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Imazethapyr absorption and fate in leafy spurge (*Euphorbia esula*)[1]

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Abstract:

Absorption, translocation, root release, and metabolism of imazethapyr by leafy spurge were determined under growth chamber conditions. ^{14}C-imazethapyr was applied to vegetatively propagated leafy spurge plants in a 1 % solution of 28% urea ammonium nitrate containing 0.25% by vol nonionic surfactant. Plants were harvested 2 and 8 days after herbicide application. Imazethapyr absorption increased from 9 % at 2 days to 20 % at 8 days. Acropetal and basipetal translocation out of the treated leaf was observed, with 3A to 4.2 % of the applied radioactivity accumulating in the root by the end of the 8-days time course. Eight days after herbicide application, radioactivity in dormant and, elongated adventitious shoot buds was twofold higher than in root tissue (compared on a dry wt basis). Two days after herbicide application, 93 % of the radioactivity remained as intact imazethapyr in the treated leaf, crown, root, and shoot buds. Eight days after application, crown, roots, and adventitious shoot buds had metabolized an average of 61, 36, and 47% of the imazethapyr, respectively, while only 14% was metabolized in the treated leaf. The primary metabolite cochromatographed with 5-hydroxyethyl-imazethapyr standard. Nomenclature: Imazethapyr, 2-[4,5-dihydro-4-(ethyl-(l-methylethyl)-5-oxo-lH-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid; leafy spurge, *Euphorbia esula L.* #[2] EPHES.

Additional index words:

Translocation, root release, rangeland weed, imidazolinone, perennial weed, adventitious shoot buds, EPHES.

Introduction

Leafy spurge is an introduced, herbaceous, perennial weed that infests large areas of rangeland in the northern and central Great Plains and the Prairie Provinces of Canada. Herbicides that are effective in management of leafy spurge are limited, and the most effective, picloram (4-amino-3,5,6-trichloro-2-pyridine-carboxylic acid), is too expensive to be used at rates that provide long-term control. The most frequently recommended treatment for leafy spurge management is an annual application of 2,4-D [(2,4-dichlorophenoxy)acetic acid] combined with low rates of picloram (0.28 to 0.56 kg ae ha^{-1}). Benefits of this annual treatment include elimination of leafy spurge seed production and increased forage yield (13). This treatment does not provide long-term control or reduce the size of existing infestations and result in suppression of desirable broadleaf species.

Imazethapyr is a relatively new herbicide that has demonstrated toxicity to leafy spurge (15) and can be used to selectively control a broad spectrum of broadleaf species in legume crops and forages. In addition, warm-season grasses have demonstrated reasonable tolerance to imazethapyr at rates up to 0.28 kg ha^{-1} (15). Imazethapyr has been developed primarily for annual cropping systems, so very little information is available on the behavior of imazethapyr in creeping perennial weeds.

Control of creeping perennials like leafy spurge requires translocation of the herbicide from the site of application to adventitious shoot buds on the root and crown. The most effective herbicides, 2,4-D and picloram, translocate sparingly to leafy spurge roots, and a large percentage, of herbicide reaching the root system is exuded from roots (8, 11). Laboratory experiments were conducted to determine absorption, translocation, root release, and metabolism of imazethapyr by leafy spurge.

Materials and methods

Plant material

Leafy spurge plants were vegetatively propagated from root cuttings from a single plant that was collected near Bozeman, MT. Root cuttings were planted in 4-cm-diam by 20-cm-long cones[3] and allowed to grow in the greenhouse for at least 3 months. Natural light was supplemented with 10 hours of 300 μE m^{-2} s^{-1} PPFD provided by metal halide lamps, and 25 g of slow-release fertilizer tablets (17-6-10) was applied to each plant[4].

Plants were removed from the greenhouse and shoots were discarded. Cones containing plant roots were placed in cold storage at 5° C for 14 days because chilling tempera-

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[2] Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 1508 West University Ave., Champaign, IL 61821-3133.

[3] Ray Leach Cone-tainer Nursery, Stuewe and Sons, Inc., Corvallis, OR 97333.

[4] Sierra 17-6-10. Grace-Sierra Horticultural Products Co., Milpitas, CA 95035.

tures stimulate shoot bud growth and development of inflorescences (7). After cold treatment, root systems were transplanted to 8-cm-diam by 30-cm-long cones[5] filled with fine, washed silica sand and placed in a growth chamber[6]. Growth chamber conditions were: 16-hour photoperiod, 25/20º C day/night temperature, 50% relative humidity, and PPFD of 650 µE m^{-2} s^{-1}. Plants were watered daily with 50 ml of half-strength Hoagland's (9) solution containing full-strength ion.

