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Absorption, Translocation, and Metabolism of ^{14}C-Thifensulfuron in Soybean (*Glycine max*), Spurred Anoda (*Anoda cristata*), and Velvetleaf (*Abutilon theophrasti*)

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Abstract. The absorption, translocation, and metabolism of thifensulfuron-methyl {methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl]-amino]-carbonyl]amino]sulfonyl]-2-thiophenecarboxylate} were investigated in tolerant "Essex" soybean [*Glycine max* (L.) Merr.], moderately tolerant "Vance" soybean, and spurred anoda [*Anoda cristata* (L.) Schlecht.], and susceptible velvetleaf (*Abutilon theophrasti* Medic.]. Radiolabeled (thiophene-2-^{14}C) thifensulfuron-methyl was absorbed readily by young seedlings of all species following a foliar spray with the herbicide. Spot-applied ^{14}C-thifensulfuron-methyl was absorbed by the treated leaf of all species. Absorption of thifensulfuron-methyl was limited when excised stems of all species were dipped into the herbicide solution for 2 h. Translocation of absorbed thifensulfuron-methyl to other plant parts was limited in all species, regardless of the method of its application. Root exudation of leaf-applied thifensulfuron-methyl was observed in all species and it was higher in seedlings of spurred anoda and velvetleaf. The two soybean cultivars metabolized 62–70% of absorbed thifensulfuron-methyl at 3 days after treatment with spot-applied ^{14}C-thifensulfuron. Velvetleaf and tolerant spurred anoda metabolized about 50% of the absorbed herbicide. The major metabolite formed in all species appeared to be deesterified thifensulfuron acid. Differential metabolism seems to be a contributing factor in the selectivity of thifensulfuron-methyl between the two soybean cultivars and velvetleaf. The metabolic basis for the moderate tolerance of spurred anoda to thifensulfuron-methyl is not understood at the present time.

Thifensulfuron-methyl, previously known by the names DPX-M6316 and thiameturon, is a selective postemergence sulfonylurea herbicide used in soybean and cereal grains for broadleaf weed control (Eberlein and Miller 1989, Green 1991, Sionis et al. 1985). Thifensulfuron-methyl provides excellent control of common lambsquarters (*Chenopodium album* L.) and velvetleaf in soybeans (Green 1991).

Differences in uptake and translocation (Hess 1985), sensitivity of target site (Brown 1990), metabolic inactivation (Brown 1990, Hatzios and Penner 1982), or placement (Hatzios and Penner 1982) have long been established as major factors contributing to the observed crop selectivity of most herbicides.

The mechanism of the herbicidal activity of all sulfonylureas, including thifensulfuron-methyl, has been identified as inhibition of acetolactate synthase (ALS, EC 4.1.3.18), a key enzyme in the production of the branched chain amino acids valine, leucine, and isoleucine (Brown 1990, Ray 1984). Mutations leading to changes in the chemistry and configuration of the ALS enzyme have been responsible for the development of biotypes of selected weeds that are resistant to these herbicides (Brown 1990) as well as for engineering sulfonylurea resistance to selected crops (Sebastian et al. 1989). In general, however, differential sensitivity of the target site has not been connected with the crop selectivity of sulfonylurea herbicides observed under field conditions (Brown 1990, Brown and

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Neighbors 1987, Brown et al. 1990, Green and Ulrich 1993).

Previous studies on the selectivity of sulfonylurea herbicides have established rapid metabolic inactivation as the basis for the observed crop selectivity of these compounds (Anderson et al. 1989, Brown 1990, Brown and Neighbors 1987, Sweetser et al. 1982). Investigations on the basis of the observed crop selectivity of thifensulfuron-methyl among crop and weed species have been published only recently. Brown et al. (1990) and Long et al. (1988) showed that differential metabolism, rather than differential absorption and translocation, was responsible for the selectivity of thifensulfuron-methyl among 'Williams 82' soybean, smooth pigweed (*Amaranthus retroflexus* L.), common lambsquarters, velvetleaf, and tall morningglory [*Ipomoea purpurea* (L.) Roth]. Similarly, rapid metabolism was found to be the basis for the selectivity of thifensulfuron in cereal crops such as wheat and corn (Cotterman and Saari 1989, Eberlein and Miller 1989, Eberlein et al. 1989).

