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Uptake, Translocation, and Metabolism of Root Absorbed Sulfentrazone and Sulfentrazone plus Clomazone in Flue-Cured Tobacco Transplants[1]

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Abstract: Research was conducted to evaluate root uptake, translocation, and metabolism of ^{14}C-sulfentrazone alone or in a mixture with clomazone in solution in flue-cured tobacco transplants. Uptake and translocation of sulfentrazone was rapid and was not affected by the addition of clomazone. Fifty-nine and 65% of the ^{14}C absorbed by the plant was translocated to the leaves within 24 h with sulfentrazone alone and in the clomazone plus sulfentrazone mixture, respectively. Differences in plant metabolism were observed between sulfentrazone alone and sulfentrazone plus clomazone. After 3 h, 66% of the ^{14}C recovered from the leaves was metabolized when sulfentrazone was applied alone, compared to 91% when sulfentrazone was applied with clomazone. The difference could indicate that metabolism of sulfentrazone by tobacco transplants was enhanced by the presence of clomazone.
Nomenclature: Clomazone; sulfentrazone; flue-cured tobacco, *Nicotiana tabacum* L. 'NC 71'.
Additional index words: Enhanced metabolism, safening, tolerance.
Abbreviations: HAT, hours after treatment; LSS, liquid scintillation spectrometry; PPI, preplant incorporated treatment; PRE-T, pre-emergence soil surface treatment.

INTRODUCTION

Tobacco farmers traditionally have used many options for weed management, including crop rotations, cultivation, hand weeding, and herbicides. Tobacco is a high-value crop, allowing growers to employ many options that are either too costly or too time consuming in other row crops (Fisher and Smith 2003). For example, North Carolina Cooperative Extension Agents estimated that nearly 9% of growers of flue-cured tobacco did not use any herbicides in the 2004 growing season. However, the increase in size of farms, decrease in labor availability, and increase in the use of mechanical harvesters have caused an increased need for herbicides (Fisher and Smith 2003).

Currently, only six herbicides are registered for use in tobacco production in North Carolina (York et al. 2004). The most widely-used herbicides include clomazone, pendimethalin, and sulfentrazone. Sulfentrazone inhibits protoporyphyrinogen oxidase (EC 1.3.3.4), an enzyme in the chlorophyll biosynthesis pathway (Dayan and Duke 1997). It was registered for use in tobacco in NC in 1997 and controls broadleaf weeds and sedges, but only suppresses annual grasses (Fisher and Smith 2003). Therefore, growers often apply sulfentrazone with pendimethalin or clomazone to control annual grasses. Sulfentrazone is the only herbicide registered for tobacco that controls of morningglory (*Ipomoea* spp.) and yellow (*Cyperus esculentus* L.) and purple nutsedge (*Cyperus rotundus* L.) (Fisher and Smith 2003). Vining *Ipomoea* spp. are especially problematic for growers using mechanical harvesters because the vines reduce harvesting efficiency and leaves are a potential source of foreign matter contamination in cured tobacco (Fisher and Smith 2003). Sulfentrazone was used on approximately 30% of all hectarage in North Carolina in a 2000 survey of North Carolina Cooperative Extension Agents by the authors.

Occasional early-season stunting from sulfentrazone has been observed (Fisher and Smith 2003). A better understanding of management practices that contribute to injury and the physiological basis for tolerance in tobacco could result in recommendations that lead to more widespread use of sulfentrazone, which could benefit growers. Injury to tobacco from sulfentrazone has been inconsistent, but is greater from preplant incorporated (PPI) treatments than

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from pre-emergence soil surface treatments (PRE-T) made prior to tobacco transplanting (Fisher et al. 2002). In soybean [*Glycine max* (L.) Merr.] and peanut (*Arachis hypogaea* L.), no differences in injury from PPI or PRE-T applications of sulfentrazone were observed (Grey et al. 2004; Vidrine et al. 1996). Flue-cured tobacco in North Carolina is typically planted on raised beds. Therefore growers prefer to apply herbicides PPI because it does not require special equipment and/or additional trips across the field to knock down beds to a similar height achieved by the transplanter so that a PRE-T application can be made.

