Chapter III

Absorption, Translocation, and Metabolism of Sulfentrazone in Potato (*Solanum tuberosum*) and Selected Weed Species

Abstract: Potato exhibits adequate tolerance to preemergence applications of sulfentrazone at rates up to 0.28 kg ai ha^{-1}. Sulfentrazone also controls several troublesome weeds in potato such as common lambsquarters but may be less effective against jimsonweed. Laboratory experiments were conducted to investigate differential tolerance to root-absorbed [^{14}C] sulfentrazone by potato, common lambsquarters, and jimsonweed. Common lambsquarters and jimsonweed absorption of [^{14}C] sulfentrazone was more than two-fold that in potato after 24 h exposure. After 48 h exposure, sulfentrazone absorption by common lambsquarters was nearly two-fold that in jimsonweed and nearly three-fold that in potato. Sulfentrazone movement from roots to shoots was also greater in common lambsquarters than in jimsonweed and potato after 6 h exposure. Both weed species exhibited nearly a two-fold increase in sulfentrazone translocation from roots to shoots compared to potato after 12, 24, and 48 h exposure. Minor differences in sulfentrazone metabolism in roots were noted between species after 6 h exposure. Metabolism in roots and shoots was similar in all species after 12, 24, and 48 h exposure. Since the site of action of sulfentrazone, protoporphyrinogen oxidase, is located in shoot tissue, translocation to shoot tissue is essential for sulfentrazone toxicity. Therefore, differential root absorption as well as differential translocation of sulfentrazone from root to shoot tissue are the proposed primary mechanisms of differential sulfentrazone tolerance between potato, common lambsquarters, and jimsonweed.

Nomenclature: common lambsquarters, *Chenopodium album* L. CHEAL; jimsonweed, *Datura stramonium* L. DATST; potato, *Solanum tuberosum* L., 'Superior'.

Key words: differential response, herbicide tolerance, metabolism, mode of action, protoporphyrinogen oxidase, root absorption, sulfentrazone, translocation.

Introduction

Sulfentrazone is a member of the phenyl triazolinone herbicide group (Theodoridis et al. 1992). Herbicides in this group function through inhibition of protoporphyrinogen oxidase in plants, a key intermediate in both heme and chlorophyll biosynthesis (Jacobs and Jacobs 1987). Unlike other members of this herbicide group such as carfentrazone and the diphenyl ethers, sulfentrazone offers excellent PRE activity (Dayan et al. 1996; Dayan et al. 1997b; Vidrine et al. 1994). Sulfentrazone is currently registered for weed control in soybean either alone or as a prepackaged mixture with chlorimuron and is registered in tobacco as a single entity product (Anonymous 2001). In previous research, sulfentrazone applied PPI or PRE has controlled monocotyledonous and dicotyledonous weed species that can be problematic in several crops (Ohmes et al. 1998; Oliver et al. 1995; Vidrine et al. 1996). Currently, there is only one known weed species that has developed resistance to protox-inhibiting herbicides (Heap 2002); therefore, these herbicides could have an important role in future resistance management programs (Swantek et al. 1998).

In addition to proven tolerance of tobacco and soybean to sulfentrazone, potato is tolerant to PRE applications of sulfentrazone at rates up to 0.28 kg ai ha^{-1} (Bailey et al. 2002). Sulfentrazone at 0.28 kg ha^{-1} controls morningglory (*Ipomeoa* spp.), nutsedge (*Cyperus* spp.), common lambsquarters (*Chenopodium album* L.), nightshade (*Solanum* spp.), and several annual grasses (Walker et al. 1992; Anonymous 2001). These weeds are among the most troublesome weed species in Virginia potato production (Bailey et al. 2001; Ackley et al. 1996). The broad spectrum weed control activity provided by

sulfentrazone coupled with potato tolerance make sulfentrazone a potential candidate as a soil-applied component in potato weed management programs.

