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Enhancement of [^{14}C]Sucrose Export from Source Leaves of *Vicia faba* by Gibberellic Acid[1]

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ABSTRACT

The effect of gibberellic acid (GA_3) on sucrose export from source leaves was studied in broad bean (*Vicia faba* L.) plants trimmed of all but one source and one sink leaf. GA_3 (10 micromolar) applied to the source leaf, enhanced export of [^{14}C]sucrose (generated by $^{14}CO_2$ fixation) to the root and to the sink leaf. Enhanced export was observed with GA treatments as short as 35 minutes. When GA_3 was applied 24 hours prior to the $^{14}CO_2$ pulse, the enhancement of sucrose transport toward the root was abolished but transport toward the upper sink leaf was unchanged. The enhanced sucrose export was not due to increased photosynthetic rate or to changes in the starch/sucrose ratio within the source leaf; rather, GA_3 increased the proportion of sucrose exported. After a 10-min exposure to [^{14}C]GA_3, radioactivity was found only in the source leaf. Following a 2 hour exposure to [^{14}C]GA_3, radioactivity was distributed along the entire stem and was present in both the roots and sink leaf. Extraction and partitioning of GA metabolites by thin layer chromatography indicated that there was a decline in [^{14}C]GA_3 in the lower stem and root, but not in the upper stem. This pattern of metabolism is consistent with the disappearance of the GA_3 effect in the lower stem with time after treatment. We conclude that in the short term, GA_3 enhances assimilate export from source leaves by increasing phloem loading. In the long term (24 hours), the effect of GA_3 is outside the source leaf. GA_3 accumulates in the apical region resulting in enhanced growth and thus greater sink strength. Conversely, GA_3 is rapidly metabolized in the lower stem thus attenuating any GA effect.

Hormones are believed to play an important role in the regulation of assimilate partitioning and translocation in plants (2, 11, 12 and references therein). In the source-path-sink continuum, phloem loading of organic solutes is a crucial step subject to regulation by phytohormones (3, 13, 16). However, the mechanism by which each hormone influences these processes is not well understood. In general, a distinction must be made between the indirect effect of hormones through their influence on growth and more direct short term effects on transport and carbon metabolism.

Based on the evidence in the literature, it is believed that GA and IAA enhance and ABA inhibits phloem loading of sucrose (4, 5). Gibberellin and kinetin influence assimilate transport at the site of hormone application (11, 13–15) whereas IAA has been shown to act at a remote site (17). Using isolated phloem tissue from celery as well as intact plants, Daie *et al.* (5) suggested that GA and IAA had a direct effect on rates of sucrose loading. Recently, Aloni *et al.* (1) reported a GA effect on phloem unloading of sucrose in stem segments of *Vicia faba*.

Regulation of assimilate transport and partitioning by phytohormones is not limited to their action on phloem loading and unloading. For example, starch synthesis and metabolism in source leaves may be subject to hormone action (7, 8). GA and IAA have also been shown to enhance the activity of sucrose phosphate synthase in sugarbeet leaves (3), therefore, altering the chemical partitioning of carbon between starch and sucrose.

With the exception of work by Daie *et al.* (4), most evidence on the hormonal regulation of phloem loading has been obtained *in vitro*. In the present study we investigated the effect of GA_3 on movement of ^{14}C assimilates from the source leaf to remote sinks (root and young sink leaves) *in vivo*. The results indicate that a short-term effect of GA_3 may be enhanced phloem loading. The observation is consistent with our combined *in vitro* and *in vivo* studies in celery (5), supporting a direct role for GA and IAA in phloem loading.

