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Rapid Changes in Translocation Patterns in Soybeans following Source-Sink Alterations[1, 2]

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ABSTRACT

The effects of source-sink alterations on the translocation patterns to soybean ("Fiskeby V") pods were studied using a pod leakage technique. The distribution of assimilates from a source leaf using double pulse experiments was followed at the pods at the source node and the node immediately below. Alterations were made by shading, illuminating, or excising two-thirds of the leaf area of the leaf at the node below. In control experiments both pulses exhibited identical time-course patterns at both nodes. Shading the lower leaf during the first half of the experiment and illuminating during the second reduced the distribution of ^{14}C-assimilate to the lower node's pods from the source leaf by approximately 30 to 50% while having no effect at the source node. Illuminating the lower leaf during the first half of the experiment followed by excision of two-thirds of that leaf's area and shading increased the import from the source leaf by 4- to 33-fold relative to the control while reducing the distribution to the source node by up to 40%. The change in distribution pattern took place in less than 30 minutes with no apparent change in the source leaf net photosynthesis or in the rate of movement to the pods. The results indicate that any alterations in the source-sink balance will quickly produce a change in the distribution patterns to the pods.

The effects of source-sink alterations upon translocation patterns within the plant have been the objects of several studies (*cf.* reviews 18 and 20). These alterations have usually been induced through shading (16), or defoliation (17). These were comparatively long term studies however (>2 h), and any rapid changes, indicators of how responsive the system might be, were overlooked.

Recent reports with wheat (*Triticum aestivum* L.) (19), and bean (*Phaseolus vulgaris*) (9, 15) have demonstrated that rapid changes are evident with sink excision or defoliation. Wardlaw and Moncur (19) measured changes in wheat in the amount of assimilate distribution to the grain from the flag leaf within 10 min after one-third to one-half of the grains were removed from the ear. Geiger (9) reported alterations in distribution patterns to a young sink leaf of bean within 30 to 40 min following the excision of the leaves between the source leaf and the sink. Using steady-state labeling techniques, Swanson *et al.* (15) observed alterations in the distribution pattern of assimilate to a developing trifoliolate of bean within 9 min after the primary source had been excised. Pickard *et al.* (14) have also reported rapid changes (<5 min) in the movement of ^{11}C-assimilate along the stems of moonflower (*Ipomoea alba* L.) following various source and sink treatments. These experiments, while yielding the highest resolution to date, have also required the most complex instrumentation.

Direct measurements of ^{14}C-assimilate translocation into the pods of soybean (*Glycine max* [L.]Merr.) are difficult due to the self-absorbancy of the pod. Through the use of a pod-leaking technique (4) a simple, inexpensive measure of the material that is being translocated to the pod can be obtained and a time-course of the translocate followed. Results of the following study indicate that alterations in translocation patterns to the pods can occur rapidly with changes in the source-sink balance.

MATERIALS AND METHODS

Soybean plants (*G. max* [L.] Merr., Fiskeby V) were grown in the greenhouse on solution culture with a modified Hoagland solution. Experimental plants were 50 to 60 days old (10–12 nodes) and at approximately mid-pod-fill. The primary source leaf, used for photosynthetic measurements and $^{14}CO_2$ assimilation, was routinely located at the sixth or seventh node. Translocated assimilate in the pod leakage was collected from two pods at both the source leaf node (source node) and at the node immediately below (node below). If present, any extra pods at these nodes were removed at least 24 h prior to the experiment.

The $^{14}CO_2$-labeling procedures were as described previously (4) except that each experiment was 500 min in length with two 10-min pulses (30 μCi each) at time 0 and 250 min followed by 240-min chase periods. At least 1 h prior to the first pulse the stylar tips of the pods at both the source node and the node below were cut off under a solution of 20 mM EDTA, disodium salt (pH 7.0) (13), and maintained under 2 ml of solution in 5-ml plastic beakers attached to the plant with caulk cord (4). For time-course experiments the solutions were changed at 15-min intervals starting at time 0. Each sample was placed directly into a scintillation vial with 15 ml of cocktail (3a80b, R.P.I. Industries) and counted.