Absorption, translocation, and root release

Leafy spurge plants were 32 to 45 cm tall and had reached the midbloom growth 14 to 18 days after being transferred to the growth chamber. All experiments were conducted with plants at this growth stage. Imazethapyr was applied in a 1% by vol solution of 28% urea ammonium nitrate liquid fertilizer containing 0.25% by vol nonionic surfactant[7]. An overhead greenhouse pot sprayer was used to apply 0.07 kg ae ha^{-1} at a volume of 187 L ha^{-1}. This is the standard field rate for weed control in soybean. Two alternate leaves, approximately 10 cm below the shoot apex, were protected from spray solution with wax paper. ^{14}C-imazethapyr (specific activity 784 kBq mg^{-1}), labeled at the number 6 carbon of the pyridine ring, was mixed with spray solution and applied to the protected leaves as 10 1-µl droplets (5 µl per leaf, 11. 1 kBq per plant).

Plants were harvested 2 and 8 DAT and divided into seven parts: shoot above the treated leaves, treated leaves, stem below the treated leaves, crown, root, and both dormant and elongated adventitious shoot buds. Plant portions were patted dry with a paper towel and weighed. Treated leaves were swirled for 30 s in a 10% by vol aqueous methanol solution containing 0.25% by vol nonionic surfactant[7]. ^{14}C in the leaf wash solution was determined by liquid scintillation spectroscopy (LSS)[8] and used to estimate herbicide absorption. The ^{14}C in each plant part was determined by sample oxidation[9] and LSS. The silica sand rooting medium was placed in a 12-cm-diam buchner funnel containing filter paper[10] Two 250-ml aqueous washes were pulled through the silica sand under vacuum and 5-ml subsamples were removed from the filtrate. The ^{14}C present in subsamples was quantified with LSS.

Metabolism

Imazethapyr metabolism in leafy spurge was examined under the conditions previously described, except that ^{14}C applied per plant was increased to 18.5 kBq. Plants were divided into the same parts as previously noted and the treated leaf, crown, root, and ad-

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[5] Deepot Nursery Containers. Stuewe and Sons, Inc., Corvallis, OR 97333.

[6] Controlled Environments, Pembina, ND 5827 1.

[7] Tween 20. ICI Americas, Wilmington, DE.

[8] Abbreviations: ACN, acetonitrile; HPLC, high-pressure liquid chromatography LSS, liquid scintillation spectrometry.

[9] Packard Tri-Carb Oxidizer. Packard Instrument Co., Downers Grove, IL.

[10] Whatman #1. Whatman Int., Ltd., Maidstone, England.

ventitious shoot buds (dormant and elongated buds combined) were analyzed for herbicide metabolites. Tissue samples were immediately frozen in liquid N_2 and stored at -20° C.

Imazethapyr and possible metabolites were extracted from tissue samples by grinding samples in 10 ml of 90% methanol/water by vol using a tissue homogenizer[11]. Samples were extracted for 24 hours and then filtered through 0.2-µm membranes[12]. The filtrate was reduced under vacuum to 200 µl. Subsamples of the reduced extract were analyzed by reverse-phase[13] (C_{18}) high-pressure liquid chromatography[14] (HPLC)[8] coupled with in-line "radioactivity detection"[15]. A binary solvent system consisting of HPLC-grade water[12] with 0.1% phosphoric acid, and HPLC-grade acetonitrile[16] (ACN)[8] was used to separate intact herbicide from metabolites. The binary gradient consisted of three linear steps: 15 to 30% ACN in 5 min, 30 to 35% ACN in 10 min, and 35 to 85% ACN in 15 min. Percent ^{14}C remaining as intact imazethapyr was determined by the ratio of the imazethapyr peak area to the total ^{14}C of the extract. Plant parts that were not subjected to methanol extraction and HPLC analysis were dried and the amount of ^{14}C present was determined by sample oxidation and LSS.

