Effect of Glyphosate on Auxin Transport in Corn and Cotton Tissues

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ABSTRACT

Basipetal auxin transport in 6-day-old dark-grown corn coleoptiles was severely inhibited by increasing levels of glyphosate applied during the transport period.

The velocity of basipetal transport of [14C]indoleacetic acid in hypocotyls from 7-day-old cotton seedlings was significantly reduced when sublethal doses of glyphosate [N-(phosphonomethyl)glycine] were applied to the cotyledonary leaves 24 hours before transport measurement. Simultaneous application of glyphosate and indoleacetic acid during transport measurement had no effect on basipetal transport in cotton hypocotyl sections.

Slowing of transport was inversely proportional to the dosage applied to both species.

Sublethal quantities of the herbicide glyphosate [N-(phosphonomethyl)glycine] have been shown to induce tillering in seedlings of sorghum [Sorghum bicolour (L.) Moench cv. Tophand] (4), wheat (Triticum aestivum L. cv. Era) (4), and quackgrass [Agropyron repens (L.) Beauv.] (9, 11). Glyphosate-induced tillering in sorghum seedlings was significantly reduced by incorporating the cytokinin M'2-isopentenyljadenine in the glyphosate treatment solution; no reduction in tillering was noted with combinations of IAA and glyphosate (2).

Histochemical examination of the meristematic region of glyphosate-treated sorghum seedlings has shown that the apical meristem is viable (4). This observation suggests that glyphosate-induced tillering is not caused by death of the meristem and concomitant reduction in auxin biosynthesis (4).

Application of DPX-1840 [3, 3a-dihydro-2-(p-methoxyphenyl)-8H-pyrazolo-[5, 1-a]isoindol-8-one], an auxin transport inhibitor, to stems of sand bluestem (Andropogon hallii Hack.) significantly increased tiller production (22). Application of Etephon [(2-chloroethyl)phosphonic acid], which readily degrades to ethylene (10), to sand bluestem and “Waldron” wheat seedlings likewise significantly increased tiller production (20, 22).

The following experiments were designed to test the hypothesis that glyphosate-induced tillering is the result of glyphosate inhibition of basipetal auxin transport.

MATERIALS AND METHODS

Plant Material. Preliminary experiments revealed that corn and sorghum seedlings have identical tillering responses to treatment with the same concentration of glyphosate. Consequently, corn coleoptiles were chosen for use instead of sorghum coleoptiles whose small diameter and flexibility made them unacceptable for use in these studies. Hypocotyls from cotton seedlings were chosen as a dicot species for a comparison.

Corn (Zea mays L. cv. single cross 5855 × 127 C) seeds were planted 2 cm deep in plastic dishes filled with 10 cm of Vermiculite that had been saturated with water and then drained. The pans were placed in a dark incubator at 28 C. Transport tissue taken for study was a 2-cm section of the coleoptile cut 0.5 cm below the apical tip of 6-day-old seedlings. The only coleoptile sections used were those that did not contain leaf tissue in the upper 1 mm after the meristematic cap had been removed.

Cotton (Gossypium hirsutum L. cv. Stoneville 213) seeds were planted 1 cm deep in Vermiculite in plastic dishes. Each pan was placed in a 56-liter plastic bag and then placed in a growth chamber. The conditions during germination and growth were 27 C day and 21 C night temperatures, 50 to 60% RH, and a 16-h photoperiod. An illuminance of 4.8 klux was supplied by a mixture of cool-white fluorescent tubes and incandescent bulbs. The growth medium was kept moist by periodic watering with a half-strength dilution of water-soluble fertilizer (8-5-16). The plastic bags were removed on the 5th day after planting, when the seedlings were 5 to 7 cm high with fully expanded cotyledonary leaves. Transport tissue taken for study was a 2-cm section of hypocotyl cut 0.5 cm below the cotyledonal node of 7-day-old seedlings.

Reaction Materials. The agar blocks used for transport studies were prepared from Difco Bacto-Agar1 that had been washed by flushing with deionized H2O once each day for 2 weeks, dried at 50 C, and ground to pass a 40-mesh screen in a Wiley mill (17).

Nonradioactive IAA was obtained from Sigma Chemical Co.; glyphosate, 99% (w/w) pure, from Monsanto Chemical Co.; and carboxyl-labeled [14C]IAA, 57.6 mCi/mmol, from the Amshersham Corporation. The radiochemical purity of the [14C]IAA was determined by paper chromatography (14) and liquid scintillation counting to be 96% (w/w). Co-chromatography of [14C]IAA with 100 μg of nonradioactive IAA followed by bioassay of 1-cm sections of the chromatogram with a wheat coleoptile straight-growth test (19) verified the growth-regulating activity of the radioactive portion of the chromatogram.

