J Plant Growth Regul (1994) 13:11–14

Journal of
Plant
Growth
Regulation

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Absorption, Translocation, and Metabolism of Flurtamone in Sicklepod (*Cassia obtusifolia*) and Peanut (*Arachis hypogea*)

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Received June 3, 1993; accepted December 20, 1993

Abstract. The basis of peanut tolerance to the bleaching herbicide flurtamone was examined. The absorption, translocation, and metabolism of ^{14}C-flurtamone were examined in peanut at 6, 24, and 48 h after root application. Differences in ^{14}C-flurtamone uptake over time were not detected. Approximately 40% of the absorbed ^{14}C-flurtamone was in the leaves at 6 h after treatment; 60% was metabolized to polar products 41% of absorbed ^{14}C in 6 h; 40% of this moved from roots to shoots; and 60% of this did not co-chromatograph with the parent; 9.8% of applied ^{14}C-flurtamone was altered in leaf tissue. The levels of metabolized flurtamone increased with time after treatment (75% and 83% of applied ^{14}C-flurtamone metabolized at 24 and 48 h, respectively). Parent ^{14}C-flurtamone was detectable with R_t of 7 min and unknown metabolites with an R_t of 3.3, 4.4, and 5.6 min, respectively, was detected in leaf tissue at 6, 24, and 48 h after treatment.

Flurtamone [(±)-5-(methylamino)-2-phenyl-4-[3-(trifluoromethyl)phenyl]-3(2*H*)-furanone] is a recently introduced herbicide (Ward et al. 1987). It is a preemergence herbicide that has activity against a wide range of annual and broadleaf weeds in several crops including peanut (*Arachis hypogea* L.), grain sorghum (*Sorghum bicolor* Moench.), and cotton (*Gossypium hirsutum* L.) (Rogers et al. 1987, Mueller and Banks 1989).

Flurtamone is a furanone derivative that reduces carotenoid and chlorophyll levels causing a bleached appearance in treated susceptible plant species (Ward et al. 1987). Sandmann et al. (1990) reported that flurtamone interferes with phytoene desaturase similar to pyridazinone herbicides.

Sicklepod is one of the most troublesome and widespread weeds in peanuts and other row crops in the southeast (Dowler 1992). In field studies, flurtamone has been shown to control sicklepod in peanuts at rates of 0.1 kg/ha, whereas peanuts have shown tolerance to flurtamone at rates greater than 15 kg/ha. The basis for the observed differential sensitivity is unknown. Differential absorption, translocation, and metabolism have long been recognized as important factors contributing to herbicide selectivity (Hatzios and Penner 1982, Shimabukuro 1985).

The objectives of this research were (1) to study the absorption and translocation of radiolabeled flurtamone in tolerant-peanut and in susceptible-sicklepod following root application of this herbicide and (2) determine the potential contribution of ^{14}C-flurtamone metabolism in tolerant-peanut.

Materials and Methods

Chemicals

Technical (97%) and radiolabeled samples of flurtamone were provided by Valent Chemical Corporation (Richmond, CA, USA). Radiolabeled flurtamone was labeled with ^{14}C- on the phenyl ring containing the trifluorocarbon group with a specific activity of 54.5 mCi/mmol (Fig. 1). Flurtamone was dissolved in methanol and made up to volume with distilled water.

Plant Material

Seeds of each species were planted in 400-ml cups containing vermiculite. Plants were grown in a growth chamber at a constant temperature of 27°C, and a photoperiod of 14 h light at 900 $\mu E \cdot m^{-2} \cdot s^{-1}$ provided by both incandescent and fluorescent lamps. At the first compound leaf stage (6–8 cm) of sicklepod and peanut, seedlings were transferred to half-strength Hoagland's solution (pH 6.0) in opaque 150-ml containers, one plant per container (Hoagland and Arnon 1950). After 2 days, plants were transferred to full-strength Hoagland's nutrient solution.