Although sulfentrazone controls common lambsquarters at rates as low as 0.11 kg/ha, this herbicide is less effective on jimsonweed (Bailey et al. 2002). Dayan et al. (1996) noted differential responses of sicklepod [*Senna obtusifolia* (L.) Irwin and Barneby] and coffee senna (*Cassia occidentalis* L.) to sulfentrazone and found that tolerance of sicklepod was primarily due to a relatively higher rate of metabolism compared to coffee senna. Although most soybean cultivars are tolerant to sulfentrazone, differential tolerance has also been noted between some soybean cultivars. Morris et al. (1993) noted that 'Centennial' soybean was more tolerant to sulfentrazone than 'Hutcheson'. Dayan et al. (1997a) later found that 'Centennial' sustained less cellular damage from sulfentrazone than did 'Hutcheson', which may, in part, be the basis for differential tolerance in these soybean cultivars. In other research, differential sulfentrazone tolerance between 'Stonewall' and 'Asgrow 6785' soybean cultivars was due to differential absorption in the early stages of growth (Li et al. 2000).

The objective of this research was to determine whether differential sulfentrazone tolerance between potato, common lambsquarters, and jimsonweed was due to differential absorption, translocation, and/or metabolism of sulfentrazone in these species.

Materials and Methods

Absorption and Translocation

Radiolabeled sulfentrazone used in all experiments was provided by the FMC Corporation[1] and uniformly labeled with [^{14}C] in the fifth position on the triazole ring (radiochemical purity of 97.2% and specific activity of 807 kBq mmol^{-1}). Seed of common lambsquarters and jimsonweed were pre-germinated on moist filter paper in a growth chamber[2] under alternating day/night

temperatures of 35/25 C with a 12-h photoperiod. Upon radical emergence, seedlings were transplanted into 10-cm square pots containing a 1:1 mixture of vermiculite[3]:sand[4]. 'Superior', a white table-stock cultivar, seed potato were divided into 40-g seed pieces and planted directly into 10-cm square pots containing the vermiculite:sand mixture. Seedlings were fertilized weekly with water-soluble fertilizer[5]. When seedlings of all species were approximately 8 to 10 cm tall, seedlings were removed from soil, roots washed, and transplanted into glass jars containing 100 mL of quarter-strength Hoagland's nutrient solution (pH 6.5). Seedlings were allowed to acclimate to this hydroponic solution for 48 h. Solutions were then replaced with a fresh 100 mL Hoagland's solution spiked with 3.7 ± 0.2 kBq [^{14}C] sulfentrazone. Roots of each species were then placed in the spiked Hoagland's solution. Plants were maintained in the greenhouse under natural light conditions. Seedlings were collected after 6, 12, 24, and 48 h exposure (HE) to the radiolabeled solution. At each collection time, six randomly selected plants of each species were harvested. Two plants of each species were used for absorption and translocation, two plants were used for X-ray autoradiography, and two plants were used for metabolism experiments. Plants harvested for absorption and translocation experiments were separated into root and shoot tissues and dried for 72 h at 60 C. Radioactivity in the roots and shoots was determined by liquid scintillation spectrometry (LSS)[6] with ^{14}C-trapping cocktail[7] after combustion in a biological sample oxidizer[8]. Preliminary experiments indicated that absorption of radioactivity by potato seed pieces was negligible (<0.01%); therefore, seed pieces were discarded at harvest prior to determining radioactivity in roots and shoots. Plants used for X-ray autoradiography were pressed for 1 wk and then placed on X-ray film[9] for 21 d.

Metabolism

Plants used for metabolism experiments were grown and harvested in the manner previously described. Extraction and metabolism procedures followed closely the procedure used by Dayan et al. (1996). Plants collected at each harvest period for metabolism experiments were separated into roots and shoots and stored at –20 C prior to extraction. For extraction, root and shoot portions of each species were homogenized in 10 mL methanol:water (1:1 by vol.), and the insoluble plant material was separated by centrifugation[10] for 10 min at 3000 rpm. Extraction and centrifugation were repeated three times and the supernatants were combined and separated into aqueous and organic phases with 10 mL volumes of methylene chloride, and the organic phases were combined. Resulting aqueous and organic phases from root or shoot portions of each plant were kept separate and concentrated to 1 mL with an N-evaporator[11]. Thin-layer chromatography (TLC) was used to separate the aqueous and organic fractions of each plant portion. TLC was performed on 20 cm by 20 cm plates coated with silica gel 60A (254 μm thickness)[12]. Fifteen μL of aqueous and organic extracts of each plant portion was spotted at the origin of TLC plates along with 10 μL of standard [^{14}C] sulfentrazone and the plates were developed in a solvent system of methylene chloride:methanol:ammonium hydroxide (84:15:1 by vol.). Following development, standards and metabolites were visualized under UV light and metabolites were tentatively identified based on R_f values of the standard [^{14}C] sulfentrazone and those of previously identified metabolites (Dayan et al. 1996). Silica gel from areas on the plates corresponding to R_f values of sulfentrazone and assumed metabolites was removed and placed in vials containing 10 mL of scintillation cocktail. All vials were then vortexed and radioactivity was determined by LSS. Radioactivity from aqueous and organic phases of each plate fraction for each species was combined for each exposure time.

