately -20 C.

**Sample Analysis.** Initially, one-dimensional paper chromatograms were run to determine the maximum possible running time for the two-dimensional chromatograms without loss of <sup>14</sup>C from the end. Aliquots of each sample were chromatographed using two-dimensional chromatography, solvent I being 1-butanol-ethanol-water (5:3:2 v/v/v) and solvent II being 1-butanol-acetic acid-water (1:1:1 v/v/v). All chromatograms were autoradiographed using Kodak blue brand x-ray film. Radioactive spots from chromatograms were cut out and counted in a Mark I Nuclear Chicago Model 70003 scintillation counter.

Preliminary identification of sugars was obtained by comparing migration of standards with the unknowns. Final identification was obtained by co-chromatography with standards of labeled materials from a duplicate set of chromatograms.

Certain compounds were identified as phosphorylated compounds by treating an aliquot of the sample with acid phosphatase before chromatography. Those spots appearing on the chromatograms from the untreated samples but not appearing on these chromatograms from acid phosphatase-treated samples are assumed to be phosphorylated compounds. Since the primary thrust of this work was to study compounds that are known to be transported through the phloem, these phosphorylated compounds were not studied further.

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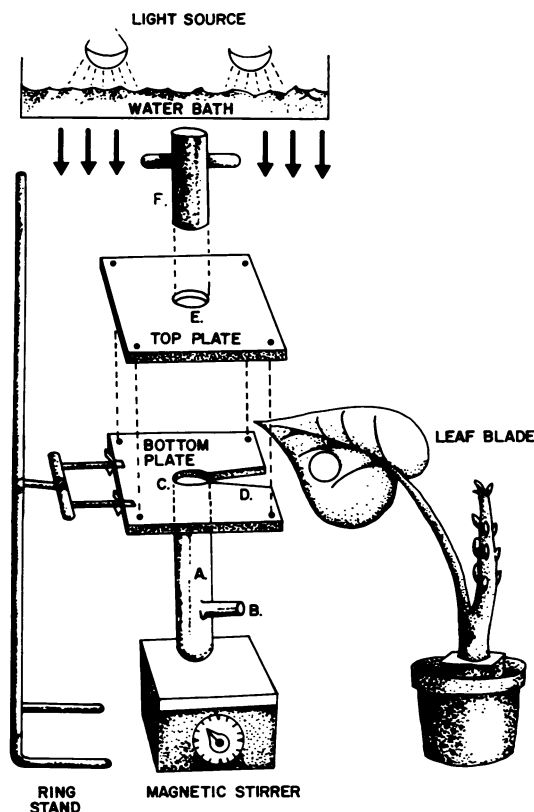


FIG. 1. Labeling apparatus—see text.

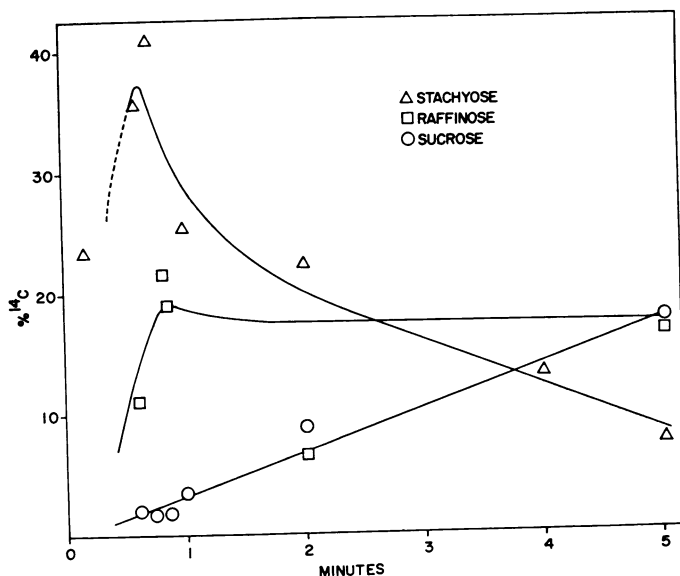


FIG. 2. Proportion of <sup>14</sup>C in various sugars from squash leaves labeled with <sup>14</sup>CO<sub>2</sub> for times indicated.

## RESULTS AND DISCUSSION

All leaves produced qualitatively similar distribution patterns of <sup>14</sup>C compounds. The number of individual compounds on each chromatogram varied as the labeling time increased from 37 sec to 5 min. Each compound, including the origin for each chromatogram was counted. Results from the acid phosphatase treatment indicated the presence of several phosphorylated compounds. This treatment eliminated all activity

from the origin, indicating that the radioactivity at the origin represented some of the phosphorylated compounds.

Percentage of the total activity contained in stachyose, raffinose, and sucrose for each labeling time is shown in Figure 2. The most important result appears to be the increase in percentage of label in stachyose as the time was decreased from 5 min to 37 sec. At 37 sec stachyose contained more than three times the activity of any other sugar represented. Between 37 sec and 2 min the percentage of label in stachyose dropped rapidly and then continued to drop more slowly, reaching 9% of the total label by 5 min. At 37 sec raffinose contained 11% of the total label, increasing to 20% at 1 min. The <sup>14</sup>C in sucrose rose steadily from slightly less than 2% at 37 sec to slightly more than 17% at 5 min. Labeled hexoses were not detected even at 5 min. It is noted that this pattern is quite different from that reported by Kandler for *Catalpa* (8). These differences may be due to the fact that these plants are quite unrelated.

A preliminary 10-sec labeling experiment was done. The results were similar to those for 44 sec, except that 12.8% of the <sup>14</sup>C was in sucrose. In attempts to duplicate these results in subsequent experiments of 11, 14, and 16 sec. no free sugars were found to contain <sup>14</sup>C. In constructing the curves (Fig. 2), we have disregarded the data from the original 10-sec experiment and feel justified in extrapolating the stachyose curve toward 0 between 10 and 20 sec.

These data indicate that stachyose becomes labeled very early, followed by raffinose and then sucrose. To get such a large proportion of the <sup>14</sup>C into stachyose so soon, it would seem that the stachyose is synthesized by the photosynthetic system or by a system that is closely coupled to it. This suggestion is further supported by the fact that the galactose moiety of stachyose becomes labeled much more rapidly than do the glucose and fructose moieties (6, 9).

Raffinose and sucrose may also be synthesized by the squash photosynthetic system; however, it seems more likely that they are derived from stachyose. The <sup>14</sup>C in raffinose rises rapidly, then reaches a dynamic equilibrium between degradation to sucrose and synthesis from stachyose; stachyose being both exported and degraded. This hypothesis is supported by the demonstration that, at any one time, the specific radioactivity of all hexose moieties in stachyose in squash leaves fed with <sup>14</sup>CO<sub>2</sub> was higher than those same hexose moieties in raffinose (5). Further support is provided by the fact that the curves in Figure 2 agree remarkably well with Zelitch's, "Hypothetical distribution of percent of <sup>14</sup>C fixed with time in three successive compounds arising in sequence" (11, Fig. 4.1). The differences between our data and the model presented by Zelitch are that the compounds of interest here are further down the biochemical sequence; therefore the time frame is different, and because of the longer time frame, some materials were translocated out of the sample tissue in this study.

From these and data previously presented (7), it appears reasonable to propose that the chemical nature of the material transported by squash plants is controlled not by the phloem loading system but rather by the system supplying nonreducing sugars to that loading system be it the endogenous synthetic system of the leaf or an exogenous supply.

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