TRANSLOCATION OF STEM-INJECTED GLUCOSE-^{3}H AND SUCROSE-^{14}C IN BEAN SEEDLINGS AS AFFECTED BY 2,4,5-TRICHLOROPHENOXYACETIC ACID TREATMENT[1]

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Sucrose-^{14}C (U) and glucose-3-^{3}H were injected simultaneously, either with or without 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), into the stem, at the cotyledonary node, of 10-day-old bush bean seedlings (*Phaseolus vulgaris* L., var. Stringless Green Pod). The effect of 2,4,5-T was to retard the rate of translocation of the sugars into, and the disappearance of label from, growing points and primary leaves. Treatment with 2,4,5-T enhanced disappearance of label from the stem, and slightly enhanced its accumulation in the roots. Disappearance of total ^{14}C label from the whole plant was somewhat accelerated by 2,4,5-T, while ^{3}H label was unaffected.

INTRODUCTION

The hypothesis that auxins produced by metabolic sinks, such as apical meristems and rapidly maturing fruits, regulate the flow of metabolites to these areas was advanced by Went as early as 1939 (1). Investigations of the movements of isotopically labeled metabolites in plants have demonstrated that such substances tend to move out of mature leaves towards actively growing regions, such as young developing leaves and fruits (2). Booth, et al. (3) attempted to ascertain the role of auxins on sugar translocation in pea plants by replacing the apical meristem with a lanolin paste containing indole-3-acetic acid (IAA) and observing the movement of ^{14}C-labeled sucrose in the plant. They noted that IAA stimulates the movement of substances to the point of application. Both ^{32}P and labeled photosynthates were shown, by Seth and Wareing (4), to move into the peduncles of beans treated with IAA.

Khan and Sagar (5) reported that treating the fruits of tomato plants with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) enhanced the movement of sugar from other parts of the plant. They further reported a 15% increase in the amount of sugar exported from a leaf treated with 2,4,5-T.

The translocation of photosynthate in young soybean plants is altered by the replacement of the apical meristem with IAA in water (6). In order to determine the distribution of the IAA applied in this manner, these workers treated the plants with ^{14}C-labeled IAA and then analyzed tissue extracts for the presence of label after treatment. They were able to find no label in the leaves and in only one case was IAA recovered from the roots. Almost all of the label was recovered from the stem tissue. This suggests that auxins exert their regulatory influence within the stem and, probably, specifically on the vascular system of the stem.

In a series of studies on the translocation patterns of 2,4,5-T injected into the stem of young bean seedlings at the cotyledon node, Long and Basler have shown that increasing the amount of 2,4,5-T injected into the stem from 0.5 μg to 3.0 μg caused a geometric increase in the amount of auxin translocated acropetally into the growing point (unpublished data). Very little auxin was recovered from the primary leaves, however. A possible explanation for this is that auxins may cause generalized changes in the semipermeability of membranes of vascular tissues which would result in the mobilization of translocatable metabolites to the most active metabolic sink. Zimmerman (2) suggested that the exchange of sugars between phloem and surrounding tissues is regulated by auxins. The increase in acropetal movement of auxin would merely reflect the change in metabolite flow. There are implications that auxins produced in the apical meristem move basipetally into the stem where they, in some manner, regulate translocation patterns. This effect would be

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produced artificially by injecting auxin into the stem.

In order to test this hypothesis ^{14}C-labeled sucrose and ^{3}H-glucose were simultaneously injected into the stem along with (or without) 2,4,5-T and translocation measurements of the label were made.

METHODS AND MATERIALS

Bush beans (*Phaseolus vulgaris* L., var. Stringless Green Pod) were germinated in perlite moistened with half-strength Hoaglands solution under Sylvania 40-watt Grolux Lifeline fluorescent lights. After the five-day germination period, the seedlings were transferred to amber glass jars containing 400 ml of half-strength Hoaglands and aeration was provided. The plants were then placed in a growth chamber adjusted for 14 hr day, 10 hr night, 90° F day temperatures, 80° F night temperatures, and humidities ranging below 30% relative humidity.