Statistical Methods

The overall experiment was arranged in a completely randomized design. The treatment design was a three by four factorial with three species (common lambsquarters, jimsonweed, and potato) and four harvest periods (6, 12, 24, and 48 h after exposure to [^{14}C] sulfentrazone). Experimental components were absorption and translocation, X-ray autoradiography, and metabolism. Two replications (one plant/replication) were used for each experimental component at each harvest period. Six plants of each species were collected at each exposure time (two for absorption and translocation, two for X-ray autoradiography, and two for metabolism). Each experiment consisted of 72 plants and each experiment was repeated three times. All data were subjected to analysis of variance (ANOVA) in SAS[13] with sums of squares partitioned to evaluate the main effects and interaction effects of experiment, species, and exposure time. ANOVA revealed no treatment by experiment interaction for absorption and translocation data; therefore, data were pooled over the three experiments. Metabolism data were pooled over two experiments. Means were separated by Fisher's protected LSD ($\propto$ = 0.05).

Results and Discussion

Sulfentrazone Absorption

ANOVA revealed that sulfentrazone absorption was influenced by time (F = 18.47, P = <0.0001) and plant species (F = 6.45, P = 0.0053). Although seedlings of all species were similar in height throughout the experiments, there were inherent differences in the mass of different species. Throughout the experiment, average fresh weight recorded immediately after each plant was harvested was 1.82 ± standard error (se) of 0.19 g for common lambsquarters, 3.12 ± se of 0.29 g for jimsonweed, and 6.13 ± se of 0.91 g for potato. To account for these differences in plant mass, absorption data are presented as kBq radioactivity absorbed per g of fresh weight for each species (Figure 3.1).

Absorption of [^{14}C] sulfentrazone was generally similar in all species after 6 and 12 h exposure. However, after 24 h exposure, absorption was 0.068 to 0.075 kBq per g fresh weight in the two weed species and was significantly greater than absorption in potato (0.032 kBq per g fresh weight). After 48 h exposure, absorption by jimsonweed did not increase significantly from 24 h exposure and was similar to absorption in potato. In common lambsquarters, however, absorption increased to 0.12 kBq per g fresh weight and was nearly two-fold that of jimsonweed and nearly three-fold that of potato. Differential root absorption may be an important factor in differential tolerance to sulfentrazone between potato, jimsonweed, and common lambquarters. Vencill et al. (1990) reported differential root uptake as a key factor contributing to differential sensitivity to clomazone between soybean and *Amaranthus* species while Mangeot et al. (1979) reported differential absorption as a contributing factor in differential response to metribuzin. In sulfentrazone research, Dayan et al. (1996) reported root uptake of radiolabeled sulfentrazone to be 74% greater in coffee senna (sulfentrazone sensitive) than in sicklepod (sulfentrazone tolerant).

Sulfentrazone translocation

Sulfentrazone translocation from root to shoot portions was influenced only by plant species ($F=40.27$, $P=<0.0001$). Regardless of time of exposure, lesser amounts of sulfentrazone were translocated from roots to shoots in potato than in either of the weed species (Table 3.1). After 6 h exposure, however, differences were also noted between the two weed species with 45.3% of the absorbed [^{14}C] sulfentrazone being translocated to common lambsquarters shoots, 37.7% translocated to jimsonweed shoots, and 21.5% translocated to potato shoots. At exposure times of 12, 24, or 48 h, sulfentrazone translocation from roots to shoots was similar in common lambsquarters and jimsonweed, ranging from 37.5 to 47.8% in both species. In potato, however, [^{14}C] sulfentrazone

translocation to shoots was never more than 23% at any exposure time. These results were verified in x-ray autoradiographs of the three species (data not presented). Since sulfentrazone inhibits protoporphyrinogen oxidase (Nandihalli and Duke 1993), an enzyme located in the chloroplast envelope and involved in chlorophyll biosynthesis in the shoots of plants, differential translocation to shoots may be the most important factor distinguishing differential sensitivity between potato and weed species. Other researchers have also attributed differential herbicide tolerance between species to differential translocation (Carey et al. 1997; Pline et al. 1999).