Liquid Scintillation Counting. Tissue or agar samples were placed in 2 ml of counting solution in 0.5-dram (0.89-g) glass vials that were subsequently placed in standard 20-ml scintillation vials for counting (3). All samples were shaken from 12 to 14 h before counting for 1 min in a Packard Tri-Carb 2420 liquid scintillation spectrometer. The counting solution contained 100 g of naphthalene, 5 g of 2,5-diphenyloxazoles, and 10 ml H2O/1 p-dioxane.

Transport Experiments. Three methods of applying glyphosate to cotton sections were used: glyphosate was incorporated with [14C]IAA (7.5 × 10-5 μ) in agar donor blocks and with nonradio-

1 Mention of trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or Texas A&M University, and does not imply its approval to the exclusion of other products that may also be suitable.
active IAA (7.5 × 10⁻⁸ m) in agar chase blocks applied to hypocotyl sections; hypocotyl sections were soaked for 60 min in aqueous glyphosate solutions before transport experiments; and seedlings were treated with 50 μl of aqueous glyphosate solution, containing 0.5% (v/v) X-77 surfactant applied as droplets to the expanded cotyledons 24 h before transport experiments. Seedlings supplying hypocotyls for the controls were treated at the same time with 50 μl of water containing 0.5% (v/v) X-77. Corn cotyledons were exposed by glyphosate incorporated in the donor and chase blocks.

The general procedure of Goldsmith (13) as modified by Dav- enport (12) was followed in all transport experiments. In each experiment, 10 corn coleoptile or 10 cotton hypocotyl sections were used for each treatment. The sections were cut with a double-bladed knife with the blades 2 cm apart. Immediately after cutting, the sections were placed in Plexiglas holders with the basipetal end (morphological base) in contact with plain agar receiver blocks (14.14 m², 1.5%, w/v). Donor blocks were immediately placed on the acropetal (apical) end of the transport sections. Where noted, acropetal transport was measured by placing the acropetal end on the receiver blocks and applying the donor blocks to the basipetal end. Donor blocks contained either [¹⁴C] IAA alone for hypocotyl sections from treated seedlings and sections treated by presoaking, or [¹⁴C]IAA plus glyphosate, in the case of certain cotton experiments and all corn coleoptile experiments. The interval between cutting of sections and placement of donor blocks seldom exceeded 3 min. All donor blocks were replaced with chase blocks (containing nonradioactive IAA alone or in combination with glyphosate, depending on the experiment) 15 min (30 min in the case of cotton hypocotyls) after initial placement of the donor blocks. Upon their removal, the 10 donor blocks from each treatment were pooled and prepared for counting. Each treatment regime was applied to paired sets of 10 sections. The first set was allowed to transport for 1 h, the second set was allowed to transport for 1.5 h (corn) or 2.0 h (cotton). Measurement of the transport period started upon placement of the donor blocks. In time course experiments, sets of 10 cotton hypocotyl sections were allowed to transport for 1, 2, 2.5, 3, and 3.5 h. At the end of the transport period, chase and receiver blocks from each set of 10 sections were pooled separately and prepared for counting. The 10 tissue sections were cut with a multibladed cutter into 2 mm segments each. All segments from the 0- to 2-mm (apical) portion of the 10 tissue sections were pooled in one scintillation vial; the segments from the 2- to 4-mm portions were likewise pooled, and this procedure was continued for the entire 2-cm length of the tissue sections. The end result was 10 vials, each containing 10 2-mm segments from the same portion of each tissue section. Throughout this paper, “section” refers to the 2 cm piece of coleoptile or hypocotyl, and “segment” refers to one of the 2-mm pieces cut from each hypocotyl or coleoptile section.

Transport velocity was calculated by dividing the distance (in mm) that the [¹⁴C]IAA pulse peak moved down the section by the duration of the transport period (in h). Transport capacity (the amount of [¹⁴C]IAA transported into new areas of tissue per unit time) was calculated by subtracting the per cent total activity of each 2-mm segment at 1 h from the per cent total activity of the corresponding 2-mm segment at the end of the transport period (2, 2.5, 3, or 3.5 h) and summing the positive values (12).