MATERIALS AND METHODS

Plant Material. Broad beans (*Vicia faba* L. cv Broadwinsor) plants were grown in a greenhouse in 3-L pots filled with a 7:3 sand:peat mixture and were irrigated daily with a complete nutrient solution. A photoperiod of 16 h was provided by extending the day length with mixed incandescent and fluorescent lamps. Light intensity was at least 300 μE m^{-2} s^{-1} at the canopy level. The average day and night temperatures were 25 and 18°C, respectively. One-month-old plants were trimmed to one source and one sink leaf 24 h before any treatment. The source leaf was at the third internode from the top, whereas the sink leaf was the top folded leaf at the plant apex, usually less than 2 cm long. Experiments were carried in three replicates and repeated three times. All experiments were conducted at midday of bright sunny days, when light intensities were at saturation level and thus nonlimiting to photosynthesis.

GA_3 Application and $^{14}CO_2$ Labeling. The lower side of the nonabraded source leaf was placed on 20 ml of a GA_3 (10 μM) solution or distilled H_2O (control) in an uncovered Petri dish. Three drops of Tween 20 were added to both solutions as a surfactant to enhance adsorption of GA_3 by the leaf. Unless otherwise stated, the standard incubation in GA_3 was for 1 h. The leaf was then allowed to dry for an additional hour after

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which it was placed in a clear acrylic plastic chamber (14 × 14 × 5.5 cm). The leaf was exposed to $^{14}CO_2$ for 10 min by injecting 50 μCi of $^{14}CO_2$ into the chamber. The air within the chamber was mixed with a small axial fan. Following a 30 min chase in air, the source leaf was excised and the plants were divided into various parts as follows: (a) 2 cm segments of stem, (b) sink and source leaves, (c) root, and (d) cotyledons. Each plant part was digested overnight in 4 ml of H_2O_2:$HClO_4$ (60:40 v/v) at 50°C. After cooling and addition of a cocktail, radioactivity was determined in a Packard[3], Tri Carb 460 CD Scintillation Counter.

Sugar Analysis. Four stem segments from each treatment were extracted in boiling ethanol. The extracts were combined and evaporated to dryness and redissolved in 0.4 ml distilled H_2O. Sugars were quantified by HPLC equipped with a differential refractometer detector on an Aminex HPX-42 column (300 mm × 7.8 mm). Sucrose, glucose, and fructose peaks were collected as separate fractions for radioactivity measurement.

Measurement of [^{14}C]GA_3 Distribution. Six plants were trimmed as described earlier. The lower surface of the source leaf of each plant was floated on 5 ml of 10 μM GA_3 containing 1 μCi [^{14}C]GA_3 (15 mCi/mmol) in a Petri dish. Following 2 h of incubation in the light, the leaf was rinsed with distilled H_2O, and three plants were harvested and divided into the following parts: source leaf, sink leaf, stem, (which was further subdivided into 2 cm segments) and root. Each part was digested in $HClO_4$-H_2O_2 (60:40 v/v) digestion medium and the radioactivity was counted as described above. Three other plants were harvested and processed identically 24 h later.

Measurement of [^{14}C]GA_3 Metabolism. Plants were trimmed as described above. The upper surface of the source leaf was gently abraded with carborundum powder and 50 μl of [^{14}C]GA_3 (0.5 μCi/ml) made up in distilled H_2O were applied in a lanolin well on the abraded surface. The treated area was then covered with a glass cover slip to prevent evaporation. Following 10 min, 2 and 24 h of incubation, the nonadsorbed [^{14}C]GA_3 was washed away with distilled H_2O and three plants were harvested and divided into the following parts: source leaf blade, sink leaf, upper stem (internodes above the attachment point of the source leaf), and lower stem (from the source leaf down to the root). The root system of each plant was washed in tap water to remove adhering soil particles. Each plant part was frozen separately in liquid N_2 and stored at −20°C. Extraction and partitioning of GA_3 was carried out according to Metzger and Zeevaart (10). In short, the frozen plant material was thawed and homogenized in 20 ml of ice-cold 80% methanol, stirred overnight at 5°C and filtered. The extract was then evaporated under reduced pressure to remove the methanol and an equal amount of 0.1 M phosphate buffer (pH 8.2) was added to the aqueous residue. The resulting mixture was partitioned two times against equal volumes of petroleum ether. The aqueous phase was adjusted to pH 2.5 with 4 M HCl and partitioned two times against an equal volume of petroleum ether. The aqueous phase was adjusted to pH 2.5 with 4 M HCl and partitioned two times against an equal volume of ethyl acetate. The organic phase was then collected and evaporated to dryness under reduced pressure. The dry residue was dissolved in 0.5 ml ethyl acetate. The purified samples were applied as bands on glass TLC plates (20 × 20 cm) coated with silica gel H. Authentic [^{14}C]GA_3 marker spots were applied to each plate. The thin layer plates were developed to 15 cm from the origin in chloroform:ethyl acetate:acetic acid (60:40:5 v/v). The resulting chromatogram was divided into 10 equal zones, and the silica gel from each zone was scraped off and eluted with 5 ml of water-saturated ethyl acetate. The ethyl acetate was evaporated, and after the addition of scintillation solution, the radioactivity was counted.