Data analyses

Each treatment was replicated from three to five times and each experiment was repeated. Each experiment was designed as a factorial with plant part and DAT as factors. Percentage data from studies were arcsin transformed and Bq g^{-1} dry weight data from the absorption and translocation study were log transformed before statistical analyses. Data transformations did not result in significant changes in statistical analyses, so nontransformed data are presented. Bartlett's test for variance homogeneity was conducted to determine if data from the two trials of each study could be pooled (19). This test revealed that the error variance associated with the percentage data from the two trials of the absorption and translocation study were not homogeneous, while error variances associated with Bq g^{-1} dry weight data were homogeneous. Percentage data from the absorption and translocation study were analyzed separately and Bq g^{-1} dry weight data were pooled for analysis. Error variances associated with percentage data from the metabolism study were determined to be homogeneous and were pooled for analysis. Mean values for parameters measured were compared using Fisher's Protected Least Significant Difference Test ($P \leq 0.05$) (19).

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[11] Polytron. Brinkmann, Westbury, NY 11590.

[12] Gelman Sciences, Ann Arbor, MI 48106.

[13] J. T. Baker, Phillipsburg, NJ 08865.

[14] Shimatzu Instruments, Kyoto, Japan.

[15] Radiomatic Instrument & Chem. Co., Inc., Tampa, Fl, 33611.

[16] Baxter Healthcare Corp., Burdick and Jackson Div., Muskegon, MI 49442.

Results and discussion

Absorption, translocation, and root release

Recovery of ^{14}C ranged from 78 to 83% in the absorption and translocation experiments. Imazethapyr absorption increased from an average of 9.3% 2 DAT to 20.1% 8 DAT (Table 1). Imazethapyr absorption by leafy spurge was similar to that reported for 2,4-D and picloram (14), but was considerably lower than the 81% absorption reported for glyphosate [*N*-(phosphono-methyl)glycine](16).

Radioactivity data for dormant and elongated adventitious shoot buds were combined with root data for analysis of translocation patterns. The majority of radioactivity remained in the treated leaf; however, ^{14}C in leafy spurge roots increased threefold during the 8-day translocation period (Table 1). ^{14}C was detected in all other plant parts, but amounts were generally less than 1% of applied ^{14}C. One exception was that greater translocation from the treated leaf occurred in plants harvested 8 DAT in trial II. This resulted in 2.6% more ^{14}C above and 2.0% more ^{14}C below the treated leaf than in trial I, while the amount of ^{14}C in the root was similar between the trials.

Table 1. Percent absorption and distribution of ^{14}C in leafy spurge and aqueous filtrate of rooting media 2 and 8 days after ^{14}C-imazethapyr application[a].

Plant part and filtrate	^{14}C present			
	Trial I		Trial II	
	2 DAT[b]	8 DAT[b]	2 DAT[b]	8 DAT[b]
	% of applied			
Above treated leaf	0.4	0.7	0.6	3.3
Treated leaf	4.6	9.3	5.1	6.8
Below treated leaf	0.6	0.6	0.7	2.6
Crown	0.3	0.6	0.3	1.1
Root'	1.1	3.4	1.3	4.2
Filtrate	0.1	0.2	0.3	0.5
LSD (0.05)	1.5		0.9	
Absorption	9.0	18.1	9.7	22.2
LSD (0.05)	2.5		3.8	

[a]Results were separated by Trials I and II.
[b]Days after treatment.
[c]Combined root and dormant and elongated adventitious shoot buds.

Research with perennial plants is challenging because of the difficulties in duplicating precise phenologic and physiologic conditions. The most important aspect of translocation, e.g. herbicide movement into the leafy spurge root system, was consistent between trials. No data are available on imazethapyr translocation in other perennial plants. Research involving annual species indicates that imazethapyr translocation is variable and

species dependent. Soybean (*Glycine max* L.) translocated 11.3% of applied imazethapyr below the treated leaf compared to only 1 % translocation in velvetleaf (*Abutilon theophrasti* L.) (12). The percent of applied ^{14}C reaching leafy spurge roots following ^{14}C-imazethapyr application was similar to amounts of ^{14}C translocated to leafy spurge roots following ^{14}C-picloram and ^{14}C-2,4-D applications (14).

The maximum amount of ^{14}C present in the filtrate from rooting media was 0.5% of ^{14}C applied, which was approximately 10% of the ^{14}C translocated to the root system Table 1). Leafy spurge released 60 to 80% of ^{14}C-picloram (8) and ^{14}C-2,4-D (11) from the root system. Since root release of picloram has been described as a physical process controlled primarily by temperature and concentration gradients (8), it is difficult to speculate about the differences between these studies. The fact that previous studies were conducted in hydroponic systems and the present study was conducted in sand culture might explain the observed differences. Lingle and Suttle (11) found that the amount of 2,4-D released declined from 80 to 40% when leafy spurge was grown in vermiculite media compared to hydroponics. It should be noted that significant root release of 2,4-D has been reported in other perennial plants (20).