A mixture of sulfentrazone and clomazone is very common in tobacco because of the broad spectrum weed control that this herbicide combination provides (Fisher and Smith 2003). Reduced injury from a mixture of sulfentrazone plus clomazone compared to sulfentrazone alone has been observed in field and greenhouse research (Fisher et al. 2000). Studies to determine the physiological basis for sulfentrazone tolerance in tobacco have not been conducted. Vaughn and Duke (1991), however, reported possible tolerance mechanisms in several crop and weed species, including modification to the site of action, metabolism, and reduced translocation to the site of action. Dayan et al. (1997) reported that primary detoxification resulted from the oxidation of the methyl group of the triazolinone ring and the resulting formation of a hydroxymethyl derivative. Theodoridis et al. (1992) concluded that this methyl group is necessary for biological activity and that its replacement resulted in a three- to six-fold decrease in biological activity. Research by Thomas et al. (2005) suggested that prickly sida (*Sida spinosa* L.) and peanut were able to metabolize sulfentrazone. In soybean, Dayan et al. (1997) reported no differences in uptake and translocation, and only small differences in metabolism between tolerant and susceptible cultivars, indicating that some other mechanism was responsible for tolerance.

An understanding of the physiological basis for tolerance of tobacco to sulfentrazone, and evaluation of the potential basis of safening of sulfentrazone from clomazone would assist in the development of recommendations to minimize the risk for injury in tobacco transplants. Therefore, the objectives of this research were to evaluate uptake, translocation, and metabolism of sulfentrazone alone and in a mixture with clomazone.

MATERIALS AND METHODS

Methods Common to All Experiments. Transplants of flue-cured tobacco 'NC 71,' a widely planted cultivar, were produced in a greenhouse float system following extension recommendations and were of normal transplant size (10 cm height) at the time of use in all experiments (Smith et al. 2004). Transplants were removed from the float tray and roots were rinsed thoroughly to remove soilless medium. They were then placed in a 125 ml flask with roots submerged in 100 ml of a 50% Hoagland's solution (Hoagland and Arnon 1950) and placed in a growth chamber. Temperature in the growth chamber was maintained at a constant 26 C and approximately 50% relative humidity. Artificial lighting was provided by both fluorescent and incandescent lamps at 500 μmol m^{-2} s^{-1}. Transplants were acclimated in the growth chamber for 48 h prior to treatment. Radiolabeled and technical-grade sulfentrazone and technical-grade clomazone were provided by FMC Corporation[3]. Sulfentrazone provided was radiolabeled with [^{14}C] in the phenyl ring (98.7% purity, specific activity of 10.55×10^8 Bq $mmole^{-1}$). Technical-grade sulfentrazone and clomazone were 91 and 90% pure, respectively. The volume of Hoagland's solution in each flask of acclimated transplants was returned to 100 ml before the start of each experiment. Two experiments were conducted and each was repeated. A randomized complete block design was used with four replications of treatments in uptake and translocation studies and three replications of treatments in metabolism studies. Data were analyzed together due to an insignificant run by experiment interaction and an insignificant effect of runs nested within clomazone. Data were subjected to an ANOVA and means were separated using Fisher's Protected LSD at $P = 0.05$.

Uptake and Translocation Experiments. Radiolabeled sulfentrazone at 1.83 kBq and 0.44 mg ai L^{-1} technical-grade sulfentrazone alone or with 1.32 mg ai L^{-1} technical-grade clomazone were added to each flask. Flasks, each containing one transplant, were then returned to the growth chamber. After 72 h, plants were removed from radiolabeled solution and transferred to a solution containing only technical-grade sulfentrazone at 0.44 mg L^{-1} alone or with 1.32 mg ai L^{-1} technical-grade clomazone. Total

[3] FMC Corporation, 1735 Market Street, Philadelphia, PA 19103.

Table 1. Uptake and translocation of ^{14}C-sulfentrazone alone and mixed with clomazone from solution in tobacco transplants.

Herbicide Treatment	Hours after ^{14}C exposure	^{14}C absorbed	Root	Stem	Leaves
		%	% of absorbed activity[a]		
^{14}C-sulfentrazone	24	6 a	26 a	15 a	59 a
	48	6 a	20 ab	17 a	62 a
	72	5 a	19 ab	20 a	62 a
	96	6 a	21 ab	15 a	64 a
^{14}C-sulfentrazone + clomazone	24	6 a	14 bc	21 a	65 a
	48	4 a	10 c	20 a	70 a
	72	4 a	11 c	18 a	72 a
	96	7 a	8 c	24 a	69 a

[a] Means within the same column followed by the same letter do not significantly differ at P = 0.05.

concentrations of sulfentrazone and clomazone in solution were based on registered rates and the volume of water at field capacity, assuming 20% moisture by weight and 50% herbicide adsorption by the soil (Weber et al. 2000). A 1.0 ml aliquot was taken from solution, diluted in scintillation liquid,[4] and the amount of nonabsorbed radioactivity remaining in the solution was quantified by liquid scintillation spectrometry (LSS).[5] Four plants were sampled at this time and four additional plants were sampled each day for the next three days. Sampled plants were dried for 48 h in a forced air oven at 40 C. Plants were divided into roots, stem, and leaves; weighed; combusted in a biological oxidizer;[6] and $^{14}CO_2$ was captured in scintillation cocktail.[7] Radioactivity in the oxidized samples was quantified by LSS. Total recovery of ^{14}C was greater than 90% for all treatments.