In the course of these experiments, the radioactivity detected in the various components of the test systems is assumed to be that of [¹⁴C]IAA. Comparison of total counts recovered at the end of each experiment with total counts applied indicated a 90 to 95% accountability. All transport measurements were made with carboxyl-labeled IAA under subdued light (available laboratory lighting) and on tissues having a relatively low photosynthetic capacity (cotton hypocotyl and darkgrown corn coleoptiles).

During the uptake and transport periods, the holders containing the tissue sections were kept in moist chambers lined with damp paper toweling. Three to five separate experiments were carried out for each part of this study. The data were subjected to analysis of variance and Student’s t-test (where applicable); means were ranked according to Duncan’s multiple range test (21).

RESULTS AND DISCUSSION

Corn Experiments. Glyphosate incorporated in donor and chase blocks at concentrations of 7.5 × 10⁻⁷, -10⁻⁷, -10⁻⁶, and -10⁻⁵ m caused varying degrees of [¹⁴C]IAA transport inhibition. After 1 h of transport (broken line, Fig. 1), the [¹⁴C]IAA peak was located in the fifth segment (8- to 10-mm) of control sections and sections treated with 7.5 × 10⁻⁶ m glyphosate. The 1 h [¹⁴C]IAA peak was slowed to the fourth segment (6- to 8-mm) in sections treated with 7.5 × 10⁻⁵ m glyphosate; whereas [¹⁴C]IAA transport during the 1st h was reduced in sections treated with the two highest concentrations of glyphosate (7.5 × 10⁻⁵ and 7.5 × 10⁻⁴ m). By 1.5 h of transport (bold line, Fig. 1), the [¹⁴C]IAA peak in control sections had moved from the fifth segment to the seventh segment, a distance of 4 mm; thus, transport velocity was 8 mm/h. The peak after 1.5 h of transport in sections treated with 7.5 × 10⁻⁵ m glyphosate was still evident at the fifth segment, which indicates that [¹⁴C]IAA transport had been restricted. No peak was observed after 1.5 h of transport in sections treated with the two highest concentrations of glyphosate which suggests that active transport had been severely restricted by these concentrations. Transport velocity between 1 and 1.5 h was calculated for control sections and sections treated at four levels from three separate experiments (Table I). Statistical analysis indicated that glyphosate at all concentrations significantly slowed transport.

No significant differences between treatments (glyphosate concentration) were noted in the recovery of [¹⁴C]IAA in coleoptile tissues or chase or receiver blocks (data not given). The percentage of total tissue radioactivity associated with the first 2-mm segment (adjacent to the donor block) tended to increase with glyphosate concentration for both transport periods (Fig. 1). This observation, along with the lack of treatment differences in chase block activity, suggests that glyphosate immobilized [¹⁴C]IAA in the first tissue segment, preventing its entry into the active transport process as well as its diffusion back into the chase block.

Glyphosate administered at the time of transport measurement had no effect on acropetal movement of [¹⁴C]IAA; very little movement was detected in either treated or untreated tissues over a 5-h transport period. There were no significant differences in radioactivity detected in the total tissue, the first and second tissue segments, and the chase and receiver blocks (data not given).

Cotton Experiments. Preliminary experiments with hypocotyl sections from 7-day-old cotton seedlings indicated that glyphosate incorporated in the donor and chase blocks did not inhibit basal-petal movement of the [¹⁴C]IAA pulse over a 2.5-h period. The concentrations of glyphosate used were 10-fold dilutions, 7.5 × 10⁻⁴ m through 7.5 × 10⁻⁷ m. Likewise, exposure of hypocotyl sections to aqueous solutions of glyphosate (7.5 × 10⁻⁴ - 7.5 × 10⁻⁷ m) for 1 h before transport measurement had no effect on basipetal transport of [¹⁴C]IAA.

Hypocotyl sections from seedlings treated with glyphosate 24 h before transport measurement had significant reductions in transport velocity computed for the final 2 h of a 3-h transport period. The reduction in the rate at which the [¹⁴C]IAA (glyphosate contained in the 2-cm section) was directly proportional to the concentration of glyphosate applied to the seedlings (Fig. 2). The only visible external effect in seedlings given the highest concentration of glyphosate (112 μg/plant) was a slight downward reflection of the cotyledonal leaves. The seedlings appeared healthy and normal in every other respect, which rules out mechanical stress or external stem necrosis as the cause of reduced transport.

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² Multi-film X-77, a mixture of alkylarylpolyethylene glycol, free fatty acids, and isopropl alcohol; Kalo laboratories, Inc., Kansas City, Missouri.
represents the base. Cross-hatched 1.5 was significantly during three periods of 2-2.5 h). Seedlings treated completely in three of because period of 2.5 from 0.05 level. Glyphosate concentration on \[^{14}C\]IAA transport in corn coleoptiles.