Studies involving herbicide movement in leafy spurge rarely determine preferential translocation of ^{14}C to adventitious shoot buds. Maxwell *et al*. (16) examined translocation of ^{14}C to roots and adventitious shoot buds of leafy spurge 5 DAT with ^{14}C-glyphosate. Comparisons on a Bq g^{-1} dry weight basis indicated that ^{14}C in roots and adventitious shoot buds were generally equivalent or somewhat greater in adventitious shoot buds. In the present study, the amount of ^{14}C accumulating in roots increased twofold between 2 and 8 DAT (Table 2). By comparison, ^{14}C accumulating in dormant and elongated adventitious shoot buds increased fourfold during the translocation period. These data suggest that translocation to dormant and elongating adventitious shoot buds was greater than translocation to roots.

Table 2. Dry weight, Bq per plant part^{-1}, and amount of ^{14}C in leafy spurge roots and dormant and elongated adventitious shoot buds 2 and 8 days after ^{14}C-imazethapyr application[a].

	Dry wt at DAT		^{14}C in plant part at DAT		^{14}C per gram dry wt at DAT[b]	
Plant part	2	8	2	8	2	8
	G		Bq			
Root	1.54 (.16)	2.05 (.14)	106 (16)	256 (26)	70	136
Dormant root buds	0.07 (.02)	0.06 (.01)	5 (l)	17 (2)	69	271
Elongated root buds	0.20 (.03)	0.22 (.03)	19 (5)	74 (11)	98	354
LSD (0.05)					105	

[a]Means (standard error).

[b]Back transformed from log Bq g dry wt^{-1}.

Substantive translocation of imazethapyr to the roots may explain the observed growth-regulating effects of the herbicide in leafy spurge (13). Imazethapyr and glyphosate produce many of the same growth-regulating effects in leafy spurge, including

stimulation of axillary and adventitious shoot bud growth (16). Abnormal leafy spurge growth caused by glyphosate could result from inhibition of indoleacetic acid (IAA) transport (1) or reductions in free IAA levels (10). Imazethapyr increased forage quality in cool-season grasses (3) and alfalfa/grass mixtures (4) by retarding stem elongation, inhibiting floral development, and inducing tiller formation (3). Physiological mechanism(s) inducing the growth-regulating properties of imazethapyr are unknown.

Imazethapyr metabolism

Recovery of ^{14}C during imazethapyr metabolism studies was greater than 90%. The HPLC gradient adequately separated intact imazethapyr from the major metabolite (Figure la). The metabolite cochromatographed with 5-hydroxyethyl-imazethapyr standards (Figure 1b) and represented 90% of the ^{14}C that was not present in the imazethapyr peak.

Greater than 90% of the ^{14}C present was intact imazethapyr 2 DAT (Table 3). The decrease in amounts of intact imazethapyr remaining 8 DAT indicated that significant imazethapyr metabolism occurred. Crown tissue metabolized imazethapyr more rapidly than other tissues examined, with 39% of extracted ^{14}C as intact imazethapyr 8 DAT. Roots and adventitious shoot buds readily metabolized imazethapyr. The amount of extractable ^{14}C remaining as intact imazethapyr in roots and adventitious root buds was 53 and 64%, respectively.

The half-life of imazethapyr was not determined specifically in leafy spurge, but based on the two sampling periods, it would appear the half-life would be less than 8 days in crown tissue and slightly more than 8 days in root and adventitious shoot buds (Table 3). For tolerant species like soybean and peanut (*Arachis hypogea* L.), the half-life of imazethapyr was approximately 6 days, while for susceptible species like sicklepod (*Cassia obtusifolia* L.) and redroot pigweed (*Amaranthus retroflexus* L.) the half-life of imazethapyr was 24 and 32 days, respectively (2). The capacity of leafy spurge to me-

