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Alteration of ^{14}C-Assimilate Partitioning in Leaves of Soybeans Having Increased Reproductive Loads at One Node[1]

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ABSTRACT

The objectives of this study were to determine if the partitioning of recently fixed carbon between starch and water-soluble compounds could be altered by increasing the pod load in the leaf axil, and if the presence of source leaves acropetal to such a node would influence the partitioning of carbon within the subtending leaf. Soybeans (*Glycine max* L. Merr. cv Hodgson 78) were grown to full-bloom in a controlled environment chamber, and then deflowered at all nodes except the eighth. This treatment resulted in an 83% increase in the number of pods at the eighth node. At 24 days after flowering, one-half of the treated plants were girdled above the untreated node. Forty-two hours later, the eighth trifoliolate was pulsed with $^{14}CO_2$ and sampled for radiolabeled starch and water-soluble compounds (WSC) at 0.5, 2, 4, 8, 12, and 24th after labeling.

When no girdling was applied above the increased pod load at the eighth node more label was accumulated by the pod walls (+6.9%) and seeds (+6.3%) when compared to the controls. Starch accumulation was not altered in the labeled leaf of the nongirdled plants. When the stem was girdled above the eighth node, significantly less starch was retained in the labeled leaf. Girdling also resulted in an increase in label accumulation by the pod walls (+5.4%) and seeds (+6.6%). These data suggest that the plant will change the distribution patterns of assimilate to supply added sink demand before altering the partitioning of recently fixed carbon in the subtending leaf.

The accumulation of TNC[2] in leaves of reproductive soybeans increases during reproductive growth. In field-grown soybeans, senescent leaf blades that were ready to abscise were found to contain from 6 to 9% TNC or starch (4, 18). Experiments designed to decrease the source/sink ratio in reproductive soybeans have resulted in decreased starch or TNC contents of the remaining or untreated leaves (2, 12, 19), stems (5, 13, 18), and pod walls (18). Although such evidence suggests that there was photosynthate available which was not converted into yield, there is disagreement as to the significance of this reserve carbohydrate (4, 18).

Foliar starch accumulation unrelated to environmental stress is influenced by daylength (3), leaf age (8), and sink demand (2, 5, 14, 18). As reproductive development progresses, the effect of daylength decreases and that of sink demand increases (1). Starch accumulated during the early to mid pod-filling stages can be mobilized during the later stages of seed-fill (8), but the extent of this contribution in the field is unknown. Starch accumulation occurring during early to mid pod-fill may result from a decrease in starch degradation or from a change in carbon partitioning, rather than from enhanced activities of the starch synthesizing enzymes. A positive correlation has been shown between SPS activity and sink demand (16). Increased SPS activity resulted in a greater labeling of sucrose in isolated cells and a reduced accumulation of starch in leaves (10, 16). Increased SPS activity has also been associated with increased translocation rates (16). Changes in the distribution patterns of ^{14}C-photoassimilates within the plant without concurrent changes in CER have been observed following source/sink manipulations (6, 7). Such evidence suggests that the biochemical partitioning of photosynthate can be altered in favor of sucrose during reproductive soybean growth.

Partitioning of recently fixed carbon in leaves on the middle and lower part of a soybean canopy may be influenced by contributions of assimilate from the more photosynthetically active leaves on the upper part of the canopy. Stephenson and Wilson (17) concluded that pods at a given node receive most of their assimilate from the subtending leaf with a minor contribution from the leaves located two nodes above and two nodes below. More recently, Fellows *et al.* (6) have suggested that secondary sources can satisfy primary sink demand if this demand is not met by the principal source for that node. They indicated that the secondary source is defined by the vascular connections present. Using Y-shaped soybeans (two main branches), resulting from removing the apex, Gent (7) has shown that at least 16% and perhaps as much as 45% of the photosynthate used to support seed growth on a previously deleafed branch was transferred from a previously depodded branch. In field-grown soybeans, most photosynthesis occurs in the upper 20% of the canopy, containing one-third of the LAI and intercepting 90% of incident PAR (9). Hence, long distance translocation is commonplace, and may overcome effects of modulations in sink demand at a given node on carbon partitioning in the subtending leaf.