RESULTS AND DISCUSSION

Short-Term Effects of GA_3. When $^{14}CO_2$ was applied 2 h after GA_3 treatment, the transport of ^{14}C-assimilates from the source leaf to the root and to the apical sink leaf was greatly enhanced (Fig. 1). An increased accumulation of ^{14}C-assimilates was observed in the root, the sink leaf, and along the entire stem. This effect of GA_3 was maximal at external concentrations of 10 μM and was less pronounced at 1 μM. When the time between GA_3 application and the $^{14}CO_2$ pulse was extended to 24 h, the pattern of GA_3 influence changed. GA_3 enhancement of the downward movement and accumulation of [^{14}C]sucrose in the root was almost abolished whereas the upward movement toward the sink leaf was significantly greater in the GA_3-treated plants than in the control (Fig. 1B).

More than 90% of the exported radioactivity in the stem segments was recovered in the sucrose fraction and essentially none in glucose or fructose (Fig. 2). This suggested a lack of sucrose hydrolysis in the stem tissue following unloading or that sucrose may not have been unloaded from the phloem to the adjacent parenchyma cells. We have previously reported that efflux of [^{14}C]sucrose from excised stem segments of *Vicia faba* was attenuated by GA_3 at concentrations as low as 0.1 μM (1) suggesting that GA_3 may inhibit sucrose unloading. However, the apparent reduction in [^{14}C]sucrose efflux from stem segments in the presence of GA may reflect enhanced reloading of sucrose back into the phloem, therefore causing a reduction in net unloading *in vitro*. This same phenomenon may have occurred *in vivo* (this study) so that sucrose was rapidly reloaded before it was hydrolyzed.

To more clearly define the GA effect, some possible sites of action were examined. For example, the greater number of counts in the receiving tissues could simply be the result of a greater supply of ^{14}C assimilates if GA_3 increased photosynthetic rates. However, similar amounts of total fixed radioactivity per plant were found in control and treated plants (Table I); therefore, GA_3 did not appear to alter the photosynthetic rate.

Alternatively, GA_3 may alter the chemical partitioning of carbon in leaves by decreasing the ratio of starch to soluble sugars (3, 6–8). Such an effect could increase the amount of sucrose available for export, therefore increasing mass flow in the phloem. ^{14}C was distributed similarly between ethanol soluble (soluble sugars) and ethanol insoluble fractions (predominantly starch) in the control and GA_3 treated plants so that the ratio of ^{14}C-sugars to ^{14}C starch was about 9.0 in both GA treated and control plants (Table I). Thus, it appeared that GA_3 enhanced the export of ^{14}C-soluble material without altering the incorporation of ^{14}C into starch. The observation ruled out any GA_3-induced alterations in the partitioning between starch and sucrose which led us to assume that the most likely site of the GA_3 action was on uptake of sucrose into the minor veins. The assumption is consistent with our *in vivo* and *in vitro* observations in celery (5), where it was shown that GA and IAA directly affect phloem loading rates.

