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Absorption, translocation, and metabolism of picloram and 2,4-D in leafy spurge¹

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Abstract:

The absorption, translocation, and metabolism of ¹⁴C-picloram and ¹⁴C-2,4-D applied alone and together to leafy spurge were evaluated. Leafy spurge absorbed 34 and 24% of the ¹⁴C-2,4-D applied alone and with picloram, respectively, and 14 and 10% of the ¹⁴C-picloram applied alone and with 2,4-D, respectively. More ¹⁴C-2,4-D was translocated in leafy spurge than ¹⁴C-picloram, and adding picloram to ¹⁴C-2,4-D decreased ¹⁴C translocation to the roots. Adding 2,4-D to ¹⁴C-picloram increased the percentage of absorbed ¹⁴C that translocated in leafy spurge from 28 to 48%. Generally, ¹⁴C-picloram and ¹⁴C-2,4-D remained as the parent acid in leafy spurge whether applied alone or together. Of ¹⁴C-picloram recovered from the roots, 83% was unmetabolized picloram when applied alone compared to 95% when applied with 2,4-D, which probably is the reason for increased leafy spurge control when these herbicides are applied together.

Nomenclature:

Picloram, 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid; 2,4-D, (2,4-dichlorophenoxy)acetic acid; leafy spurge, *Euphorbia esula* L. #² EPHES.

Introduction

Leafy spurge is an introduced perennial weed that infests over 1 million hectares in North America (3, 20). Herbicides have been the most successful method for leafy spurge

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control; dicamba (3,6-dichloro-2-methoxybenzoic acid), 2,4-D, and picloram are the ones most commonly used (15). The cost of high rates of dicamba and picloram needed for long-term leafy spurge control is often 50% or more of the total land value and 8 to 10 times higher than the cash rent value of pasture and rangeland and thus has limited their usage (12, 20). 2,4-D provides only short-term shoot control even when applied twice per year for several years (15, 17).

At 0.3 to 1.1 kg ae/ha, 2,4-D with picloram at 0.3 to 0.6 kg ae/ha applied annually provides greater leafy spurge control than picloram applied alone at similar rates and is cost effective (15, 17). Control averaged 85% following three annual applications of Picloram plus 2,4-D at 0.28 plus 1.1 kg/ha compared to 48% with picloram at 0.28 kg/ha alone. Forage production increased 71% while leafy spurge production decreased by 96% compared to the untreated control (16). Leafy spurge control did not increase by increasing the rate of 2,4-D or picloram to more than 1.1 or 0.6 kg/ha, respectively (15). Leafy spurge control was not improved when dicamba, triclopyr {[3,5,6-trichloro-2-pyridinyl)oxy]acetic acid}, or fluroxypyr {[4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy]acetic acid} was applied with 2,4-D or picloram compared to the herbicides applied alone (15, 18, 19).

Picloram translocation can be influenced by phenoxyalkanoic herbicides. Picloram translocation in bean (*Phaseolus vulgaris* L.) was greater when ¹⁴C-picloram was applied with 2,4-DB [4-(2,4-dichlorophenoxy)butanoic acid] than when applied alone (10). However, ¹⁴C-picloram translocation in field bindweed (*Convolvulus arvensis* L.) was similar when picloram was applied alone or with 2,4-D (1).

The purpose of this research was to evaluate the absorption, translocation, and metabolism of 2,4-D and picloram applied alone and together in leafy spurge and to determine the reasons for increased control when 2,4-D is applied with picloram compared to either herbicide alone.

Materials and methods

Absorption and translocation

Leafy spurge roots were obtained from a natural infestation (accession 1984 ND 001) near Fargo, ND, divided into 2- to 3-cm sections and planted in greenhouse flats containing Fairdale silty loam and peat (1:1, v/v). Plants were propagated in the greenhouse at 21 to 27° C with supplemental fluorescent light (100 $\mu\text{Em}^{-2}\text{s}^{-1}$) when necessary for a 16-hour light and 8-hour dark photoperiod. Plants were transplanted to 0.5- and then to 1-L pots when approximately 10 and 20 cm tall, respectively. Leafy spurge topgrowth was removed at the soil surface after the second transplanting, and one stem per pot was allowed to regrow to a mid- to late-vegetative growth stage (20 to 30 cm tall) before treatment. Foliar absorption and translocation of ¹⁴C-picloram were greatest at this growth stage compared to the early vegetative or flowering stages in preliminary experiments (data not shown). Plants of uniform size were selected for use in each experiment.

One leaf midway on the stem of each plant was enclosed with a protective envelope made from two small strips of paper bound with tape. Picloram potassium salt or 2,4-D

alkanolamine salt was applied at 0.28 and 0.56 kg/ha, respectively, to the topgrowth except the covered leaf with a moving-nozzle pot sprayer delivering 140 L/ha at 230kPa. The protective envelope was removed from the leaf and 5 μ l of 0.15% (v/v) nonionic surfactant³ in H₂O was applied to 1 cm² of the leaf followed immediately by 0.023 μ Ci of uniformly pyridyl ring-labeled ¹⁴C-picloram (specific activity 16.85 mCi/mmol) or 0.023 μ Ci of uniformly phenyl ring labeled ¹⁴C-2,4-D (specific activity 55 μ Ci/mmol) in 5 μ l of 80 or 50% (v/v) ethanol, respectively. The molar concentration of the ¹⁴C-2,4-D applied was approximately 30% of the ¹⁴C-picloram due to the higher specific activity of the ¹⁴C-2,4-D. However, since both were applied at sublethal rates, it's unlikely the comparative movement was affected. Unlabeled herbicide was added to the surfactant solution to achieve an application rate equivalent to the broadcast rate. The treatments consisted of: a) ¹⁴C-picloram, b) ¹⁴C-picloram plus 2,4-D, c) ¹⁴C-2,4-D, and d) ¹⁴C-2,4-D plus picloram.