Sulfentrazone Metabolism

As observed with regard to translocation, differences in metabolism between species also occurred. After 6 h exposure, roots of potato contained higher levels of parent [^{14}C] sulfentrazone than the roots of either weed species (Table 3.2), a possible indication of decreased metabolism in roots and increased levels of sulfentrazone in the roots of potato. In shoots, however, parent [^{14}C] sulfentrazone in common lambsquarters and jimsonweed shoots was similar to that in potato shoots. After 12, 24, and 48 h exposure, amounts of parent [^{14}C] sulfentrazone were similar between species. Comparison of the amounts of parent [^{14}C] sulfentrazone between roots and shoots each species over time were similar to trends seen in translocation, with more parent herbicide in the shoots of jimsonweed after 6 h exposure and in the shoots of common lambsquarters after 6, 12, and 24 h exposure. Amounts of parent [^{14}C] sulfentrazone in potato roots and shoots were similar at all exposure times. These results are generally in agreement with those of Li et al. (2000), who reported similar levels of metabolism in sulfentrazone-tolerant and –sensitive soybean cultivars.

Based on R_f values of previously reported sulfentrazone metabolites (Theodoridis et al. 1992), the primary metabolites found in this experiment

were assumed to be a 3-hydroxymethyl derivative (R_f=0.35) followed closely by what was assumed to be a 3-carboxylic acid derivative (R_f=0.08) (data not presented). Formation of these derivatives is known to be achieved through a stepwise oxidation of the methyl group on the triazolinone ring of the parent molecule (Dayan et al. 1996). These metabolic derivatives are known to be less toxic than sulfentrazone (Dayan et al. 1998). It has been reported that the methyl group on position three of the triazolinone ring is necessary for maximum biological activity of sulfentrazone and that replacement by other substituents results in dramatic decreases in biological activity (Theodoridis et al. 1992).

Collectively, the results of these experiments suggest that differential sulfentrazone tolerance between potato and weed species is primarily due to differential root absorption as well as differential translocation of the herbicide from root to shoot portions. Absorption by the two weed species was more than two-fold that in potato after 24 h root exposure while common lambsquarters absorbtion was nearly two-fold that of jimsonweed and three-fold that of potato after 48 h exposure. Although differences in translocation were noted between the more-sensitive common lambsquarters and the less-sensitive jimsonweed after 6 h exposure to sulfentrazone, sulfentrazone translocation to shoot tissue in either weed species was nearly two-fold that of potato at any time of exposure. Since the site of action of sulfentrazone is found primarily in the shoot tissue of plants, adequate translocation to these areas is essential for adequate effectiveness of sulfentrazone. Sulfentrazone tolerance in potato coupled with the broad-spectrum weed control activity of this herbicide makes it a suitable candidate for use in future potato weed management programs.

Sources of Materials

[1]FMC Corporation, Agricultural Products Group, Philadelphia, PA 19103.

[2]Conviron model E7 growth chamber. Conviron Controlled Environments Limited, Winnipeg, Manitoba.

[3]Horticultural vermiculite sterile growing media, medium grade. The Schundler Company, P. O. Box 513, Metuchen, NJ 08840-0513.

[4]Quikrete all-purpose sand. The Quikrete Companies, Atlanta, GA 30329.

[5]Peters Professional General Purpose 20-20-20. Scotts-Sierra Horticultural Products Company, 14111 Scottslawn Rd., Marysville, OH 43041.

[6]Liquid scintillation counter, Beckman LS 5000TA model, Beckman Instruments, 4300 N. Harbor Boulevard, Fullerton, CA 92634.

[7]Scintiverse® BD scintillation cocktail. Fisher Scientific, Fair Lawn, NJ 07410.

[8]Packard Model 307 Biological oxidizer, Packard Instrument Co. 2200 Warrenville Road, Downer's Grove, IL 60515.

[9]X-OMAT diagnostic film, Eastman Kodak Company, Rochester, NY 14650.

[10]Sorvall RC-58 refrigerated superspeed centrifuge with SS-34 rotor. Sorvall-Kendro Laboratory Products, L.P., 31 Pecks Lane, Newtown, CT 06470-2337.