After about three days in the growth chamber, the plants were transferred to amber jars containing fresh one-fourth-strength Hoaglands solution. On the day following this latter transfer, the plants were treated by simultaneously injecting 0.146 nanomoles of sucrose-^{14}C (U) (specific activity 360 mc/mmole) and 0.722 nanomoles D-glucose-3-^{3}H(n) (specific activity 1100 mc/mmole) into the stem of the intact plant with a Hamilton 1-μl syringe at the cotyledonary node, with the needle penetrating down the pith area about 1 cm from where the sugars and 2,4,5-T were deposited. The sugars were injected alone in 1 μl of 85% ethanol per plant or along with 3.0 μg 2,4,5-T.

The plants were harvested at 1, 2, 4, and 6 hr and were separated into primary leaves, including petioles, growing points (all tissue above the primary leaf node), stems (all tissue from the root collar up to the primary leaf node), and roots. The plant parts were quickly frozen at -40° C and freeze-dried. The plant parts were then homogenized in 95% ethanol in a Virtis high-speed homogenizer. Small aliquots of the homogenate were transferred to vials containing 15 ml Bray's cocktail and analyzed for radioactivity in a Packard Tri-Carb Model 3320 Liquid Scintillation Spectrometer, which was calibrated for simultaneous dual-label counting. Carbon-14 and ^{3}H counts were corrected for efficiency by external standardization and comparison against chlorophyll-quenched standards. Five milliliter samples of the nutrient solution in which the plants were growing were pipetted into counting vials. The water was removed by lyophilization, 15 ml of Bray's solution was added and the solution was counted as described above.

RESULTS AND DISCUSSION

Figure 1A shows the acropetal movement of the label from sucrose-^{14}C and glucose-3-^{3}H to the growing point in the presence and in the absence of 2,4,5-T. The rate of movement of sucrose label to the growing point was decreased by 2,4,5-T, but the total amount translocated essentially was equivalent.

The pattern of translocation of glucose label to the growing point was much the same as that of sucrose, but the total amount was much less. Sucrose and glucose labels reached a maximum at 2 hr and then rapidly disappeared. 2,4,5-T delayed the peak

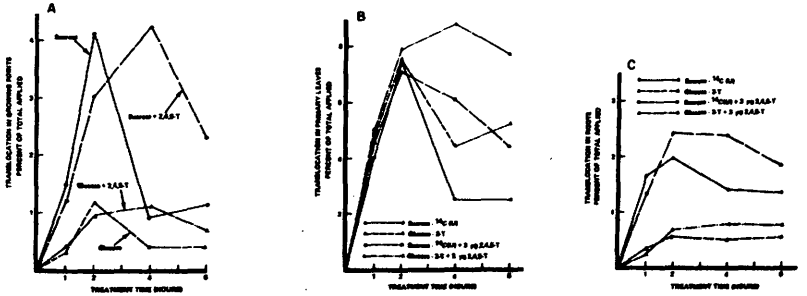

FIGURE 1. The translocation of ^{14}C sucrose label and ^{3}H glucose label after injection with or without 2,4,5-T at the cotyledonary node to (A) growing points, (B) primary leaves, and (C) roots.

times for the translocation of both sucrose and glucose by approximately 2 hr. Movement to the growing point of stem-injected sugars is not enhanced by 2,4,5-T and, thus, the previously noted 2,4,5-T translocation response is not a generalized mobilization of nutrients in the stem with a concomitant movement of 2,4,5-T.

The translocation pattern to primary leaves (Fig. 1B) was moderately different from that to the growing points, although the peak times in both plant parts were similar. Whereas grossly dissimilar amounts of glucose and sucrose moved into the growing points, the rate of movement into the primary leaves was very similar for sucrose and glucose, both in the presence and in the absence of 2,4,5-T, for the first 2 hr. This may be an indication that both sugars reached the leaves via the same pathway (possibly the xylem), while translocation to the growing points, as shown in Figure 1A, may have been via separate pathways for sucrose and glucose. Comparison of translocation patterns to the growing points and leaves shows that the patterns of translocation of glucose and glucose with simultaneous 2,4,5-T treatment are very similar in the two plant parts. The sugar concentration reached a maximum in primary leaves at 2 hr (except in the case of glucose-3-^{3}H + 2,4,5-T) after which time the label rapidly disappeared. Apparently 2,4,5-T delayed the disappearance of label, as it did in growing points, possibly by causing incorporation of the sugars into a more stable pool.