Uptake and Distribution of [^{14}C]-Flurtamone

At the first compound leaf stage of sicklepod and peanut, plants were exposed to radiolabeled flurtamone following root application. Seedlings of all species were placed in nutrient solution

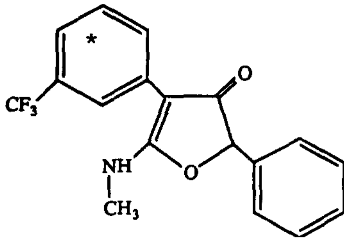

Fig. 1. Chemical structure of flurtamone. The asterisk represents the phenyl ring that contains the ^{14}C-label.

containing 5 μCi/L radiolabeled flurtamone adjusted to 100 μM concentration with technical flurtamone as this was found to be an effective concentration in research conducted by Sandmann et al. (1990).

Plants were harvested at 6, 24, and 48 h after treatment. The roots of treated plants were washed with distilled water to remove any radioactivity from their surface. Radioactivity in root washes was determined by liquid scintillation spectrometry (Beckman LS 5100). At the end of each harvesting period, a 1-ml sample of the remaining nutrient solution was used to determine the amount of radioactivity remaining in the solution by means of liquid scintillation spectrometry. Harvested plants were separated into roots and shoots. Plant parts were weighed and ground in a mortar under liquid nitrogen. The plant parts were extracted twice with 50-ml aliquots of 80% acetone. Extracts were concentrated under nitrogen, and 1-ml aliquots were taken to determine the amount of radioactivity in harvested roots and shoots to quantitatively determine absorption and translocation of the radiolabeled flurtamone. These data will be presented as a percent and on a dpm/g basis. The remaining dry plant material was combusted in a biological sample oxidizer (Model OX500, R. J. Harvey Instruments, Hillsdale, NJ, USA), and released $^{14}CO_2$ was trapped and counted by liquid scintillation spectrometry to quantitatively determine absorption and translocation of the radiolabled flurtamone. Greater than 95% of the absorbed flurtamone was extracted at each harvest interval (data not shown).

Autoradiography

A set of seedlings treated with radiolabeled flurtamone was used for analysis by autoradiography following the procedures of Crafts and Yamaguchi (1964). The plant samples were dried after harvest and placed on X-ray film (Kodak X-Omat AR). Three weeks later, the film was developed for qualitative analysis of flurtamone translocation.

Separation and Identification of Flurtamone Metabolite Peaks

Seedlings of peanut were grown hydroponically as described earlier. ^{14}C-Flurtamone was supplied to the peanut through their roots and was added to the nutrient solution at a concentration of 100 μM containing 5.0 μCi/L of nutrient solution. Plants were again harvested at 6, 24, and 48 h after treatment. Harvested peanuts were divided into roots and shoots plus leaves, and weighed and ground to a fine powder with liquid nitrogen. Radiolabeled herbicide and its metabolites were extracted with two aliquots of 50 ml of 80% acetone. The acetone aliquots were combined and concentrated to 1 ml by rotoevaporation at 40°C. A 0.1-ml sample of each extract was then counted by liquid scintillation spectrometry. To analyze peanut extracts for flurtamone and its metabolites, we utilized high-performance liquid chromatography (HPLC). A 50-μl aliquot was injected into a HPLC system (Shimadzu LC-6) with a reversed-phase C_{18}-column (Econosphere, Alltech Associates, Deerfield, IL, USA) using an isocratic mobile phase that consisted of methanol/water (80:20, v/v) at 1 ml min^{-1} flow rate. Samples were detected utilizing a UV/VIS detector at 275 nm. A fraction collector (Retriever II, ISCO Corporation) was used to collect sample effluent at 30-s intervals. Radioactivity in collected fractions was quantitatively determined by liquid scintillation spectrometry.

Statistical Analysis

Each experiment was repeated twice with three replications of all treatments in each experiment. Means were separated by Fisher's protected least significance (LSD) test at the 5% level.

Results

Flurtamone Absorption and Translocation

Flurtamone absorption did not significantly increase from 6 to 48 h after application in either susceptible-sicklepod or tolerant-peanut. Sicklepod absorbed significantly more flurtamone at each harvest interval than did peanut by approximately 20% (Table 1). In both sicklepod and peanut, root-absorbed [^{14}C] flurtamone remained in the root tissue of sicklepod (53%–69%) and peanut (68%–72%) at 6, 24, and 48 h after application. Sicklepod roots contained 64% more flurtamone per gram of tissue than did peanut at 48 h after application (Table 1). Sicklepod shoots contained 75% more flurtamone per gram of shoot tissue than peanut shoot tissue. Autoradiography (not shown) qualitatively showed that a considerable portion of applied ^{14}C-flurtamone remained in the roots. The movement of absorbed radioactivity from roots to shoots was independent of the time after treatment with root-applied ^{14}C-flurtamone in sicklepod and peanut. A differential species response in flurtamone translocation was not observed.