To examine further this assumption, the source leaf was exposed to GA_3 for only 10 min followed immediately by a 10 min $^{14}CO_2$ pulse and 15 min chase period. Under these conditions. [^{14}C]sucrose export was enhanced both towards the root and the apical sink leaf (Fig. 3). A more pronounced accumulation of ^{14}C counts in the sink leaf as compared to that in the root was probably a result of proximity to the point of ^{14}C application (14 cm for the sink leaf and 42 cm for the root). In that short time period, GA_3 could not induce any growth change in the site of application (being a fully mature leaf). GA-induced growth was also unlikely to occur in the sink region since during that short time (total elapsed time was 35 min) GA_3 could not have reached

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

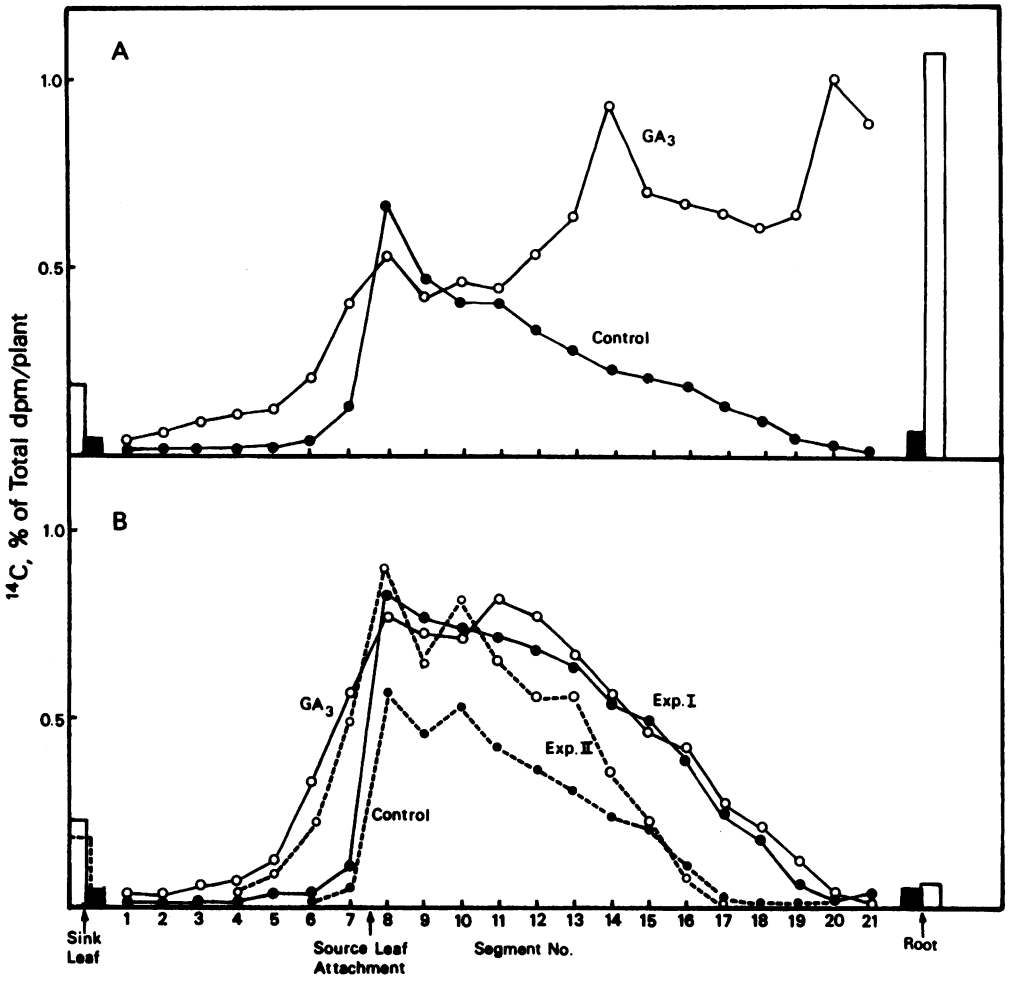

FIG. 1. The effect of GA_3 (10 μM) applied to the source leaf on the export of ^{14}C-assimilates to the root and upper sink leaf. GA_3 was applied 2 h (A) or 24 h (B) before $^{14}CO_2$ gassing (10 min pulse and 30 min chase). A, A typical experiment; B, variability between two identical experiments.