The present study was conducted to determine 1) the growth responses of Essex and Vance soybean, spurred anoda and velvetleaf to foliarly applied thifensulfuron and 2) the potential differences in absorption, translocation, and metabolism of thifensulfuron-methyl among the two soybean cultivars, spurred anoda, and velvetleaf.

Materials and Methods

Chemicals

Formulated, analytical, and radiolabeled samples of the methyl ester of thifensulfuron were provided by E.I. DuPont de Nemours & Co., Inc., Wilmington, Delaware. The radiochemical purity of [thiophene-2-^{14}C]thifensulfuron was 98%, as determined by TLC analysis, and its specific activity 4.15 mCi/mmol.

Growth Response Studies

Greenhouse studies were conducted to determine the effect of thifensulfuron-methyl on the growth of the two soybean cultivars, spurred anoda, and velvetleaf. Seedlings of all species were grown in 960-ml styrofoam containers (four plants per container) containing 1.3 kg of State sandy loam soil (Typic Hapladults), which was 67% sand, 28% silt, 5% clay, and 1% organic matter with a pH of 6.1. All seedlings were grown in a greenhouse with a temperature of 25 ± 5°C, a 16-h photoperiod, and a photosynthetic photon flux density (PPFD) of about 550 μE m^{-2} s^{-1}.

Plants were watered as necessary, and each container was fertilized weekly with 125 mg of a water-soluble fertilizer (Peter's all-purpose 20-20-20, W.R. Grace & Co., Fogelsville, PA 18051) in 50 ml of water. Formulated thifensulfuron-methyl was applied to the foliage (postemergence) with a propane-pressurized backpack sprayer delivering 190 L ha^{-1} spray mix at 220 kPa pressure with flat fan tips (Teejet 8003, Spraying Systems Co., Wheaton, IL 60827). Plant stages at treatment time were the third trifoliate leaf stage for soybean and the four- to five-true leaf stage for the two weed species. Thifensulfuron was applied at 0, 1.1, 2.3, 4.5, and 9.1 g ha^{-1}. A nonionic surfactant (X-77) was included at 0.125% of the spray volume.

Treated plants were arranged on greenhouse benches in a completely randomized design. Treatments were replicated three times, and the study was repeated in time. Shoot fresh weights were determined at 10 days after treatment (DAT). Injury was expressed as percentage (%) reduction of shoot growth compared to untreated controls. Homogeneity of variance allowed combination of experiments over time. Data were converted to percentage of control, transformed to the arcsine, and subjected to analysis of variance. Means were separated by the LSD test ($p = 0.05$).

Absorption and Translocation Studies

Seeds of Essex and Vance soybean, spurred anoda, and velvetleaf were germinated in sterilized sand and grown in a greenhouse with a temperature of 25 ± 5°C, a 14-h photoperiod and 550 μE m^{-2} s^{-1} PPFD. At 7 days prior to the application of ^{14}C-thifensulfuron-methyl, seedlings were transferred to 150-ml cups filled with full-strength Hoagland's solution. When the soybean seedlings were at the third trifoliate stage and the weed species were at the four- to five-true leaf stage, ^{14}C-thifensulfuron-methyl was applied to seedlings of all plant species by using each of the following three methods.

1. ^{14}C-thifensulfuron-methyl (90 nCi) dissolved in 1 ml of 80% acetone was applied as foliar spray with an atomizer using 0.125% of X-77 surfactant. Seedlings were harvested at 8 and 72 h after treatment.
2. Five 2-μl droplets of ^{14}C-thifensulfuron-methyl (90 nCi) dissolved in 80% acetone and combined with 0.125% X-77 surfactant were applied to the center leaflet of the first trifoliate leaf of soybeans and to the third true leaf of spurred anoda and velvetleaf. Treated seedlings were harvested at 24 and 120 h after treatment.
3. Seedlings were carefully removed from the hydroponic solution and placed in a water bath at 20°C. Roots were excised under water with a razor blade. Excised stems were then transferred to bottles containing 130 ml of Hoagland solution treated with ^{14}C-thifensulfuron-methyl (90 nCi) and exposed to the herbicide for 2 h. Following exposure, the excised stems were dipped in 5 ml of 80% acetone for 1 min, and a set of seedlings was harvested, while the rest were returned to fresh, untreated nutrient solution and harvested at 12 and 72 h after treatment.