Three types of experiments were conducted. For a control, the pulses were given with no alterations in the quantity of light on the source node leaf and the leaf at the node below (650 and 350 $\mu E\ m^{-2}\ s^{-1}$, respectively). In the second type of experiment there was a constant light intensity on the source leaf while the leaf on the node below was shaded (<20 $\mu E\ m^{-2}\ s^{-1}$) for 24 h prior to and during the first half of the experiment (250 min). The shaded leaf was subsequently illuminated (500 $\mu E\ m^{-2}\ s^{-1}$) during the second half of the experiment. In the third type of experiment there was again a constant light on the source leaf, while the leaf immediately below was given supplemental light (500 $\mu E\ m^{-2}\ s^{-1}$) during the first half of the experiment followed by excision of the two lateral leaflets and shading of the remaining leaflet for the remainder of the experiment. All experiments were conducted at least in triplicate and the results described here (Figs. 1–3) are typical examples.

RESULTS AND DISCUSSION

The distribution patterns of ^{14}C for the experimental plants were reported previously (4). Briefly, the principal sinks for the

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source leaf were the pods at the source node and at the node immediately below (approximately 50 and 20% respectively of the total ^{14}C translocated out of the source leaf). The leakage of ^{14}C-assimilate from both of the pods at the source node and node immediately below was followed. Both pods at each node gave essentially identical results; for the sake of clarity the results from only one of the pods at each node are plotted in Figures 1 to 3.

As shown in Figure 1 (control experiment), the leakage curves obtained from the two pulses given at time 0 and 250 min are quite similar in shape both at the source node and node below and resemble those reported by Fisher (7) in soybean petioles and stems. The small oscillations may be due to the sampling technique, changes in the profile caused by the nodes (spreading of the curves) (5), or perturbation of the steady-state photosynthetic rate of the source leaf during the labeling period (11). These perturbations are not considered to be serious artifacts, and are reduced in amplitude at the node below (Figs. 1–3).

While some spreading, or flattening, of the profiles is evident between the two nodes (Fig. 1), this is not unexpected since some lateral distribution is bound to occur (1). The areas under the curves (dpm values/recorded pulse) are also considered to be representative of the total label arriving in each pod. It was observed previously that 1.5 to 4.0% of the total ^{14}C translocated to the pods at mid- and late-pod-fill was routinely found in the leakage (4).

In the control experiments the amount of label recovered from the second pulse was usually greater than that of the first at both nodes (Fig. 1 and Table I). Equal amounts of $^{14}CO_2$ were generated for each pulse and the net photosynthetic rates during these pulses differed by less than 1.0 ± 0.5 mg CO_2 dm^{-2} h^{-1}. The increase in the second pulse is apparently due to the tail, or remaining assimilate from the first pulse being loaded into the translocation stream along with the more recently assimilated second pulse. This delayed loading from various pools can be substantial and continue for an extended period of time (2).

The observed velocities of translocation (0.24 and 0.28 cm/min, respectively) to the source node and node below for the experiment in Figure 1 were somewhat low (6). These values represent only a rough estimate because of use of a 15-min sampling time. While such measurements of velocities may indicate trends, they cannot be considered as exact figures. In all of the control experiments there were no significant differences in the observed velocities of translocation to the two nodes.

Alterations of the source-sink balance between the two nodes were accomplished by selective illumination, shading, or excision of the leaf at the node below the source. Changes induced by these alterations were then reflected in the leakage component.

When the leaf at the lower node was shaded for 24 h prior to and during the first pulse, and then illuminated during the second pulse, there was a significant decrease in the amount of label

Table I. *Ratio of dpm Recovered from Second Pulse to Those Recovered from the First Pulse at the Source Node and Node Below*

The dpm measured for the first pulse were from initial arrival of the first pulse (>50 dpm above background) to initial arrival of the second pulse (>300 dpm above tail of first pulse). The second pulse was measured from initial arrival to the end of the experiment. Data are the averages (±SD) of three experiments. The selection of pods as right or left depended on which side of the petiole they were situated coming outward from the stem.

Experiment No.	Treatment			
	Source leaf		Leaf below	
	1st pulse	2nd pulse	1st pulse	2nd pulse
1	Illuminated		Illuminated	
2	Illuminated		Shaded	Illuminated
3	Illuminated		Illuminated	2/3 Leaflets excised, shaded
	Second Pulse dpm/First Pulse dpm			
	Source node		Node below	
	Right pod	Left pod	Right pod	Left pod
1	1.33 ± 0.39	1.59 ± 0.57	1.12 ± 0.15	1.33 ± 0.10
2	1.25 ± 0.28	1.06 ± 0.15	0.72 ± 0.18	0.58 ± 0.09
3	0.63 ± 0.08	1.16 ± 0.68	14.22 ± 5.90	25.32 ± 14.70