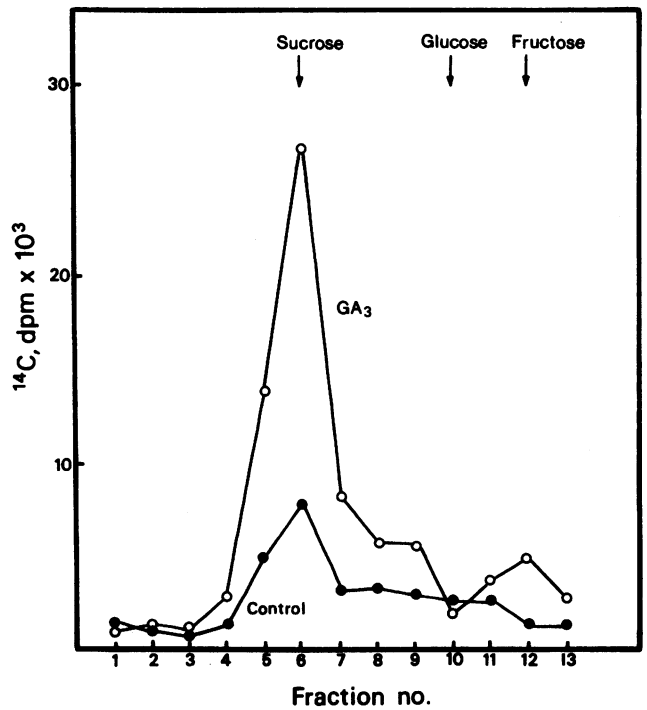

FIG. 2. Identification of sucrose as the major exported ^{14}C-sugar in the control and GA_3 treated *Vicia faba* stem by HPLC. Four-stem segments were excised following 2 h treatment of the source leaf with either water (control) or 10 μM GA_3 solution. $^{14}CO_2$ gassing was as described in Figure 1.

Table I. *Effect of 10 μM GA_3 on the Presence of ^{14}C Material in Ethanol Soluble and Insoluble Fractions of Vicia faba Source Leaves*

GA_3 was applied 2 h prior to a 30-min pulse and 45 min chase of $^{14}CO_2$.

	Control		GA_3 Treated	
	Soluble	Insoluble	Soluble	Insoluble
	dpm × 10^3			
^{14}C-material in source leaf	9,405	1,139	8,800	1,118
^{14}C-exported (stem, sink leaf, and root)	117	5	747	8
Total exported	122		755	
Total soluble ^{14}C (leaf soluble + ^{14}C exported)	9,527		9,556	
Total ^{14}C fixed	10,666		10,674	
Export[a]	1.3%		7.9%	

[a] Export was calculated as $\frac{\text{Total } ^{14}C \text{ exported}}{\text{Total soluble } ^{14}C} \times 100$.

sink regions in appreciable quantities. Therefore, the increased export of [^{14}C]sucrose from the source leaf to the rest of the plant appeared to result from an effect of GA_3 on either phloem loading or a process which increased the availability of sucrose at the site of phloem loading, *i.e.* increased sugar efflux from the mesophyll cells.