Harvested seedlings from all application methods were washed with 80% acetone to remove any unabsorbed herbicide and separated into leaves, stems, roots, and treated leaves, as appropriate according to the application method used. Plant tissues were weighed and dried in an oven at 50°C for 48 h. Absorption and translocation of ^{14}C into plant tissues were quantified by combustion in a biological sample oxidizer (Tricarb Model BO306, Packard Instruments, Dowers Grove, IL 60515) and counting of the radioactivity, trapped as $^{14}CO_2$, in a liquid scintillation spectrometer (Beckman LS 5000TA Model). Radioactivity in the acetone rinsates and in the nutrient solution was measured by liquid scintillation counting (LSC). Distribution of ^{14}C in plant tissues was determined as dpm mg^{-1} and then ex-

Table 1. Shoot growth reduction of 'Essex' and 'Vance' soybeans, velvetleaf, and spurred anoda, 10 days after foliar treatment with thifensulfuron-methyl

Plant species or cultivar	Percentage (%) reduction[a] of shoot fresh weight as affected by thifensulfuron-methyl			
	1.1[b]	2.3[b]	4.5[b]	9.1[b]
Essex soybean	21	16	26	47
Vance soybean	23	33	40	53
Spurred Anoda	21	31	44	47
Velvetleaf	78	79	85	84
LSD (0.05)	3	3	3	3

[a] Based on shoot fresh weight of untreated plants.
[b] g ha^{-1}.

pressed as a percentage (%) of applied radioactivity. A completely randomized design with two replications was used and each experiment was duplicated.

Metabolism Studies

Plants were grown and treated with 90 nCi of ^{14}C-thifensulfuron-methyl by using the second method described in the previous section. Seedlings were harvested at 3 days after treatment and treated leaves were washed with 5 ml of 80% acetone. Metabolite and parent herbicide extraction followed the methodology of Brown et al. 1990. Plant tissues were frozen in liquid nitrogen, ground with mortar and pestle, and homogenized with 20 ml of 80% acetone. The homogenates were centrifuged at 1200 g for 10 min, and the supernatant was removed and saved. The pellets were extracted two more times in 80% acetone and once more with 90% acetone. The combined supernatants (ca. 80 ml) were concentrated by rotoevaporation to 2 ml.

The concentrated extract was then separated into two 1-ml samples, and the pH of one of the samples was adjusted to 3.0 with 1*N* HCl to determine the potential effect of acidity on the stability of the parent herbicide and any of its metabolites. Aliquots (15 µl) from both samples were then spotted onto silica gel reverse-phase TLC plates [Si-C_{18}-F(19C), J.T. Baker Chemical Co., Phillipsburg, NJ, USA] and developed in acetonitrile/water/formic acid (75:24.9:0.1, v/v/v). Plates were scraped into 1-cm sections, and radioactivity was determined by LSC. Metabolites were separated by their Rf values, and the distribution of radioactivity detected in each metabolite was expressed as percentage of the total radioactivity recovered during the TLC analysis of the plant extracts. A completely randomized design with two replications was used and each experiment was repeated.

Results and Discussion

Growth Response Studies

'Essex' soybean was the most tolerant of all species treated with rates equal or lower than the recommended rate (5 g ha^{-1}) of thifensulfuron-methyl (Table 1). At 10 DAT, shoot fresh weight of 'Essex' soybean treated with 4.5 g ha^{-1} or lower rates of thifensulfuron-methyl was reduced by about 16–26%. The highest rate of the herbicide (9.1 g ha^{-1}) reduced the shoot fresh weight of 'Essex' seedlings by 47% (Table 1). Velvetleaf was very sensitive, exhibiting 78–85% reduction in shoot fresh weight 10 DAT after treatment with all rates of the herbicide. 'Vance' soybean and spurred anoda were intermediate in their response to thifensulfuron-methyl. Compared to 'Essex' soybean, the growth of 'Vance' and spurred anoda was affected more by the 2.3 and 4.5 g ha^{-1} of thifensulfuron-methyl. However, their shoot fresh weights were reduced to the same extent as that of 'Essex' soybean, following treatment with the highest rate of the herbicide (Table 1).

