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Uptake, Translocation, and Metabolite Partitioning of ^{14}C-Labeled Metribuzin in Plant Growth–Regulated Soybean (*Glycine max*)

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Abstract. Plant growth regulator (PGR) application decreased uptake of 10^{-6} M ^{14}C-labeled metribuzin (4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one) into leaf interveinal areas of 21-day-old soybean seedlings. BAS 140 810, (*N*-allyl-*N*-2-(2,4,6-trichlorophenoxy)ethyl-piperidinium-bromide), as a seed treatment or 10^{-6} M triapenthenol or RSW 0411 (*B*-(cyclohexalmethylene)-gamma-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol) in nutrient solution slowed interveinal unloading of metribuzin and altered metabolite pools. Stems and roots of PGR-treated plants exhibited significantly greater water-soluble metabolite pools than untreated controls. TLC metabolite identification indicated an increase in metribuzin conjugates. This may contribute to the mode of action involved in the apparent safening mechanism. Furthermore, floating leaf disk studies with metribuzin showed plant growth regulation figured prominently in safening against the cessation of oxygen evolution.

Genetically defined tolerance to metribuzin (Souza-Machado et al. 1978, Edwards et al. 1976) resides in the ability of the plant to detoxify the herbicide moiety before it reaches the chloroplast (Souza-Machado and Ditto 1982). Recently, however, Vavrina and Phatak (1986) and Vavrina (1986) have shown that even susceptible soybean cultivars received some safening against metribuzin injury after treatment with plant growth regulators (PGR). Differential rates of metribuzin metabolism appear to determine intraspecific soybean tolerance; polar metabolites play the major role (Falb and Smith 1984, Mangeot et al. 1979). Frear et al. (1985) have identified a homoglutathione-metribuzin conjugate as the major polar metabolite in soybean; in tomato, a beta-D-(N-glucoside) conjugate appears the dominant moiety (Frear et al. 1983). Phatak et al. (1985) have shown that the growth regulator daminozide (butanedioic acid

mono (2,2-dimethylhydrazide)) applied foliarly in potato (*Solanum tuberosum* L.) can safen against metribuzin injury while correspondingly increasing plant-soluble solids.

The objective of this study was to characterize the uptake, translocation, and metabolite partitioning in ^{14}C metribuzin-treated soybean seedlings previously treated with PGRs. A metribuzin-tolerant soybean cultivar, Braxton, was used to determine possible alterations in the genetically defined tolerance of soybean to metribuzin.

Materials and Methods

Greenhouse studies were conducted in Athens and Griffin, Georgia (1985, 1986), with certified Braxton soybean seed obtained from the Georgia Seed Development Commission. The use of the tolerant variety Braxton insured the survival of plants after the application of metribuzin in the greenhouse and complemented ongoing field studies. Greenhouse temperatures were maintained between 26 and 30°C throughout the studies. Supplemental light (200 $\mu E/m^2/sec$) was added when necessary to produce a 16-h/8-h light/dark photoperiod.

Soybeans germinated in quartz sand were transplanted when the cotyledons stood erect to floating styrofoam mats (40 plants per mat) in 8-L containers of one-quarter strength modified Hoagland's solution (Hoagland and Arnon 1950). Prior to planting, some seeds received a seed treatment of BAS 140 810 at 3.63 ml/454 g of seed (100 g/1000 ml active material). Triapenthenol at 10^{-6} M was added to the nutrient solution, specific to that treatment, at the time of transplanting. Three treatments were thus established: a control, triapenthenol-treated, and BAS 140 810–treated. Plants were thinned to 24 per mat at cotyledon leaf stage. Deionized water was used to replace transpiration losses.

Twenty-one days after planting, all seedlings received fresh nutrient solution containing 10^{-6} M 0.51 μCi/L, ring-labeled, ^{14}C metribuzin. The specific activity of the radioactive metribuzin was 4.44 mCi/mmol. Test plants had two trifoliolate leaves; however, PGR treated plants were generally smaller.