The amount of sucrose available for export is regulated at several sites within the source leaf. Enhancement of photosynthetic rates or sucrose synthesis through increased sucrose phos-

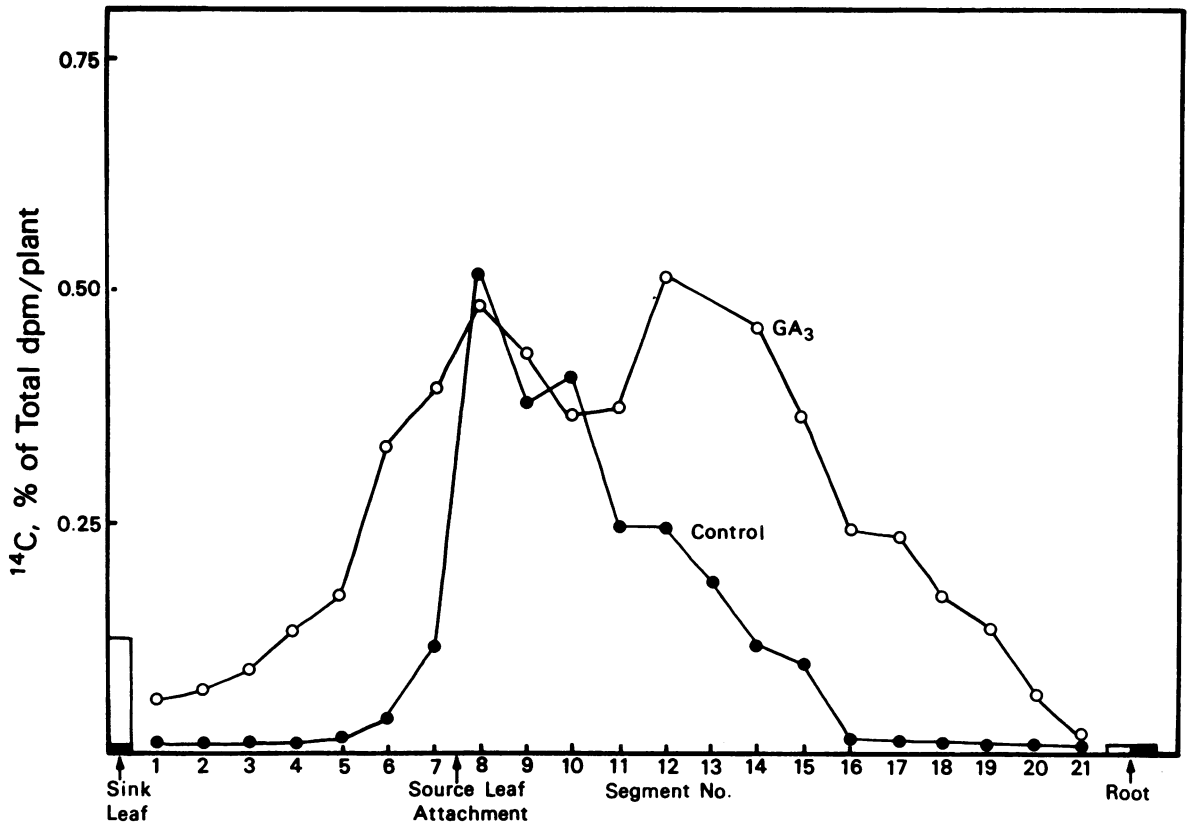

FIG. 3. Short-term effect of GA_3 (10 μM) on the export of ^{14}C-assimilates. GA_3 was applied 10 min prior to the $^{14}CO_2$ gassing (10 min pulse, 15 min chase) treatment as described in "Materials and Methods."