Basipetal transport to the roots is shown in Figure 1C. Amounts of ^{14}C label recovered from the roots were very small even when compared to those of the growing points and leaves. There appears to be a slight increase in the accumulation of label in the roots as a result of the 2,4,5-T treatment. Analysis of the nutrient solution showed essentially no label present in the nutrient solution in which the plants were growing under any treatment conditions.

Only 10% to 15% of the label applied was accounted for in growing points, primary leaves, and roots. The amount recovered from the stem (Figure 2A) shows that considerably more than this disappeared from the stem. Fifty per cent of the ^{14}C of sucrose and 75% of the ^{3}H of glucose

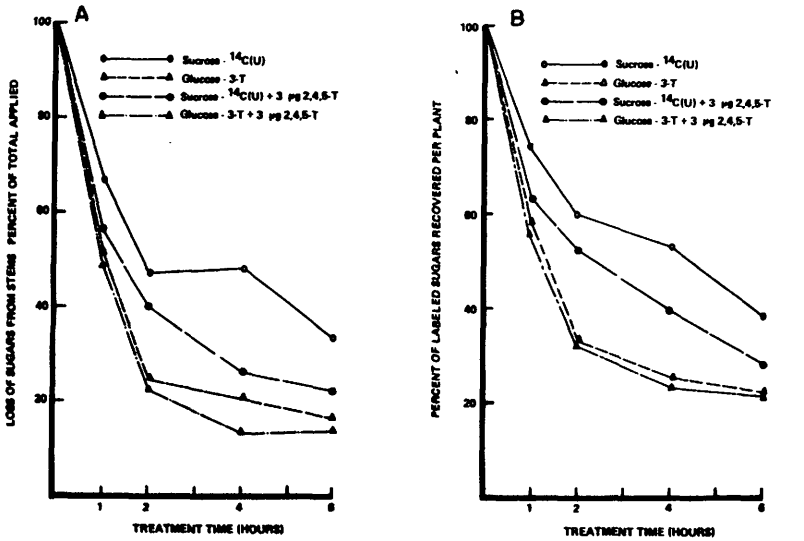

FIGURE 2. The recovery of ^{14}C of sucrose and ^{3}H of glucose after injection with or without 2,4,5-T at the cotyledonary node in (A) stems and (B) entire plants.

disappeared 2 hr after treatment. A slight enhancement of disappearance of both ^{14}C and ^{3}H label was caused by 2,4,5-T. Figure 2B represents the total label recovered per plant. Obviously both ^{14}C and ^{3}H disappeared very rapidly. Although 2,4,5-T enhanced the disappearance of both sucrose and glucose from the stems (Figure 2A), in the total plant only the disappearance of sucrose was enhanced by 2,4,5-T.

The pattern of disappearance of label from the total plant very closely parallels the disappearance of label from the stem, which suggests that a considerable portion of the label is lost, probably by $^{14}CO_2$ and $^{3}H_2O$ evolution, due to the metabolic activities of the stem. This very rapid metabolism which occurred in the stem was totally unexpected and could be a wound reaction brought about by the injection of the sugars.

It is clear from these investigations that most if not all of the translocatable pool of labeled sugars had disappeared from the stems at 2 hr, or at least no net accumulation of label occurred in any of the other organs after 2 hr.

As we have shown in previous, unpublished work, auxins such as 2,4,5-T appear to cause changes in permeability or in other factors which cause enhanced acropetal 2,4,5-T transport. The present data show that these changes do not result in enhanced movement of all common metabolites; thus, the changes must be specific for the movement of auxin-like molecules. The effect of 2,4,5-T seems, rather, to be on the incorporation or accumulation of the label into more stable metabolic pools, in terms of compartmentalization of the sugars, or incorporation of the label into other cell constituents and, to some extent, on the disappearance of label from the plant.

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