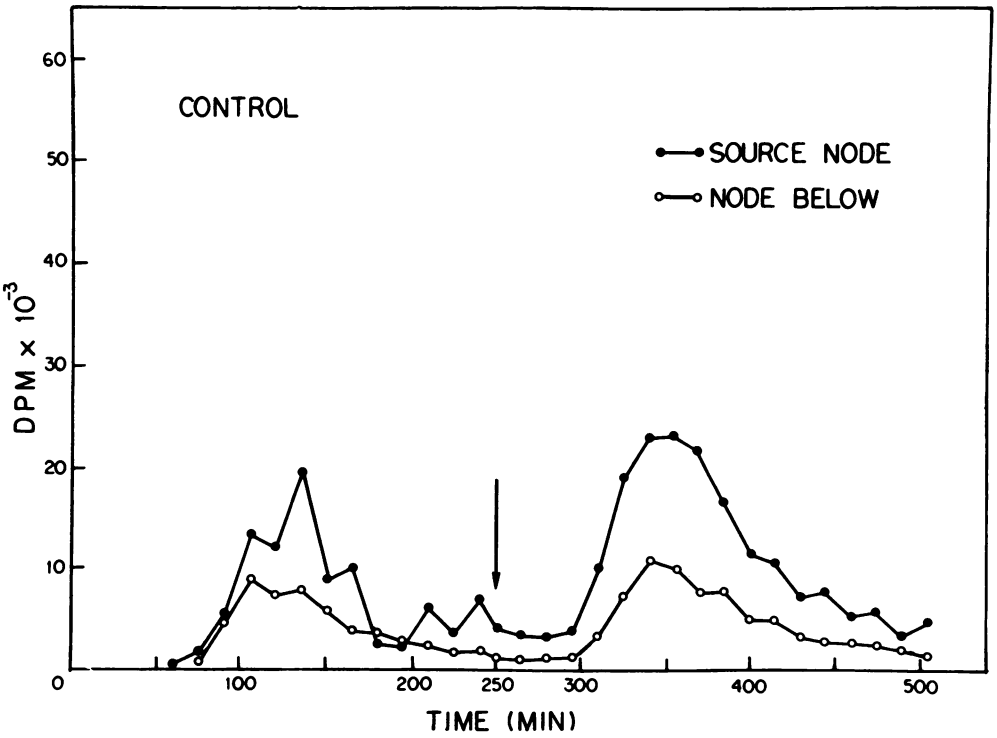

FIG. 1. Time-course of ^{14}C-assimilate leaked from pods at the source node (●) and node immediately below (○). In this control experiment the leaves at both nodes were left undisturbed during the two pulses of $^{14}CO_2$ given at time 0 and 250 min (arrow).

received from the source leaf during the second pulse (Fig. 2 and Table I). While there might appear to be a delay in the arrival of the label at the node below during the first pulse the approximate velocities for both nodes were similar, 0.37 *versus* 0.32 cm/min for the source node and node below, respectively.

The net photosynthetic rate of the source leaf declined about 25% during the final 250 min of the experiment shown in Figure 2 from 15.7 to 12.8 mg CO_2 dm^{-2} h^{-1}. This may account for the decrease in the total label recovered from the second pulse at the source node when compared with the control (Table I). Even if this is taken into account the decrease in total ^{14}C-translocate to the node below is still significant.

No measurement was made of the photosynthetic rate of the leaf at the node below. It is assumed, with reason, that the increased light intensity, from <20 to 500 μE m^2 s^{-1}, increased the leaf's photosynthetic rate, and in turn its supply of assimilate to the pods at its node. If the sink demand of the pods at the node was fairly constant, as has been indicated by a linear rate of dry weight accumulation over most of the filling period (3), then the increase in supply from its own source leaf would reduce the demand on the secondary source, as was observed. This reduction in demand appeared to occur in less than 50 min (Fig. 2), the time of illumination before onset of leakage of any ^{14}C-translocate.

To strengthen the idea that a definite balance of assimilate partitioning exists, the opposite experiment was conducted in which an illuminated leaf on the lower node was shaded (light intensity reduced to <20 μE m^2 s^{-1}) during the second pulse to promote an increase in translocation from secondary sources. In several preliminary experiments a trend was observed, but no significant increase could be demonstrated. This was thought to be due to the mobilization of recently assimilated materials from the shaded leaf, similar to that observed at the end of the light period (10). The more radical treatment of lateral leaflet excision combined with shading of the remaining leaflet was therefore employed.