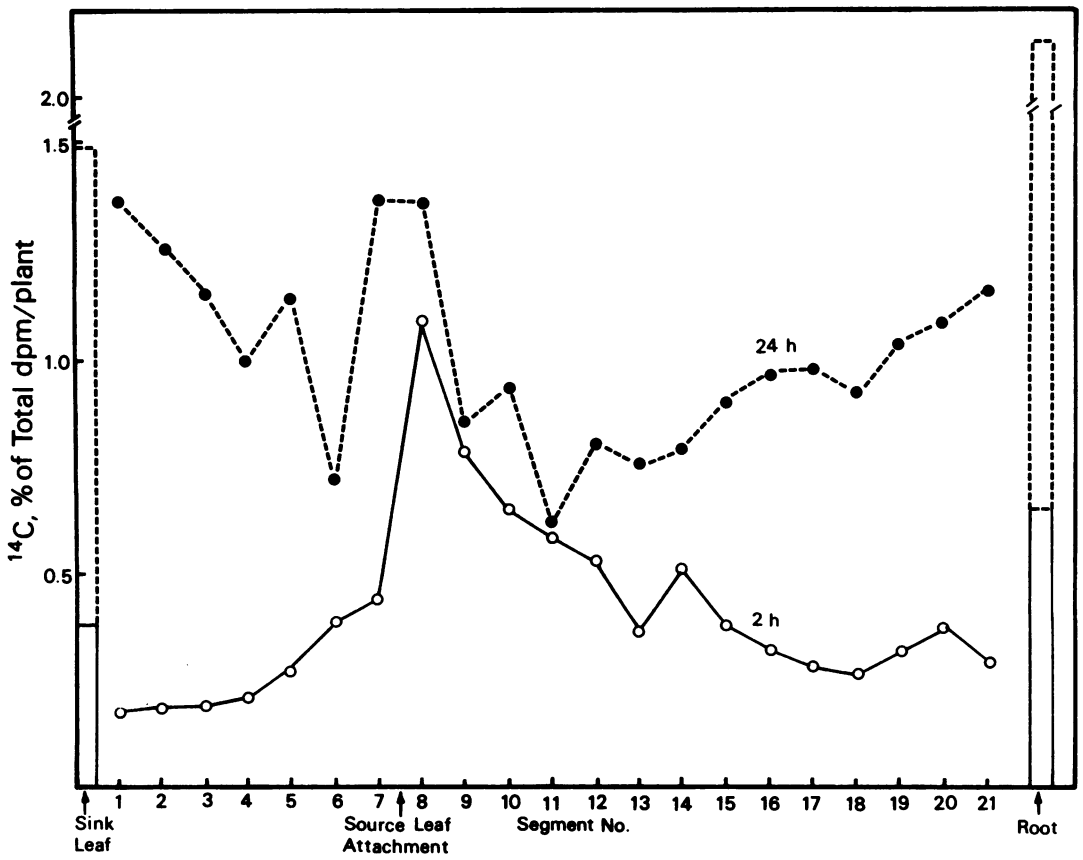

FIG. 4. Distribution of ^{14}C in the plant following application of [^{14}C]GA_3 to the source leaf which was allowed to incubate for 2 h (O) or 24 h (●).