Time Course

A time course study of uptake and translocation was undertaken via harvests at 12, 24, 48, and 96 h of six plants from each container. One plant from each replication was autoradiographed, and five were separated into leaf, stem, and root segments and lyophilized. Some lyophilized tissue was combusted by a Packard Tri-Carb model B306 sample oxidizer for total ^{14}C, and some tissue was extracted with 80% ethanol via procedures of Falb and Smith (1984) and Smith and Wilkinson (1974) to delineate polar and nonpolar metabolite pools. Modifications in the extraction procedure involved metabolite identification from fluorescent TLC plates under 245-nm UV light rather than color tests or the radiochromatogram scanner identification and ammonium hydroxide:ethanol:n-butanol (1:1:2) rather than water:ethanol:n-butanol as the TLC solvent.

The experiment was repeated three times during the winter and spring of 1985 and 1986. Each experiment was analyzed as a split-plot design (main plots = treatment; subplots = time) with four replications per treatment.

Floating Leaf Disk Study

A laboratory study was carried out to ascertain if PGR application might safen soybean leaf tissue against metribuzin when in close contact with the chloroplast. The methods of Truelove et al. (1974) were used to assay the effect of metribuzin at 10^{-6} M on floating leaf disks of soybean cv. Braxton. The disks were carried for a period of 72 h, and though continuous agitation was not supplied, vigorous swirling was employed for 5 min each hour for 12 h of each 24-h cycle. The experiment was a randomized complete block design with four replications.

Results

Autoradiographs

Autoradiographs of the plants from the time course study indicate increased sequestering of ^{14}C from the ^{14}C-metribuzin in the veins of Braxton soybean plants treated with either growth regulator, particularly through the first 48 h. By 96 h, the PGR-treated plants had accumulated more total ^{14}C than the controls, perhaps as a result of continued transpiration. Control plant damage from parent metribuzin at 96 h was extensive enough to have curtailed transpiration, whereas reduced photosynthetic apparatus injury in triapenthenol and BAS 140 810–treated plants could have fostered continued transpiration. Comparatively more ^{14}C-metribuzin or ^{14}C-metabolite occurred in leaf margins and interveinal areas (site of major herbicide action) of control plants than in PGR-treated plants. This increased the probability of injury. Falb and Smith (1984) also observed greater sequestering of metribuzin in veins of tolerant vs. metribuzin-susceptible lines.

Time Course ^{14}C Uptake

The progressive accumulation of metribuzin and metabolites viewed in the autoradiographs was further defined by the combustion analysis shown in Table 1. Soybean roots contained similar amounts of ^{14}C-metribuzin/metabolite per gram tissue across treatments throughout the time course.

The accumulation of ^{14}C in soybean stems of PGR-treated plants remained significantly higher throughout the time course study. Frear et al. (1985) indicated that vascular localization of metribuzin was a primary factor influencing tolerance in soybean. The antigibberellin effect of these PGRs, by increasing vascular sequestering of metribuzin and its metabolite moieties within Braxton

Table 1. Accumulation of total ^{14}C (metribuzin/metabolites) from combustion analysis over a 96-h period in PGR-treated soybean seedlings 21–25 days after planting (μg/g tissue).

Time (h)	Organ	Control	Triapenthenol	BAS 140 810	LSD 5%
12	Leaf	3.439	1.392	2.040	0.753
	Stem	1.842	2.398	2.682	0.347
	Root	4.693	4.171	4.337	N.S.
24	Leaf	5.367	2.007	3.298	0.882
	Stem	3.430	4.291	5.226	0.751
	Root	5.512	5.624	6.881	N.S.
48	Leaf	10.340	4.801	7.170	1.469
	Stem	5.763	7.343	8.526	1.284
	Root	10.404	9.123	9.849	N.S.
96	Leaf	17.238	12.144	14.266	2.321
	Stem	10.294	13.655	18.194	3.458
	Root	14.797	20.090	16.792	N.S.

soybean, appears to play a significant role in the PGR antidoting effect seen in the field (Vavrina 1986).

Visual injury at 96 h had advanced to >50% necrosis in control leaves while remaining <25% in BAS 140 810– and <5% in triapenthenol-treated plants. Total ^{14}C accumulation within the leaves of PGR-treated plants was lower than that of the control plants throughout the study.

Total plant ^{14}C uptake on a per-gram tissue basis resulted in no significant difference as a function of treatment. Metribuzin-susceptible plants are known to utilize more water (Srobarov et al. 1983, Vavrina and Phatak 1984). Cumulative water use in seedling control plants was shown to be greater than that of seedling PGR-treated plants (Vavrina et al. 1986), basically as a result of greater leaf area. However, on a water use per-gram tissue basis, no difference occurs between control and PGR-treated plants (Vavrina 1986). This supports the fact that increased ^{14}C levels in metribuzin-treated control leaves was not merely a function of a bulk flow of water.