These results confirm earlier reports characterizing velvetleaf as a sensitive weed to thifensulfuron and other sulfonylurea herbicides and soybean as a tolerant crop (Brown et al. 1990, Long et al. 1988). Although significant growth reduction occurred at 10 DAT, field trials have shown that 'Essex' soybean and spurred anoda recovered within 21–30 DAT with high rates of thifensulfuron-methyl (Walker 1991). 'Vance' soybean, however, was unable to fully recover (Walker 1991).

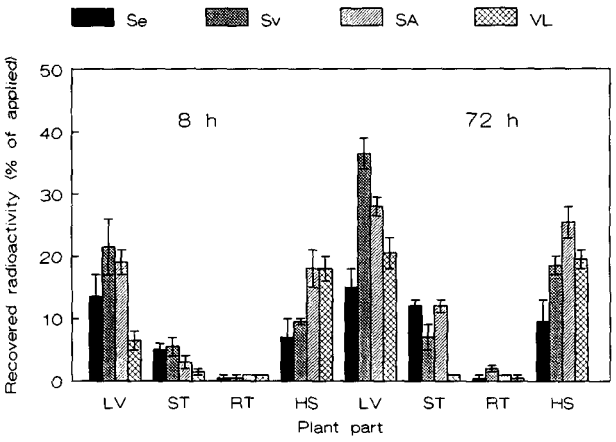

Fig. 1. Distribution of total ^{14}C recovered in leaves (LV), stems (ST), roots (RT), and Hoagland's solution (HS) at 8 and 72 h after treatment of seedlings of 'Essex' (Se) and 'Vance' (Sv) soybean, spurred anoda (SA), and velvetleaf (VL) with foliarly-applied ^{14}C-thifensulfuron-methyl. Scale bars represent mean standard deviations.

Absorption and Translocation of Thifensulfuron

At 8 h after foliar spray with ^{14}C-thifensulfuron-methyl, the respective percentages of applied ^{14}C recovered in the leaves of 'Essex' and 'Vance' soy-

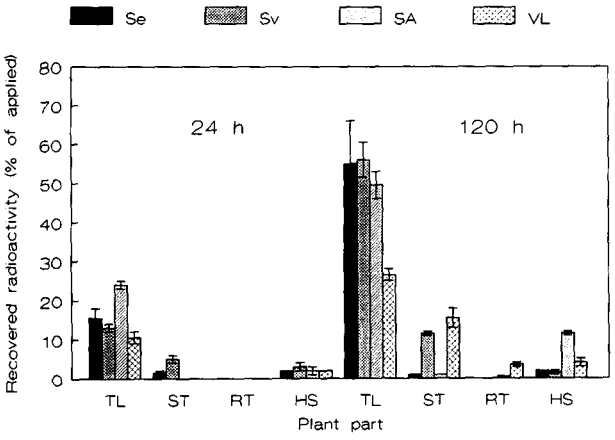

Fig. 2. Distribution of total ^{14}C recovered in treated leaf (TL), stems plus other leaves (ST), roots (RT), and Hoagland's solution (HS) at 24 and 120 h after foliar spot application of ^{14}C-thifensulfuron-methyl to seedlings of 'Essex' (Se) and 'Vance' (Sv) soybean, spurred anoda (SA), and velvetleaf (VL). Scale bars represent mean standard deviations.