The objectives of this study were to determine: (a) whether partitioning of recently fixed carbon between starch and WSC could be altered by increasing the pod load at one node and (b) if the presence of source leaves acropetal to such a node would influence the partitioning of carbon in the subtending leaf.

MATERIALS AND METHODS

Plant Culture. Soybeans (*Glycine max* L. Merr. cv Hodgson 78) were grown in plant growth chambers (Conviron model PGW-36) with a 14-h photoperiod, and day/night temperatures

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[2] Abbreviations: TNC, total nonstructural carbohydrates; WSC, water-soluble compounds; CER, carbon exchange rate; LAI, leaf area index; SPS, sucrose phosphate synthetase.

of 25/21°C. A combination of incandescent and fluorescent lamps provided a photosynthetic photon flux density of 500 μE m^{-2} s^{-1}. The rooting medium was a 3:2:1 mixture of soil:sand:zorb-all (IMC Chemicals, Des Plaines, IL) in 12.5- × 12.5-cm plastic pots. At the time of planting, seeds were inoculated with *Rhizobium japonicum* (USDA strain 140). Plants were thinned to one plant per pot at the unifoliolate leaf stage. Branches were removed from plants as soon as they began to form to ensure uniform sampling material. Sufficient one-fifth strength Hoagland solution was provided hourly during the daylight hours to provide leaching of excess nutrients. Completely randomized designs were used in these studies with five replicates per treatment.

Treatments. Previous work in our laboratory showed that it was possible to increase the number of pods at a given node by removing all the flowers on the plant but at that node (unpublished data). In this study, flower buds and recently opened flowers were removed as they appeared on all nodes but the 8th. Control plants were left intact. The 8th node flowered on all plants from 38 to 42 d after planting, and 24 d later when the 8th node was at the seed-filling stage, girdling treatments were applied to the internode above the 8th node. The girdling procedure consisted of killing the living tissue in a 1-cm segment of the stem with a 3-min exposure to a jet of steam. This treatment had previously been shown to effectively inhibit photosynthate translocation (15).

Carbon Partitioning and Translocation. At 66 d after planting (42 h after girdling), the leaf of the 8th node on all treated and control plants was pulse-labeled with $^{14}CO_2$. At 0900, 1 h after the photoperiod began, the 8th trifoliolate leaf from the base of the plant was sealed in a 27- × 28-cm clear plastic bag (Ziploc, Dow Chemical). Attached beneath this bag and opening into it was a 20-ml poyethylene scintillation vial preloaded with 4 ml 2 N lactic acid. The area around the petiole was sealed with florist's adhesive, and 1 ml 0.1 N NaOH containing 3.7×10^5 Bq $NaH^{14}CO_3$ (Research Products Int. Corp.; 2.1×10^9 Bq $mmol^{-1}$) was injected into the scintillation vial via a serum stopper. After 15 min, 4 ml of 2 N KOH was injected into the vial. Another 15 min was then allowed for the leaf to assimilate the remaining $^{14}CO_2$ within the bag. After pulsing, the bag was removed and one leaf disc (2.3 cm^2) was removed from each leaflet of the trifoliolate at 0.5, 2, 4, 8, 12, and 24 h after labeling. The samples were taken sequentially from opposite sides of the midrib and basipetal to the previous sample. The leaf discs were immediately frozen in liquid N_2, transported to the laboratory, and freeze-dried. The removal of three leaf discs at each sampling time represented 2.4% of the total area of the labeled trifoliolate.