Previous reports have shown a rapid alteration in the translocation patterns to developing leaves of bean with excision of primary (15) and secondary (9) sources. Similar rapid changes in translocation patterns were observed in soybean pods (Fig. 3). During the first 250 min while the lower node's source leaf was illuminated with an increased light intensity (509 μE m^2 s^{-1} *versus* 300 μE m^{-2} s^{-1} for the controls) there was very little import of ^{14}C-labeled assimilate from the source node (Fig. 3 and Table I). The lower node's source leaf was apparently meeting most of the sink demand at this time which would have been placed upon the source leaf as in the control experiment.

With the excision of the lower node's lateral leaflets and darkening the remaining leaflet there was a 6.5-fold increase in label recovered at the lower nodes pods and a concomitant decrease of 30% in distribution to the source node pods when compared with the preexcision pulse (Fig. 3). In similar experiments the increase to the pods at the lower node ranged from 4- to 33-fold with a decrease to the source node of up to 40% (Table I).

Such increases in ^{14}C-translocate to the lower node was observed in less than 50 min. The velocities of translocation to both nodes were essentially identical (approximately 0.37 and 0.39 cm/min, source node and node below, respectively) which may indicate that the distribution change may have occurred much sooner, *i.e.* at the source node itself, immediately upon excision.

The photosynthetic rate of the source leaf in the experiment of Figure 3 did show a slight decline similar to that in Figure 1, but there was no significant change within 2 h of excision of the lower nodes lateral leaflets. This is comparable to the observations of Swanson *et al.* (15), who reported alterations in the translocation patterns of beans in less than 9 min while the source leaf's steady-state photosynthetic rate was virtually unchanged.

As indicated in Table I the data contain some plant-to-plant variability. This is to be expected in that the photosynthetic rates or developmental stages of secondary sources and sinks relative to the two nodes studied are difficult to control. In addition, changes in photosynthetic rates within the individual leaflets of the source trifoliolate itself may contribute to alterations in label distribution patterns (Fellows, unpublished data). All of the observations reported here, however, were statistically significant and demonstrate that the technique is valid in this type of study.

The data of Figures 1 to 3 and Table I indicate that there exists a delicate balance between sinks and sources, and that changes in this balance can be compensated for rapidly. Wardlaw and Mon-

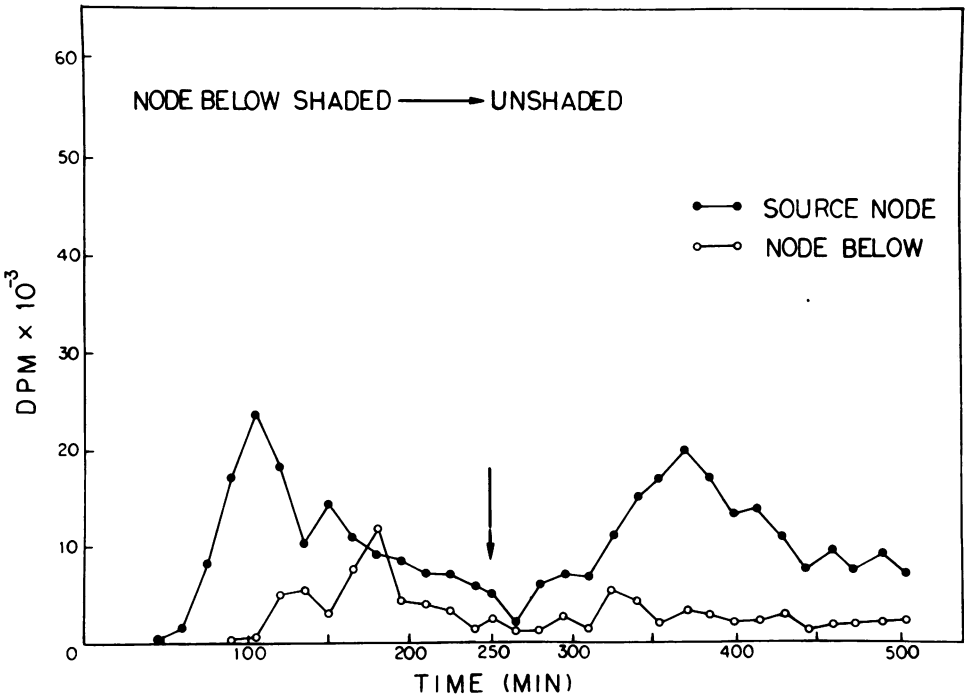

FIG. 2. Time-course of ^{14}C-assimilates leaked from pods at the source node (●) and node immediately below (○). The leaf at the lower node was shaded for 24 h prior to, and during, the first 250 min of the experiment following the first pulse (time 0). At time 250 min (arrow) the leaf was uncovered and illuminated while the source leaf received the second pulse of $^{14}CO_2$.