Flurtamone Metabolism

Approximately 40% of absorbed ^{14}C-flurtamone was unmetabolized, and 60% was metabolized by the leaf tissue of tolerant-peanut 6 h after treatment (Table 2). The levels of metabolized flurtamone increased with time after treatment (75% and 83% of applied ^{14}C-flurtamone metabolized at 24 and 48 h, respectively). Parent ^{14}C-flurtamone was detectable

Table 1. Absorption and distribution of total ^{14}C recovered in shoot and root of sicklepod and peanut 6, 24, and 48 h after root application of ^{14}C-flurtamone.[a]

Species	Time after application (h)	Absorption (% of applied)	Distribution of radioactivity: % of absorbed[b] Root	Shoot	LSD	dpm/g[b] Root	Shoot	LSD
Sicklepod	6	62	53	47	45	16396	14741	NS
	24	68	69	31	17	46849	23000	NS
	48	69	59	41	12	37578	25968	NS
LSD								
Time within species		NS	NS	NS		NS	NS	
Peanut	6	41	72	28	16	3902	1460	2181
	24	44	75	25	19	6675	2178	NS
	48	37	68	32	NS	13619	6503	NS
LSD								
Time within species		NS	NS	NS		NS	3640	
Between species		8	NS	NS		18969	12300	

LSD, Fisher's Protected least significance difference; NS, not significant.
[a] Data are means of two experiments with three replications each.
[b] Between organs.

Table 2. Metabolism of root-applied flurtamone in leaf tissue of peanut, 6, 24, and 48 h after treatment.

Metabolite	R_t value[b]	% of Radioactivity injected[a] 6	24	48
Unknown	3.3	27	35	22
Unknown	4.4	20	20	31
Unknown	5.6	13	28	23
Flurtamone	7	40	17	25
LSD (0.05)		NS	NS	NS

LSD, Fisher's protected least significant difference; NS, not significant.
[a] Data are means of three replications repeated in time.
[b] Metabolites were separated by HPLC using a mobile phase of methanol/water (72:28, vol/vol) on C_{18} column at 1 ml/min.

with an R_t of 7 min. Unknown metabolites with an R_t of 3.3, 4.4, and 5.6 min, respectively, were also detected in root tissue at 6, 24, and 48 h after treatment. Significant differences between each metabolite were not observed.

Discussion

Data obtained from the root absorption studies and autoradiography indicates that flurtamone is a xylem-mobile herbicide. However, flurtamone is poorly translocated in both susceptible-sicklepod and tolerant-peanut. These data provide evidence that one mechanism of differential selectivity of flurtamone may be based on differential absorption. Susceptible-sicklepod absorbed significantly more flurtamone than did tolerant-peanut.

Flurtamone is rapidly metabolized in tolerant-peanut (<6 h), and metabolism increases with time until about 80% of the applied ^{14}C-flurtamone is metabolized. Three metabolite peaks of flurtamone in peanuts were recognized. Since their R_t values are less than that of parent flurtamone, the metabolites are probably polar metabolites. The three metabolite peaks of flurtamone assayed in tolerant-peanut tissue were found in roughly equal concentrations, so no one metabolic pathway seems to predominate.

Our data indicate that flurtamone is not absorbed as rapidly in tolerant-peanut as in susceptible-sicklepod and is approximately 80% metabolized within 24 h. These data provide some basis for differential selectivity between flurtamone-susceptible and -tolerant species, but probably do not account for the two order of magnitude difference in susceptibility of sicklepod and peanut to flurtamone. A difference at the site of action between susceptible and tolerant plants may account for the differences in flurtamone tolerance.

Acknowledgments. Provision of partial financial support and technical and radiolabeled flurtamone by Valent Corporation is gratefully acknowledged.

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