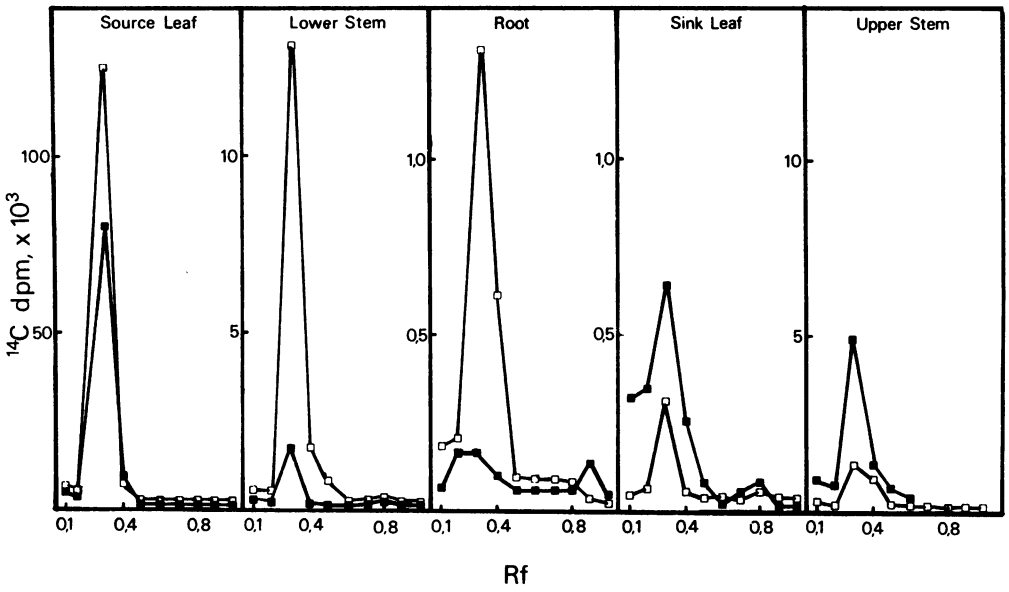

FIG. 5. TLC partitioning of [^{14}C] GA_3 containing acidic fractions of various plant parts following 2 (□) or 24 h (■) of [^{14}C]GA_3 application to the source leaf. Each part was detached, extracted, and after volume reduction the extract was separated by TLC. Note the different dpm scales for each tissue. The R_F for authentic GA_3 was 0.3.

phate synthase activity would yield more sucrose to be exported and, therefore, would increase the pressure gradient in the phloem which, in turn, increase assimilate flux. The present data (Table I), show that in the short term (2 h), GA_3 neither affects photosynthetic rates nor does it affect ^{14}C partitioning between soluble sugars and starch. However, in longer (24 h) studies, GA_3 has been shown to alter rates of sucrose synthesis by enhancing sucrose phosphate synthase activity (3) or inhibit starch synthesis (9).

GA_3 Distribution and Metabolism. To determine patterns of GA_3 distribution and metabolism, [^{14}C]GA_3 was applied to the source leaf. After a 10 min exposure none of the [^{14}C]GA_3 had moved out of the treated leaf. Movement of GA_3 from the site of application to the rest of the plant continued over time as indicated by the presence of greater total amounts of radioactivity in plants exposed for 24 h as compared to the 2 h treatment (Fig. 4).

The enhancement of ^{14}C-assimilate movement towards the root, observed after the 2 h treatment with GA_3, appeared to be a transient event because it disappeared after 24 h (Fig. 1B). The transient nature was correlated with a decline in GA_3 concentration as a consequence of its metabolism in that region. Although radioactivity derived from [^{14}C]GA_3 accumulated in all plant parts after 24 h, less radioactivity was associated with GA_3 fraction in the lower stem and root compared to the first 2 h (Fig. 5). In the root and the mature lower stem [^{14}C]GA_3 was almost entirely metabolized after 24 h, whereas in the source leaf, only 40% of the GA_3 was metabolized or exported (Fig. 5). Conversely, in the young upper stem tissue and sink leaf [^{14}C] GA_3 accumulated progressively from 2 to 24 h indicating lesser degree of GA_3 metabolism in these tissues. Therefore, we believe that it was GA_3 degradation rather than its distribution patterns which caused the disappearance of a GA_3 effect on assimilate accumulation in the lower parts of the plant after 24 h.

The mechanism for the enhanced export towards the sink leaf (remote site) after 24 h in GA_3-treated plants is not clear. Mulligan and Patrick (11) showed that GA_3 increased accumulation of ^{14}C assimilates when applied at the cut stem of decapitated *Phaseolus vulgaris* plants. They suggested that this effect was due to some transfer mechanism at the site of hormone application. In our system, the long-term GA_3 exposure might enhance growth in the sink leaf (symplastic unloading) where sucrose is rapidly utilized in growth and metabolism. In this matter, GA may enhance export to the sink leaf by increasing the assimilate concentration gradient between the source and sink leaf. Another possibility (which was not addressed in this study) is that the increased accumulation of [^{14}C]sucrose in the young upper part of the stem after 24 h was due to the enhancement of growth that GA_3 might have caused in this region.

In conclusion, we suggest that in the short term, GA_3 enhances assimilate export from source leaves through increased rates of phloem loading by a yet unknown mechanism. In the long term, the GA_3 effect is controlled to a large extent by its metabolism and accessibility to the site of action.

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