Extraction and Analysis Procedure. The three freeze dried leaf discs from each sampling time were placed in 50-ml polyethylene centrifuge tubes. The tissue was then homogenized (Brinkman Polytron, model PT 10 20 350D with PTV-2 generator) in 10 ml distilled H_2O for 30 s at 9000 rpm. The homogenate was centrifuged (IEC, model K) in a horizontal rotor for 15 min at 1650 *g* and the supernatant decanted into 25-ml volumetric flasks. The residue was resuspended in 10 ml of distilled H_2O and recentrifuged another 10 min at 1650*g*. The supernatant was decanted, combined with the original extract, and brought up to 25 ml with distilled H_2O. Five ml aliquots were removed and combined with 15 ml of Aquisol-2 (New England Nuclear) and subjected to liquid scintillation spectroscopy. This fraction was designated ^{14}C-labeled WSC.

Each pellet remaining from the second WSC centrifugation was combined with 6 ml distilled H_2O and transferred to a 25-ml Erlenmeyer flask. After adding 1.5 ml of ethanol, the mixture was boiled for 1 to 2 min, and then allowed to cool to room temperature. To this mixture was added 3 ml of a sodium acetate buffer solution (pH 4.45), and 3 ml of a 0.5% α-amylase solution (Clarase 40,000; Miles Laboratories, Inc.). The mixture was then incubated at 38°C for 44 h to allow complete enzymic degradation of the starches. After incubation, the extracts were filtered through Whatman No. 1 filter paper into volumetric flasks and brought up to 25 ml with distilled H_2O. Five-ml aliquots were removed and combined with 15 ml of Aquisol-2 and subjected to liquid scintillation spectroscopy. This fraction was designated ^{14}C-labeled starch.

The filter paper used in the starch filtration was dried and pelletized. The filter paper plus residue was then combusted in a tissue oxidizer and the $^{14}CO_2$ released was absorbed in 9 ml of Carbosorb II and 13 ml of Permafluor V. This fraction was counted directly and designated ^{14}C-residual material.

At 24 h, the subtending pods of the labeled leaf were harvested for the measurement of the numbers of pods and seeds, dry weights, and accumulation of label in the pod wall and seed fractions. These separate fractions were then dried and ground in a cyclone sample mill (Udy Analyzer Co., Boulder, CO). Subsamples were removed and weighed. These subsamples were combusted using a tissue oxidizer (Packard model 306) and the released $^{14}CO_2$ was absorbed in 6 ml of Permafluor V (Packard Instrument Co.) and counted on a liquid scintillation spectrometer (Beckman, model LS-8000).

Total radioactivity (Bqs) present in the labeled leaf at 0.5 h was calculated as:

$$\frac{(^{14}\text{C-WSC} + {}^{14}\text{C-Starch} + {}^{14}\text{C-residual})}{\text{Area of three leaf discs}} \times \text{Area of the labeled leaf}$$

Radioactivity accumulated in the pod walls and seeds in the axil of the labeled leaf was expressed in terms of the total amount translocated, and as a percentage of the total radioactivity present at 0.5h. The retention and partitioning of label as starch, WSC, and residual material within the leaf was expressed as a percentage of the total activity found in the sum of these three fractions at 0.5 h. The starch/WSC ratio was calculated by dividing the amount of label incorporated into starch by that incorporated into WSC and then averaging these ratios for the six intervals after labeling. The effect of girdling on the accumulation of label by the pod walls and seeds was calculated by subtracting the mean of the nongirdled plants from that of the girdled plants, and comparing this number with the control mean.

Statistical Analysis. Significant differences between treatment means were detected using Duncan's multiple range test ($P \leq 0.05$). Comparisons of the profiles of ^{14}C-labeled starch, WSC, and residual between treatments were accomplished using the same multiple range test at each interval after labeling.

RESULTS

Removing all the flowers on the soybean plant except those at the 8th node resulted in almost a 2-fold increase in the reproductive structures at that node. The numbers of pods and seeds and their corresponding dry weights are given in Table I. With flower removal, total number of pods was increased 83%, while number of seeds was increased 87%. Pod wall dry weight increased 117%, and seed dry weight increased 80%. Girdling above the 8th node had no effect on the measured parameters.