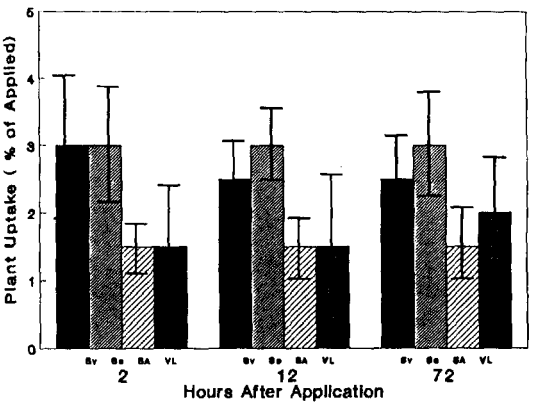

Fig. 3. Absorption of ^{14}C by 'Essex' (Se) and 'Vance' (Sv) soybean, spurred anoda (SA), and velvetleaf (VL) at 2, 12, and 72 h after cut seedling stems were exposed for 2 h to a nutrient solution containing ^{14}C-thifensulfuron-methyl. Scale bars represent mean standard deviations.

bean, spurred anoda, and velvetleaf were 13.5, 21.5, 19, and 6.5% (Fig. 1). Translocation of ^{14}C from the treated leaves to roots was limited in all species, but the two soybean cultivars translocated more radioactivity to their stems than either of the two weed species. A sizeable amount of the applied radioactivity (7–18%) was recovered in the hydroponic nutrient solution, suggesting a strong potential for root exudation of leaf-applied thifensulfuron-methyl (Fig. 1). Preliminary analysis of radioactive exudates from all plant species by TLC showed that unmetabolized thifensulfuron-methyl accounted for the great majority of radioactivity exuded into the hydroponic solution.

At 72 h after foliar spray with ^{14}C-thifensulfuron, the amount of the applied ^{14}C that was recovered in the leaves of 'Vance' soybean and spurred anoda was again greater than that recovered in the leaves of 'Essex' soybean and velvetleaf (Fig. 1). Movement of ^{14}C from the leaves to stems was considerable in the two soybean cultivars and spurred anoda, but translocation of ^{14}C to the roots was again very limited in all species examined. Root exudation of foliarly applied radioactivity was again significant in all species, particularly 'Vance' soybean, spurred anoda, and velvetleaf.

Figure 2 shows the absorption and distribution of ^{14}C at 24 and 120 h following spot application of five 2-μl droplets of radiolabeled thifensulfuron-methyl to the center leaflet of the first trifoliate of soybean and the third true leaf of spurred anoda and velvetleaf. Almost all of the recovered radioactivity remained in the treated leaf. Absorption of thifensulfuron-methyl was greatest in the two soybean cultivars and spurred anoda and lowest in sensitive velvetleaf.

At 24 h after the spot-application of ^{14}C-thifensulfuron-methyl, translocation of radioactivity out of the treated leaf was limited in all species. At 120 h after treatment, 10–15% of the applied radioactivity had translocated to stems plus other leaves or roots of 'Vance' soybean and velvetleaf (Fig. 2). At the same time period, translocation of radioactivity to other parts was quite limited (1.5% of applied) in the tolerant 'Essex' soybean and spurred anoda (Fig. 2).

At 24 h after the spot-application of ^{14}C-thifensulfuron, about 2% of the radioactivity spotted on the treated leaf of all species was recovered in Hoagland's solution, indicating movement of radioactivity and exudation through the root system (Fig. 2). At 120 h, root exudation of ^{14}C did not increase significantly in the two soybean cultivars and velvetleaf, but in spurred anoda a significant amount of the applied radioactivity (11.5%) was recovered in Hoagland's solution (Fig. 2). Exudation of nicosulfuron, another sulfonylurea herbicide from the roots and rhizomes of johnsongrass [*Sorghum halepense* (L.) Pers.] has been reported recently by Nagabhaushana et al. (1992).

At 2 h after treatment, excised stems of the two soybean cultivars that were dipped into Hoagland's solution containing ^{14}C-thifensulfuron-methyl, absorbed about 3% of the absorbed radioactivity (Fig. 3). At the same period, spurred anoda and velvetleaf absorbed only 1.5% of the applied radioactivity. The levels of uptake remained approximately the same at 12 and 72 h after the 2 h exposure to radiolabeled herbicide (Fig. 3).

Table 2. TLC analysis of acetone-soluble substances extracted from leaves of 'Essex' and 'Vance' soybean, spurred anoda, and velvetleaf at 3 days after treatment with ^{14}C-thifensulfuron-methyl

Metabolite number	Rf value[a]	% of Radioactivity recovered			
		Essex	Vance	S. Anoda	Velvetleaf
1	0.0	8	9	5	12
2	0.5	8	7	5	7
3	0.6	7	8	4	5
4	0.7	8	8	6	8
5	0.8	31	40	32	21
6	0.9	38	30	48	47

[a] The methyl ester of ^{14}C-thifensulfuron chromatographed at Rf 0.9.