Metabolism Experiments. Radiolabeled sulfentrazone at 16.67 kBq and 0.44 mg ai L^{-1} technical-grade sulfentrazone alone or with 1.32 mg ai L^{-1} technical-grade clomazone were added to each flask. Flasks, containing one transplant, were then returned to the growth chamber. After 3 h, three plants were sampled and the remaining plants were transferred to a solution containing only technical-grade sulfentrazone at 0.44 mg L^{-1} alone or with 1.32 mg ai L^{-1} clomazone. A 1.0 ml aliquot was taken from solution and quantified by LSS to determine unabsorbed radioactivity left in solution. Three additional plants were sampled 6 and 9 h after treatment (HAT). Plants were separated into roots, stem, and leaves; weighed; and frozen at −30 C prior to extraction. The extraction procedure was based on experiments by Dayan et al. (1996). Plants were ground in a tissue homogenizer[8] with 10 ml methanol, rinsed with an additional 10 ml of methanol and vacuum filtered. Residue and filter paper were wrapped in aluminum foil and stored. Filtrate was concentrated under an oxygen gas stream and 200 µl of each sample were spotted on one lane of a 20 by 20 cm silica gel thin layer chromatography plate[9] containing 10 lanes. A standard of 1 µl of stock radiolabeled solution dissolved in 15 ml of methanol was also spotted on one lane of each plate. Plates were developed to a 16 cm solvent front. The solvent consisted of methylene chloride:methanol ammonium hydroxide (84:15:1, v/v). Plates were air dried and scanned using a radiochromatogram scanner[10] to determine radioactive positions and corresponding R_f values. Peak area was calculated using Win-Scan software[11] with smoothing set at 14-point cubic and background excluded from calculation. Parent herbicide was identified from standard on each plate. Total recovery was greater than 94% for all treatments.

RESULTS AND DISCUSSION

Uptake and Translocation. ^{14}C was absorbed by the roots of tobacco transplants and translocated to the leaves (Table 1). Uptake of ^{14}C ranged from 4 to 7% of total ^{14}C available in solution and did not change over the time of the experiment. After 24 h, 59% or

[4] Scintiverse BD, Fisher Scientific, Fair Lawn, NJ 07410.
[5] Liquid Scintillation Analyzer, Model Tri-Carb 2100TR, Packard Instrument Company, Downers Grove, IL 60515.
[6] Biological Oxidizer, Model OX500, R. J. Harvey Instrument Corporation, Hillsdale, NJ 07642.
[7] Carbon-14 Cocktail, R. J. Harvey Instrument Corporation, Hillsdale, NJ 07642.
[8] Pyrex Tissue Homogenizer No. 7727-40, Corning Incorporated, Corning, NY 14381.
[9] Watman K6F Silica Gel 60A Thin Layer Chromatography Plates, Watman Incorporated, Clifton, NJ 07013.
[10] Bioscan System 200 Imaging Scanner, Bioscan, 4590 MacArthur Boulevard NW, Washington, D.C. 20007.
[11] Lablogic Win-Scan Radio TLC Version 2.2 (5) 32-bit, Distributed by BioScan, 4590 MacArthur Boulevard NW, Washington, D.C. 20007.

Table 2. Distribution of ^{14}C-sulfentrazone alone and mixed with clomazone in root, stem, and leaves in tobacco transplants.

	Hours after ^{14}C exposure	^{14}C absorbed	Root	Stem	Leaves
		%	% of absorbed activity[a]		
^{14}C-sulfentrazone	3	4.1 a	73 a	16 b	12 c
	6	1.1 b	25 c	28 a	47 a
	9	1.0 b	25 c	20 b	55 a
^{14}C-sulfentrazone + clomazone	3	1.3 b	35 b	28 a	37 b
	6	0.9 b	34 b	31 a	35 b
	9	1.2 b	68 a	21 b	12 c

[a] Means within the same column followed by the same letter do not significantly differ at P = 0.05.

more of the absorbed ^{14}C had been translocated to the leaves in all treatments. Time, or the addition of clomazone, had no effect on the absorption or translocation of ^{14}C in the stem or leaves. However, at all time comparisons, the roots of transplants exposed to sulfentrazone alone contained a significantly greater amount of the ^{14}C than those of tobacco plants exposed to the mixture of sulfentrazone and clomazone. The greatest portion of ^{14}C sulfentrazone over time in both species was also found in the leaves, followed by the stem and the roots. Similar results were reported by Thomas et al. (2005) in peanut and prickly sida (sulfentrazone-tolerant and -susceptible species, respectively). Dayan et al. (1996) also reported that sulfentrazone-tolerant and -susceptible weed species did not differ in root uptake of sulfentrazone. Therefore, the uptake and translocation of sulfentrazone observed in tobacco was consistent with other research and is apparently not the mechanism of tolerance in tobacco.