The size of the individual reproductive organs was not decreased as their numbers increased. Weights per pod were 134, 155, 164 mg, and weights per seed were 52, 53, and 55 mg for the control, nongirdled, and girdled treatments, respectively.

No significant differences were found for the leaf area of the trifoliolate at the 8th node, or in the initial radioactivity found in the leaf at 0.5 h (data not shown).

In the girdled plants, significantly less starch was accumulated and retained in the leaf (Fig. 1). The ratio of starch to WSC was

Table I. *Effect of Flower Removal (at all but Node 8) and Stem Girdling (Just Above Node 8) on Pod and Seed Numbers and Weight at Node 8*

Plants were sampled 66 d after planting.

Treatment	Pod no.	Total pod Dry wt	Seed no.	Total seed Dry wt
		g		*g*
Control	7.2 a[a]	0.89 b	11.0 b	0.56 b
Flower removal without girdle	13.2 a	1.93 a	20.6 a	1.01 a
Flower removal with girdle	14.0 a	2.11 a	22.3 a	1.15 a

[a] Within each parameter, means with same letter are not significantly different at the 5% level according to Duncan's multiple range test.

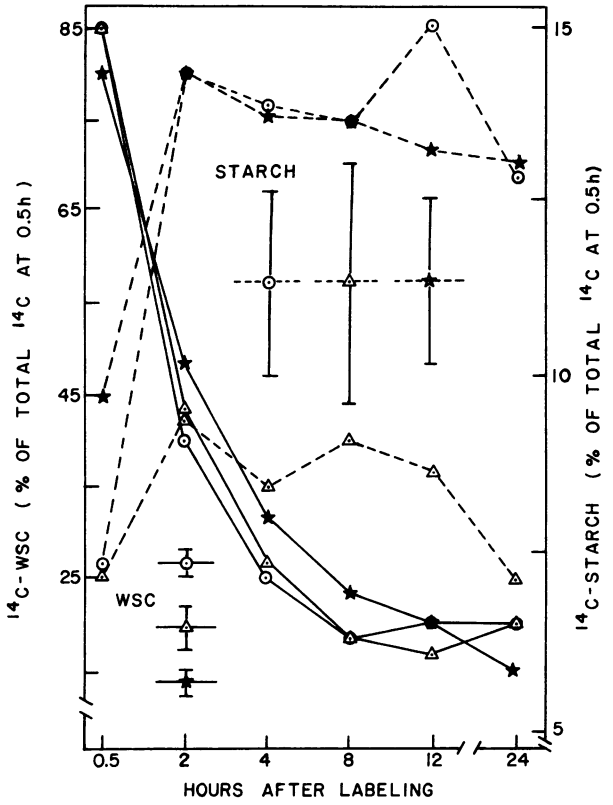

FIG. 1. Effect of flower removal (at all but node 8) and stem girdling (just above node 8) on the labeled WSC and starch content of the subtending leaf at node 8. Each data point represents the mean of five replicates ± the SE. Control (⊙); deflowered (★); deflowered and girdled (△). Treatments applied 64 d after planting.

0.55, 0.47, and 0.34 for the control, nongirdled, and girdled treatments, respectively. This represented a 15% decrease for the nongirdled treatment, and a 38% decrease for the girdled plants. The decrease in this ratio for the nongirdled plants was due to a slightly higher amount of WSC present 2 to 8 h after labeling. The 38% decrease in the starch/WSC ratio for the girdled plants was a direct result of decreased starch accumulation, as the WSC profile for this treatment was almost identical to that of the control.

Eliminating the acropetal source of assimilate supply resulted in less residual accumulation in the leaf supporting the increased pod load (data not shown). The amount of label observed in the residual fraction was not significantly different for the control and nongirdled plants, except at 0.5 h. On the average, the labeled leaf on those plants treated with a girdle retained 2 and 4% less residual material than the control and nongirdled plants, respectively.