Metabolite Partitioning

Table 2 displays the partitioning of polar and nonpolar metabolites from ethanol extracts of soybean plants treated 96 h with metribuzin. Nonpolar metabolite pools did not vary among treatments. Only the PGR-treated plant stem polar metabolite pool showed significantly higher levels of ^{14}C accumulation when compared to the control plants. No difference occurred in polar metabolite partitioning between PGR treatments themselves.

The production rate of polar metabolite has been cited (Falb and Smith 1984) as a major factor contributing to differential tolerance in soybean cultivars. The inherent tolerance of soybean cv. Braxton appeared heightened by the addition of the growth regulator treatments. The significance of increased levels of polar metabolite in the stem, a major site of metribuzin detoxification

Table 2. A comparison of polar and nonpolar metribuzin/metabolite pools in growth-regulated and non-growth-regulated soybean plants treated 21 days after planting with 10^{-6} M ^{14}C metribuzin for 96 h (μg/g).

Organ	Control	Triapenthenol	BAS 140 810	LSD 5%
Polar metabolite				
Leaf	0.37	0.34	0.33	N.S.
Stem	0.20	0.32	0.42	0.12
Root	0.55	0.72	0.58	N.S.
Nonpolar metabolite				
Leaf	0.27	0.30	0.27	N.S.
Stem	0.07	0.09	0.09	N.S.
Root	0.14	0.15	0.14	N.S.

Table 3. Partitioning of metribuzin and metabolites as a percent of the total radioactivity by thin-layer chromatography separation.

Moiety	R_f	Control (%)	Triapenthenol (%)	BAS 140 810 (%)	LSD 5% (%)
Metribuzin	0.83–1.0	21.71	17.43	18.71	N.S.
DA, DK[a]	0.73–0.83	15.00	12.42	13.57	1.37
DADK	0.63–0.73	16.00	13.00	15.29	N.S.
Residual A[b]	0.43–0.63	17.29	21.86	19.00	2.92
Residual B	0.23–0.43	17.43	21.86	21.00	1.78
Residual C	0.0–0.23	12.57	13.57	12.42	N.S.

[a] DA = deaminated; DK = diketo; DADK = deaminated-diketo metribuzin metabolites.
[b] Conjugate forms were inseparable by TLC method; however, conjugate R_f values coincided with previously published reports of an "unknown" metabolite.

(Falb and Smith 1984, Frear et al. 1985), further contributes to PGR antidotal capacity.

Leaf and root concentrations of polar metabolite were similar across treatments, yet PGR-treated plant visual injury was reduced. This points to a metabolite-partitioning phenomenon; although less ^{14}C was accumulated in PGR-treated leaves (Table 1), more polar metabolite was produced.

Metabolite Identification

TLC separation of the ethanol extractions revealed a modification in metabolite partitioning (Table 3). Although no difference occurred in parent metribuzin, the deaminated-diketo (DADK) moiety, and conjugate C, PGR-treated plants produced more conjugate A and B metabolites and less deaminated (DA) and diketo (DK) moieties than the non-growth-regulated control plants.

Frear et al. (1985) have shown the primary metabolites of metribuzin in soybean to be a homoglutathione conjugate and an N-glucoside conjugate. Separation of the predominate conjugate in this work was impossible with methods at our disposal, and conjugate R_f values in our study differed slightly from pre-