<table>
<thead>
<tr>
<th>Glyphosate Concentration</th>
<th>[^{14}C]IAA Transport Velocity</th>
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<tbody>
<tr>
<td>M</td>
<td>mm/h</td>
</tr>
<tr>
<td>0</td>
<td>8.3 a</td>
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<tr>
<td>7.5 \times 10^{-7}</td>
<td>2.7 b</td>
</tr>
<tr>
<td>7.5 \times 10^{-5}</td>
<td>0 b</td>
</tr>
<tr>
<td>7.5 \times 10^{-4}</td>
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</table>

A progressively slower rate of \[^{14}C\]IAA transport was noted during three intervals of a 3-h transport period in hypocotyls from seedlings treated with glyphosate (112 \mu g/plant) (Table II). The velocity of \[^{14}C\]IAA transport in sections from treated seedlings was lower (nonsignificantly) than that in sections from control seedlings during the first two periods of measurement (1-2 h and 2-2.5 h). Transport velocity in hypocotyls from treated seedlings was significantly lower than in control hypocotyls during the third period of measurement (2.5-3 h). The velocity for control sections from 2.5 to 3 h has been conservatively estimated at 8.0 mm/h because in three of the four experiments, the \[^{14}C\]IAA peak had completely traversed the 2-cm hypocotyl section.

The time course of accumulation of radioactivity in the basal 2-mm segment (the segment resting on the receiver block) during a 3.5-h transport period clearly demonstrates reduced \[^{14}C\]IAA transport in hypocotyl sections from seedlings treated with glyphosate (112 \mu g/plant) 24 h before transport measurement (Fig. 3). After 2.5 h of transport, slightly higher (nonsignificantly) levels of \[^{14}C\]IAA began to accumulate in the basal segment of sections from untreated (control) seedlings than in the basal segment of

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**Fig. 1.** Effect of glyphosate concentration on the transport of \[^{14}C\]IAA by corn coleoptiles after 1 h (---) and 1.5 h (----) of transport. Each bar represents the percentage of total coleoptile \[^{14}C\]IAA activity found in each 2-mm segment, progressing from the morphological apex toward the morphological base. Cross-hatched area represents \[^{14}C\]IAA peak after 1 h of transport; stippled area represents additional accumulation between 1 and 1.5 h of transport. Data presented are from one of three replicate experiments.

**Fig. 2.** Velocity of \[^{14}C\]IAA transport in 7-day-old cotton hypocotyls treated with three concentrations of glyphosate 24 h before measurement. Velocity measurements were computed over a 2-h transport period. Each data point represents the mean of three separate experiments. Data points marked with the same letter are not significantly different at the 0.05 level.
sections from treated seedlings. The differences after 3 and 3.5 h of transport were highly significant (P = 0.01). The pattern of [14C]IAA accumulation was the same in the receiver blocks except that the differences were not significant until 3.5 h of transport (Fig. 3, inset). There were no significant differences in total radioactivity detected in hypocotyls from treated and untreated seedlings after any of the five transport periods. Consequently, the reduced detection of [14C]IAA from the basal segment and the receiver blocks for the glyphosate-treated hypocotyls cannot be attributed to any effect of glyphosate on [14C]IAA uptake.

Evaluation of the time course of distribution of [14C]IAA within the components of the total system (i.e., tissue, chase blocks, and receiver blocks) on a per cent-of-total-system basis revealed that hypocotyls from treated seedlings retained a significantly smaller portion of the [14C]IAA than did hypocotyls from control seedlings. The higher percentage of [14C]IAA in chase blocks from treated hypocotyls suggested that glyphosate restricted entry of [14C]IAA into the transport system and thus allowed the auxin to diffuse into the chase blocks. The significantly higher percentage of [14C]IAA in receiver blocks for control hypocotyls compared with treated hypocotyls supported this conclusion. A comparison of the activity detected in the acropetal 2-mm segments (adjacent to donor and chase blocks) of hypocotyl sections from glyphosate-treated and control seedlings indicated that these segments from treated seedlings retained a higher percentage of the radioactivity than did comparable segments from control seedlings.

The time course of acropetal transport over a 5-h period indicated that very little [14C]IAA was transported out of the first 2-mm segment of hypocotyls from untreated or treated seedlings (data not given). There were no significant differences between hypocotyls from treated and untreated seedlings in the amount of radioactivity detected from the total tissue, first and second tissue segments, and chase or receiver blocks. These results, and those for acropetal transport in corn coleoptiles, indicated that the effect of glyphosate on auxin transport was not due to a loss of polarity of the transport mechanism in either species.