[11]Meyer N-EVAP analytical evaporator. Organomation Associates, Inc., P. O. Box 5 Tpk. Sta., Shrewsbury, MA 01545.

[12]Silica Gel 60F_{254} precoated plates for thin layer chromatography. EM Science, 480 Democrat Road, Gibbstown, NJ 08027.

[13]Statistical Analysis Systems (SAS) software, Version 7.0, SAS Institute, Inc., Box 8000, SAS Circle, Cary, NC 27513.

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Table 3.1. Translocation of [^{14}C] sulfentrazone from roots to shoots in common lambsquarters, jimsonweed, and potato after 6, 12, 24, and 48 h root exposure.

	[^{14}C] sulfentrazone absorption[ab]					
	Common lambsquarters		Jimsonweed		Potato	
Time	Roots	Shoots	Roots	Shoots	Roots	Shoots
HE[c]	% of absorbed[d]					
6	54.7 ± 4.3	45.3 ± 4.3 aA	62.3 ± 3.6	37.7 ± 3.6 aB	78.5 ± 7.3	21.5 ± 7.3 aC
12	61.1 ± 3.4	38.8 ± 3.4 aA	62.5 ± 5.3	37.5 ± 5.3 aA	83.2 ± 6.9	16.8 ± 6.9 aB
24	57.8 ± 3.5	42.2 ± 3.5 aA	58.5 ± 4.6	41.5 ± 4.6 aA	77.0 ± 5.6	23.0 ± 5.6 aB
48	52.2 ± 5.4	47.8 ± 5.4 aA	56.8 ± 4.4	43.2 ± 4.4 aA	78.3 ± 4.5	21.7 ± 4.5 aB

[a]Values represent the pooled average of three experiments as a percentage of absorbed [^{14}C] sulfentrazone ± standard errors.

[b]Means of radioactivity in shoots followed by the same lowercase letter are not significantly different within species. Means of radioactivity in shoots followed by the same uppercase letter are not significantly different between species.

[c]HE = h exposure.

[d]Translocation data presented as percentage of absorbed radioactivity.

Table 3.2. Sulfentrazone metabolism by common lambsquarters, jimsonweed, and potato seedlings as influenced by time and plant portion.

Root absorption time	Species	Parent [^{14}C] sulfentrazone[a]	
		Plant portion	
		Roots	Shoots
HE[b]		% of recovered ^{14}C[c]	
6	Common lambsquarters	26.8 ± 8.5 bB	75.0 ± 8.2 aA
	Jimsonweed	30.8 ± 8.1 bB	80.8 ± 3.0 aA
	Potato	56.5 ± 5.8 aA	69.5 ± 7.9 aA
12	Common lambsquarters	52.3 ± 6.5 aB	67.8 ± 6.4 aA
	Jimsonweed	45.0 ± 7.0 aA	61.5 ± 10.2 aA
	Potato	53.3 ± 5.9 aA	52.5 ± 14.5 aA
24	Common lambsquarters	24.0 ± 6.1 aB	64.8 ± 10.2 aA
	Jimsonweed	36.5 ± 10.8 aA	46.0 ± 7.4 aA
	Potato	45.3 ± 9.4 aA	54.3 ± 8.7 aA
48	Common lambsquarters	54.5 ± 8.9 aA	73.5 ± 6.1 aA
	Jimsonweed	47.0 ± 6.8 aA	65.8 ± 2.1 aA
	Potato	53.3 ± 14.0 aA	77.5 ± 6.4 aA

[a]Means represent the pooled average of two experiments as a percentage of the total radioactivity attributed to parent sulfentrazone ± standard errors. Means of radioactivity followed by the same lowercase letter are not significantly different within plant portion. Means of radioactivity followed by the same uppercase letter are not significantly different between plant portions.

[b]HE = h exposure.

[c]Metabolism data presented as a percentage of recovered radioactivity attributed to parent sulfentrazone.

CAPTIONS FOR FIGURES

Figure 3.1. Absorption of [^{14}C] sulfentrazone per g fresh weight of common lambsquarters (*Chenopodium album* L.), jimsonweed (*Datura stramonium* L.), and potato (*Solanum tuberosum* L.) after 6, 12, 24, and 48 h root exposure. Error bars represent the standard error of the mean of three experiments. Asterisks denote significant difference between species at the P = 0.05 significance level.

Bailey, *Figure 3.1.*