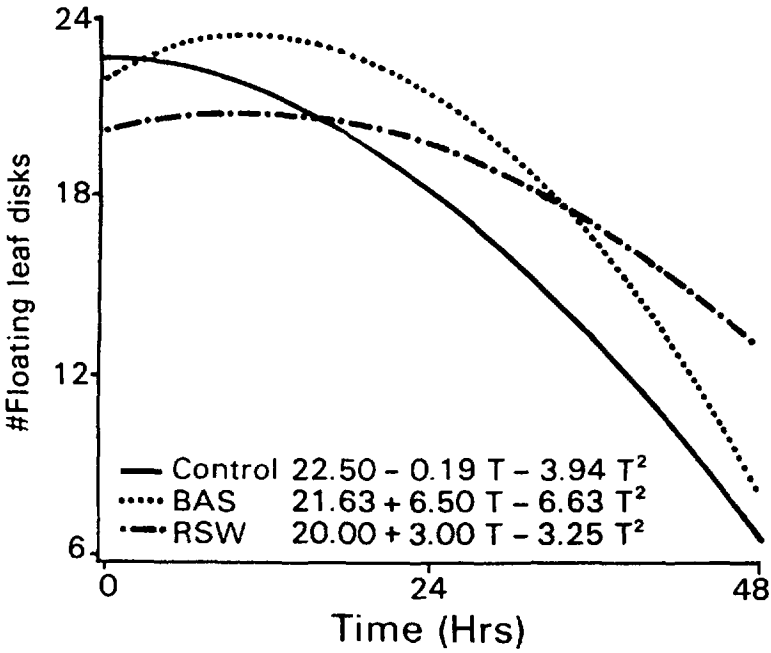

Fig. 1. The effect of plant growth-regulating chemicals on floating leaf disks of soybean immersed in a solution containing 10^{-6} M metribuzin.

viously published reports by virtue of a modified solvent system. However, the relative positioning of those metabolites in the conjugate portion of the separation coincided closely with previously published reports of a possible conjugate or "unknown" (Falb and Smith 1984, Mangeot et al. 1979). Vascular sequestering of the herbicide and its metabolites increase the probability of the occurrence of a "washing out" phenomenon (Duke 1985) and conjugation formation.

Floating Leaf Disk Study

Figure 1 depicts the response of soybean leaf tissue, with and without growth regulation, to metribuzin at 10^{-6} M in a floating disk assay. Both triapenthenol- and BAS 140 810–treated tissues required longer periods of time before oxygen evolution ceased and tissue subsequently sank. Differing area-to-weight ratios (Vavrina 1986) may be partially involved in this phenomenon, as may differences in metabolism resulting from PGR treatment (Vavrina 1986).

Discussion

In general, four classes of antidote action (Hatzios 1983) are considered: (1) interference with herbicide uptake and/or translocation; (2) competitive inhibi-

tion at the site of action with the herbicide; (3) stimulation of herbicide metabolism; and (4) a combination of mechanisms.

The PGR-treated system described here appears to involve interference with herbicide translocation and stimulating herbicide metabolism. As tolerance and susceptibility to metribuzin are governed by detoxification and immobilization of the herbicidal moiety (Frear et al. 1983, 1985), the amount of parent compound available to the choloroplast determines the level of injury (Mangeot et al. 1979).

Plant growth regulation with antigibberellins can offer antidotal properties against the photosynthetic inhibiting herbicide metribuzin through (1) increased vascular localization of parent compound and metabolites, (2) increased polar metabolite production, and (3) altered metabolite pools favoring metribuzin conjugates.

The isolated floating leaf disk system insures the herbicide will swamp out the photosynthetic apparatus, resulting in eventual cessation of oxygen evolution. However, if a treatment can extend the length of time to that cessation of oxygen evolution in the disk system, it may provide enough time to deactivate the herbicidal moiety and provide effective herbicide safening in the dynamic plant system.

Beyond the metabolic effects of plant growth regulation on metribuzin detoxification are the physiological effects. Soybean leaf structure component and chlorophyll content were increased by PGR treatment (Vavrina 1986), and this may have provided increased binding sites for the polar metabolite moieties also. Increased chlorophyll and carotenoid levels (Vavrina 1986) could aid in quenching free radicals generated in the photosynthetic process or during lipid peroxidation from herbicide action. Furthermore, the stoichiometry of herbicide molecule to photosynthetic unit could be altered by increased chlorophyll level, resulting in less damage from the same amount of herbicide.

Smaller, thicker leaves (Vavrina 1986) may provide increased barriers to herbicide movement. Water use modifications (Vavrina 1986) may affect metribuzin uptake. Reduced seedling water use will result in longer residence of metribuzin in the soil/water column, where microbial and physical factors could contribute to chemical degradation. All these factors may contribute to greater survival of PGR-treated seedling plants.

Regardless of the economic impact of the PGR/herbicide antidote consideration, PGR/herbicide combinations may provide some interesting concepts for agriculture and plant physiology.

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