Table II. *Effect of Flower Removal (at all but Node 8) and Stem Girdling (Just Above Node 8) on Radioactivity in Pod Walls and Seeds at Node 8*

Sampled 24 h after labeling the subtending leaf with $^{14}CO_2$. Plants were sampled 66 d after planting.

Treatment	Total Activity		% of Total Present in Labeled Leaf at 0.5 h	
	Pod wall	Seed	Pod walls	Seed
	Bq (× 10^5)			
Control	1.14 b[a]	1.78 c	1.6 b	2.6 c
Flower removal without girdle	6.61 ab	6.76 b	8.5 a	8.9 b
Flower removal with girdle	10.98 a	12.32 a	13.9 a	15.5 a

[a] Within each parameter, means with same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Accumulation of ^{14}C-assimilate by the pod walls and seeds was greatly increased when the pod load was doubled at that node. The effect of increasing the pod load alone was indicated by a 480% increase in the total Bqs accumulated by the pod wall, and a 280% increase in the seed (Table II). When expressed as a per cent of total radioactivity present at 0.5 h, these increases were at least 6.9% and 6.3% for the pod wall and seed fractions, respectively. The added effect of girdling plants with an increased pod load was shown by a 383% increase in the total Bqs accumulated in the pod wall, and a 312% increase in the seed (Table II). As a per cent of total radioactivity, the additional label accumulation was 5.4% and 6.6% for the pod walls and seeds, respectively.

DISCUSSION

The result of increasing the sink load at node 8 was a greater accumulation of label in the pods at that node. In both flower removal treatments, doubling the seed dry weight at node 8 resulted in a 3-fold increase in the amount of radioactivity accumulated in those seeds (as a per cent of the total ^{14}C present in the labeled leaf at 0.5 h). Similar increases in label accumulation occurred in the pod walls (Table II). This 3-fold increase in label accumulation was probably due to the fact that these plants were supporting no other reproductive sink but at the 8th node. In the nongirdled plants, the increase in the accumulation of radioactivity by the pods in the axil of the labeled leaf occurred without a change in starch accumulation (Fig. 1). The similarity of the starch profiles from the control and nongirdled plants and the fact that there was an unproportionate accumulation of label in the pods of the latter suggests that some of the label from the pulsed leaf in the control plants went to pods at nodes other than the 8th. This observation is consistent with the results of the study by Stephenson and Wilson (17), and may indicate that such contributions are more important than previously thought.

Elimination of acropetal sources by girdling resulted in an additional accumulation of radioactivity by pods in the axil of the labeled leaf almost equal to that gained by increasing the pod load alone (Table II). Since the number of pods and seed weights were increased by a similar proportion in the girdled and nongirdled plants, sink demand would presumably be the same in each case (Table I). However, with the girdle, only the trifoliolate leaf at node 8 and those leaves below it could supply the increased sink demand. The lower leaves in both situations were undoubtedly supplying the roots with assimilate as well (11, 17, 20), but this contribution has been shown to decrease as reproductive

development advances (11, 16). Comparing flower removal treatments, the fact that significantly more label accumulated in the pod walls and seeds of the girdled plants implies that acropetal sources supplied the assimilates necessary to meet the added sink demand in the nongirdled plants.

Starch accumulation within the leaf was decreased as a result of girdling the internode above the increased pod load. Apparently, a similar change in starch/WSC partitioning was not necessary in the nongirdled plants due to the aforementioned contribution of nonlabeled assimilate from source leaves above the increased pod load. In cases of added sink demand, it appears that the plant will change the distribution patterns of assimilate to supply that demand before altering the partitioning of recently fixed carbon in the leaf. The observations made by Fellows *et al.* (6) and Gent (7) are consistent with such a hypothesis. Their studies also showed that changes in the distribution patterns of assimilate occurred without concurrent changes in source leaf photosynthetic rate, or in the rate of export from those leaves (6, 7). Based on the results of our study, it appears that photosynthetic starch/WSC partitioning will also remain unaltered in cases where changes in the distribution patterns of assimilate can supply added sink demand.

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