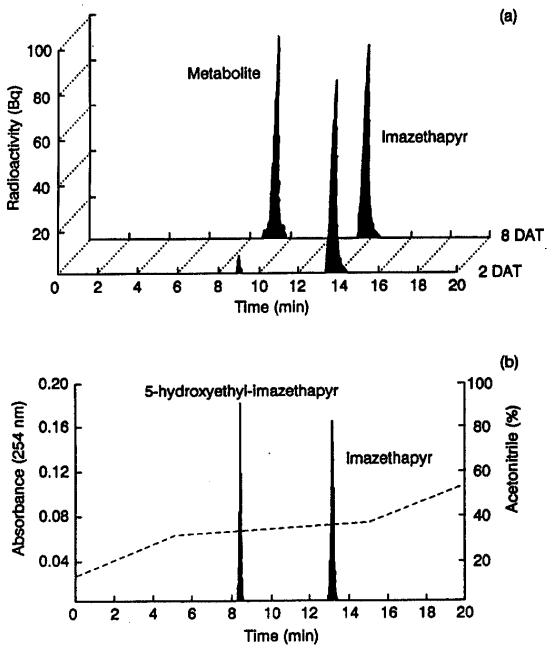

Figure 1. Reverse-phase HPLC chromatogram of 90% methanol extract from leafy spurge crown tissue 2 and 8 days after ^{14}C-imazethapyr application (a) and imazethapyr and 5-hydroxyethyl imazethapyr standards (b). HPLC solvents were 0.1 % phosphoric acid and HPLC-grade acetonitrile. A binary gradient consisting of three linear steps was used to separate imazethapyr from metabolites. The linear steps were 15 to 30% ACN in 5 minutes, 30 to 35% ACN in 10 minutes, and 35 to 85% ACN in 15 minutes.

tabolize imazethapyr to 5-hydroxyethyl-imazethapyr appears to be very rapid; however, tolerant species like soybean and peanut rapidly conjugate 5-hydroxyethyl-imazethapyr to glucose (18). This step is critical because 5-hydroxyethyl-imazethapyr is phytotoxic, with an acetohydroxyacid synthase I_{50} value of 2.5 µM compared to 1 µM for imazethapyr (17). There was little evidence for glucose conjugation of 5-hydroxyethyl-imazethapyr in leafy spurge because 90% of the ^{14}C extracted and fractionated by HPLC was associated with the imazethapyr or 5-hydroxyethyl-imazethapyr peaks 8 DAT (Figure 1). In whole plant studies, 5-hydroxyethyl-imazethapyr does not appear to be highly phytotoxic because the compound does not translocate to meristematic areas (19). Therefore, in leafy spurge, imazethapyr was translocated intact and then metabolized to 5-hydroxyethyl-imazethapyr. This suggests that the rapid disappearance of imazethapyr does not reduce herbicidal activity but could affect translocation patterns.

Table 3. Percent of extracted ^{14}C remaining as intact imazethapyr in treated leaf, crown, roots, and adventitious shoot buds of leafy spurge 2 and 8 days after ^{14}C-imazethapyr application.

Plant part	^{14}C extracted	
	2 DAT[a]	8 DAT[a]
	%	
Treated leaf	91	86
Crown	94	39
Buds[b]	96	64
Root	96	53
LSD (0.05)	12	

[a]Days after treatment.
[b]Combined dormant and elongated adventitious shoot buds.

It is difficult to compare the half-life of imazethapyr to that of other herbicides used to control leafy spurge. The half-lives of picloram, 2,4-D, glyphosate, and dicamba (3,6-dichloro-2-methoxybenzoic acid) have not been adequately determined in leafy spurge. The literature contains conflicting reports on the rate of picloram metabolism. Frear *et al*. (5) reported that 62% of foliar-absorbed picloram. remained intact 72 hours after application, while Lym and Moxness (14) found 94% intact picloram 96 hours after foliar application. In addition, Lym and Moxness (14) reported that 92% of the foliar-absorbed 2,4-D remained intact 96 hours after treatment, while Lingle and Suttle (11) found significant amounts of 2,4-D metabolism. Glyphosate has limited effectiveness on leafy spurge but was completely intact 168 hours after application (6). Based on this evidence, herbicide half-life may not be a good indicator of herbicide efficacy in leafy spurge.

Imazethapyr absorption and translocation in leafy spurge appeared to be similar to that of picloram and 2,4-D. Detailed studies of translocation patterns indicated significant movement of imazethapyr to adventitious shoot buds. Unlike 2,4-D and picloram, only 10% of imazethapyr reaching the root system was released into the rooting media. Significant amounts of imazethapyr were metabolized 8 DAT; however, there is no way to

compare imazethapyr metabolism to that of picloram and 2,4-D because definitive studies have not been conducted. Imazethapyr has many desirable properties that could be useful in leafy spurge management programs.

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