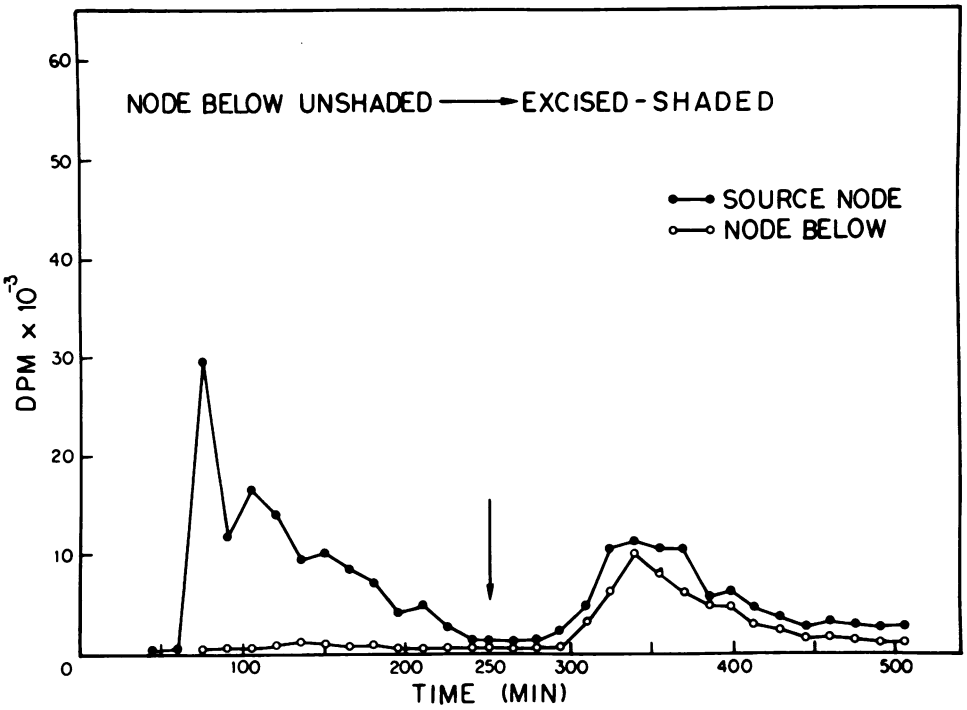

FIG. 3. Time-course of ^{14}C-assimilate leaked from pods at the source node (●) and node immediately below (○). The leaf at the lower node was unshaded and illuminated during the first 250 min following the first pulse of $^{14}CO_2$ (time 0). At 250 min (arrow) two-thirds of the lower leaf's area was excised and the remainder shaded while the source leaf received the second pulse of $^{14}CO_2$.

cur (19) reported that alterations in the sink demand promoted changes in the velocity of assimilate movement through the peduncle of wheat with an increase in movement when ear photosynthesis was inhibited and a decrease when half of the grains were removed. When they shaded the rest of the plant below the flag leaf, thus reducing or eliminating any secondary sources, the velocities and distribution patterns of the ^{14}C-assimilate were, however, essentially unchanged. In the experiments reported here the principal sinks, *i.e.* the pods, were not disturbed and alterations were made on the primary sources of the lower node. While the patterns of distribution from the source leaf were altered, the approximate velocities of assimilate movement did not change significantly.

There seems to be a constant demand issued by the sinks, in this case the pods at their respective nodes, which may be the major factor involved. When the primary sink demand is not met by the principal source for that node, it will rapidly create a demand on a secondary source, probably determined by vascular connections (18). A change in the concentration gradient of assimilates, most likely sucrose, within the sieve elements could account for the rapid changes in distribution, especially if these changes occurred in closely linked vascular traces. Housley and Fisher (12), and more recently Fisher (6) have given excellent evidence that such a gradient can exist in soybean, and, while performed on vegetative tissue, Fritz (8) has shown that connecting vascular traces can exist between the differing nodes of broad bean (*Vicia faba*).

In conclusion, the simple inexpensive pod leakage technique as employed in this study can demonstrate the rapid effects of source-sink alterations in soybean on the translocation patterns to the pods. The results indicate that the strength of the individual sink demand can be considerable and that the translocation stream possesses a definite homeostatic ability to meet the demand from primary and secondary sources.

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