Metabolism of Thifensulfuron

A set of six radiolabeled substances were detected by TLC analysis of extracts from all species (Table 2). The Rf values of these metabolites in the acetonitrile/water/formic acid (75:24.9:0.1 v/v/v) developing system were as follows: metabolite no. 1, 0.0; metabolite no. 2, 0.5; metabolite no. 3, 0.6; metabolite no. 4, 0.7; metabolite no. 5, 0.8, and metabolite no. 6, 0.9 (Table 2).

A sample of the standard radiolabeled herbicide migrated to an Rf value of 0.9. Thus, metabolite no. 6, which was found in all species, is likely the unmetabolized methyl ester of thifensulfuron. A major metabolite with an Rf value of 0.8 (metabolite no. 5) was also detected in all species and it is believed to be the deesterified acid of thifensulfuron (H. M. Brown, 1991, personal communication. DuPont de Nemours & Co., Stine/Haskell Research Center, Newark, DE 19714). All other metabolites were minor, and their identity is currently unknown.

Acidification (pH 3) of plant extracts before TLC analysis reduced the levels of radioactivity corresponding to the parent herbicide (methyl-ester of thifensulfuron, metabolite no. 6) by 10–20% causing a concomitant increase in the levels of the acid form of the herbicide (metabolite no. 5) in all species (data not shown). This finding supports further the view that metabolite no. 5 is the deesterified acid of thifensulfuron.

Quantitative differences in the metabolism of thifensulfuron-methyl among the four species were evident. 'Vance' and 'Essex' soybean were more efficient in metabolizing thifensulfuron-methyl than either spurred anoda or velvetleaf (Table 2). The percentages of unmetabolized thifensulfuron-methyl recovered in Essex and Vance soybeans were 38 and 30%, respectively. By comparison, the percentage levels of unmetabolized herbicide in spurred anoda and velvetleaf were 48 and 47%, respectively (Table 2). The percentage levels of the major metabolite of thifensulfuron formed in all species were as follows: 40% in 'Vance' soybean, 31% in 'Essex' soybean, 32% in spurred anoda, and 21% in velvetleaf.

The obtained results support the data reported earlier by Brown et al. (1990) and Long et al. (1988) on the metabolism of thifensulfuron-methyl by soybean. Both studies showed that 'Williams 82' soybean metabolized 80% of thifensulfuron-methyl within 10 h of treatment through excised stems, and that greater than 95% of the recovered radioactivity was present as the deesterified acid of thifensulfuron. In the same study, Brown et al. (1990) showed that the sensitivity of velvetleaf to thifensulfuron-methyl correlated with its reduced ability to metabolize this herbicide.

An alternative basis for the differential tolerance of 'Essex' and 'Vance' soybean, spurred anoda, and velvetleaf to thifensulfuron may be differential sensitivity of the ALS enzyme, which is the target site affected by all sulfonylurea herbicides. Previous studies by Brown et al. (1990), however, have shown that ALS preparations from soybean and velvetleaf were equally sensitive to thifensulfuron-methyl. Thifensulfuron acid had no effect on ALS activity extracted from soybean or velvetleaf (Brown et al. 1990). The sensitivity of ALS preparations from spurred anoda to thifensulfuron-methyl or other sulfonylurea herbicides has not been investigated. Nevertheless, similar to the situation of other sulfonylurea-sensitive or tolerant weeds it is doubtful that differential ALS sensitivity would be responsible for the observed tolerance of spurred anoda to thifensulfuron-methyl.

Overall, our results indicate that differential metabolism may be an important factor contributing to the selectivity of thifensulfuron-methyl between soybean and velvetleaf. Metabolism may be involved in the tolerance of spurred anoda to this herbicide. The potential contribution of root exudation in the observed tolerance of spurred anoda to foliarly applied thifensulfuron-methyl needs to be explored further in the future.

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