Metabolism. Total absorption of ^{14}C from solution ranged from 0.9 to 1.3% of that available, except at 3 h when tobacco was placed in sulfentrazone alone, when total uptake from solution was 4.1% of available (Table 2). No apparent reason for the difference in uptake is offered and this difference is likely an anomaly, based on uptake and translocation data (Table 1).

Significant herbicide treatment differences were observed in distribution of ^{14}C over time in the metabolism experiments and were similar to differences observed in the uptake and translocation experiments, even though the time interval was much shorter (Table 1 and Table 2). When sulfentrazone was used alone, 12% of the radioactivity absorbed by tobacco transplants 3 HAT was in the leaves. Over the next 6 h, ^{14}C was rapidly translocated from the roots to the leaves, with 55% reaching the leaves with sulfentrazone alone after 9 h. Translocation of ^{14}C from roots to leaves was faster in a sulfentrazone plus clomazone mixture. Additionally, the percentage of absorbed activity found in the leaves of plants exposed to the mixture of sulfentrazone and clomazone decreased over the time of the experiment, while levels in the roots increased; this is opposite of the sulfentrazone alone treatments. Because plants were only exposed to ^{14}C-sulfentrazone for the first 3 h of the experiment, the decrease in concentration in the leaves indicates translocation from the leaves to the roots.

Sulfentrazone was rapidly metabolized by tobacco transplants (Table 3). When sulfentrazone was used alone, a total of 66% of the ^{14}C in the leaves had been metabolized after only 3 h. The percentage of metabolized ^{14}C in the leaves did not significantly change over the remaining 6 h of the experiment. One metabolite with an average R_f value of 0.45 was common

Table 3. Thin layer chromatography separations of ^{14}C-sulfentrazone and its metabolites, alone and in mixtures with clomazone.

	Hours after ^{14}C exposure	^{14}C-sulfentrazone			3-hydroxymethyl metabolite			Conjugated metabolites		
		Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves
		% of absorbed activity[a]								
^{14}C-sulfentrazone	3	72 a	46 a	34 a	6 d	18 b	26 b	22 b	37 b	40 b
	6	12 c	13 b	32 a	36 a	32 a	33 ab	52 a	55 a	41 b
	9	21 b	9 bc	28 a	32 b	37 a	25 b	47 a	54 a	48 ab
^{14}C-sulfentrazone + clomazone	3	28 b	12 b	9 b	25 c	33 a	37 a	47 a	45 a	57 a
	6	9 c	6 c	10 b	37 a	38 a	35 ab	54 a	56 a	55 a
	9	10 c	6 c	11 b	36 a	36 a	28 ab	54 a	58 a	61 a

[a] Means within the same column followed by the same letter do not significantly differ at P = 0.05.

in all treatments. Based upon research by Dayan et al. (1996) with tolerant weeds, this R_f value corresponds to a 3-hydroxymethyl, a primary metabolite of sufentrazone. A large percentage of metabolites were conjugated and did not have consistently distinguishable peaks, which is also similar to previous research (Dayan et al. 1996). The addition of clomazone significantly and consistently increased metabolism of the ^{14}C sulfentrazone in the leaves. At 3, 6, and 9 HAT, the total percentage of ^{14}C in the leaves that had been metabolized was greater with sulfentrazone plus clomazone than with sulfentrazone alone. In the stem and roots, the percentage of metabolized ^{14}C was significantly reduced by the addition of clomazone after 3 h, but results were inconsistent at 6 and 9 h. The inconsistency is likely because metabolism of sulfentrazone occurs in the leaves and levels of metabolite in the stem and roots would be dependent upon rate of metabolism in the leaves and subsequent movement of metabolite out of the leaves.

Our data suggest that tolerance of tobacco to sulfentrazone is a result of metabolism. The observed reduction in injury to tobacco transplants from the mixture of sulfentrazone plus clomazone compared to sulfentrazone alone might be the result of the enhanced metabolism of sulfentrazone observed in this study. However, ''safening'' of sulfentrazone by clomazone and tolerance of tobacco under field conditions has been inconsistent. Inconsistencies in tolerance and safening could be related to environmental factors that influence rate of uptake, translocation, and/or metabolism. Specifically, conditions that result in rapid uptake of soil solution and/or decrease plant metabolism increase the risk of injury to tobacco but could make differences in stunting difficult to detect visually.

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