The results of these experiments suggest that glyphosate affected [14C]IAA transport in both species examined. The major difference between the two species appeared to be one of degree with cotton showing more tolerance than corn. This pattern agrees with the inherent sensitivity of the two species to glyphosate. The level of glyphosate necessary to stimulate tillering in corn (and sorghum) was 11.2 µg/plant (2), whereas in cotton, 12 µg/plant was necessary to produce mild epinasty. In cotton, glyphosate treatments were ineffective unless they were applied to the test seedling 24 h before [14C]IAA transport measurements were begun; in corn, however, simultaneous application of glyphosate and [14C]IAA affected transport of the auxin. The delayed effect of glyphosate on auxin transport in cotton is similar to that of ethylene. Auxin transport is not altered when it is measured in the presence of ethylene (1, 8); however, transport is significantly reduced by fumigation of plants with ethylene 4 to 18 h before transport is measured (8).

Beyer and Morgan (6) demonstrated that auxin transport capacity was severely reduced in stem sections from cotton seedlings pretreated with ethylene. These workers also noted that the transport velocity sometimes was slightly reduced, but not enough to account for the observed reduction in auxin transport capacity (6). In the present study, reductions in both auxin transport capacity and velocity may have been due to glyphosate-induced ethylene production. The tendency for [14C]IAA to accumulate in the apical 2-mm segments of glyphosate-exposed cotton hypocotyls and corn coleoptiles is suggestive of an ethylene effect. A similar pattern of accumulation has been noted in stem sections from cotton plants pretreated with ethylene (7). The observation in the present study that cotton seedlings treated with glyphosate developed a downward reflection (mild epinasty) of the cotyledonary leaves also suggests that glyphosate induced ethylene production. Similar symptoms of mild epinasty have been noted for cotton (18), and for honey mesquite (Prosopis juliflora) (Swartz) DC. cv. glandulosa

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Table II. Effect of glyphosate on [14C]IAA transport in cotton hypocotyls.

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<thead>
<tr>
<th>Transport Period</th>
<th>Transport Velocity</th>
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<td></td>
<td>Treated</td>
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<td>h</td>
<td></td>
</tr>
<tr>
<td>1.0 to 2.0</td>
<td>8.0</td>
</tr>
<tr>
<td>2.0 to 2.5</td>
<td>6.3</td>
</tr>
<tr>
<td>2.5 to 3.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Mean</td>
<td>6.4</td>
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</tbody>
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Fig. 3. Effect of glyphosate on the accumulation, during a 3.5-h transport period, of [14C]IAA in the lowest (basal) segment and the receiver blocks (inset) from 7-day-old cotton hypocotyls treated with glyphosate at 112 µg/plant 24 h before measurement (see Table II). Data points are percentages of total hypocotyl radioactivity that were found in the basal segment. Inset: radioactivity (cpm) recovered in the receiver blocks; each bar represents total cpm in 10 blocks. Values shown for each transport period are means of four separate experiments; t-tests were run on data pairs (treated versus control) before averaging. **: significantly different at the 0.01 level.
(Torr.) Cockerell] seedlings fumigated with ethylene (5, 18).

In corn coleoptiles, glyphosate severely inhibited [14C]IAA transport when applied during the course of transport measurement. The absence of [14C]IAA diffusion into chase blocks during the course of measurement, as well as the tendency of [14C]IAA to accumulate in the upper (apical) portions of the coleoptile, suggests that glyphosate may be inhibiting transport via an immobilizing or binding effect. Transport inhibition by 1-N-1-naphthylphthalamic acid (16) and by the morphactin 2-monochloro-9-hydroxyfluroene-9-carboxylic acid methylester (15) has been attributed to immobilizing or binding effects.

The inhibiting effect of glyphosate on [14C]IAA transport in corn provides a possible explanation for the glyphosate-induced tillering noted in sorghum (2, 4). Tiller buds are normally suppressed by endogenous auxin produced by vegetative shoots. Any reduction in the supply of auxin to the basal meristematic zone will cause tiller buds to be released. Application of glyphosate could reduce basipetal transport of auxin below the level needed for suppression of tiller buds.

The similarity in the effects of glyphosate on [14C]IAA transport in corn coleoptile and cotton hypocotyl tissues justifies the suggestion that glyphosate may exert a similar effect on the transport of auxin from other vegetative tissues.

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