Plants were harvested 72 hours after treatment and sectioned into the treated leaf, shoot, and roots. The roots were washed with water to remove soil. The treated leaf was rapidly dipped 10 times into 15 ml of scintillation fluid 'A' [1:1, v/v, toluene:ethanol plus 5 g/L PPO (2,5-diphenyloxazole)] and 0.5 g/L dimethyl-POPOP [1,4-bis-2-(4-methyl-5phenyloxazolyl)benzene] to remove unabsorbed ¹⁴C-herbicide. Plant sections were dried at 60° C for 24 hours, and root and shoot sections were ground in a Wiley mill (#10 mesh) and weighed.

The treated leaf and two or more 120- to 150-mg root or shoot subsamples equaling at least 10% of the sample weight were each combusted in a biological tissue oxidizer.⁴ The ¹⁴CO₂ was collected in 15 ml of scintillation fluid 'B' (10:7:3, v/v/v, toluene:2-methoxyethanol:ethanolamine plus 5.0 g/L PPO and 0.5 g/L dimethyl-POPOP). Samples were assayed using liquid scintillation spectrometry. Oxidizer efficiency was determined using methyl-¹⁴C-methacrylate, and liquid scintillation counting efficiency was determined using an external standards ratio and standard corrections.

Metabolism

Leafy spurge plants were propagated as previously described except plants were transplanted from trays of vermiculite to 4-cm-diameter by 20-cm-long conical pots, which contained medium of peat, perlite, and vermiculite. Leafy spurge topgrowth was removed, and the plant was allowed to regrow to 20 to 30 cm tall.

Two leaves midway on the stem of each plant received 5 μ l of 0.15% (v/v) nonionic surfactant³ followed by 0.138 μ Ci of ¹⁴C-picloram or ¹⁴C-2,4-D, respectively, or 0.69 μ Ci of ¹⁴C-picloram plus 0.69 μ Ci of ¹⁴C-2,4-D as previously described. An additional 5 μ l of surfactant solution was then applied to maximize absorption and the plants were returned to the greenhouse.

The plants were harvested after 96 hours, beginning by removing the treated leaves and recovering unabsorbed ¹⁴C as previously described. The plants were sectioned into

³ Surfactant WK (dodecyl ether of polyethylene glycol). E.I. duPont de Nemours and Co., Wilmington, DE.

⁴ Model 0X100. R. J. Harvey Instrument Corp., Hillsdale, NJ.

treated leaves, stem and leaves above the treated leaves, stem and leaves below the treated leaves, and roots. All tissues were stored frozen at -20° C until extraction, generally 2 to 3 weeks. Previous research has shown picloram and 2,4-D conjugates remain stable at least 3 to 4 months when frozen.⁵

Herbicides and metabolites were extracted by grinding in 80% ethanol:water (v/v) in a motor-driven tissue homogenizer, followed by centrifugation and re-extraction of the pellet twice in the same solvent mixture to increase recovery. The extracts were combined and extracted with ether once. The pellet was dried and combusted. The pellet contained <0.3% of the applied ¹⁴C-picloram regardless of plant section and an average of 3% and <0.8% of the applied ¹⁴C-2,4-D or ¹⁴C-picloram plus ¹⁴C-2,4-D in the treated leaf and other plant sections, respectively. The ether extract contained less than 1% of the total ¹⁴C applied and when chromatographed separated with the same R_f values and in the same proportions as the ¹⁴C in the ethanol:water extract. Thereafter the ether extract was discarded.

The aqueous extract was concentrated with a stream of air to 0.2 ml. The ¹⁴C herbicide and metabolites were quantified by spotting on precoated 20- by 20-cm silica 60 thin-layer chromatography plates. The plates were developed to a 15-cm solvent front using two-way development. Solvent system I (benzene:acetone: acetic acid:methanol, 80:40:10:5, v/v/v/v) separated unmetabolized ¹⁴C-picloram and ¹⁴C-2,4-D. Then the plate was rotated 90 degrees and developed using solvent system II (chloroform:water, 65:25:4, v/v/v) for further separation. The developed plates were cut into four 5-cm-wide bands, and the ¹⁴C was detected with a radiochromatogram scanner. R_f values were calculated and compared to those of the standard ¹⁴C herbicides and to reported values for picloram metabolites in leafy spurge⁵. The plates were reassembled and autoradiographed for visual determination of ¹⁴C herbicide and metabolites. The plates were pressed against X-ray film⁶ for a 60-day exposure.

Areas of the chromatography plate that contained ¹⁴C were then scrapped and quantified using liquid scintillation spectrometry or scrapped and rechromatographed using HPLC⁷, and the fractions were collected and quantified (two replications of treated leaf and root sections only). The ¹⁴C-labeled herbicides and metabolites were redissolved in 3 ml of acetonitrile:water (60:40, v/v) prior to HPLC analysis. The solution was concentrated to 100 µl, filtered, and a 10-µl injection was chromatographed using a C-18 reverse-phase cartridge⁸. The solvent system consisted of eluent 'A', 1% acetic acid in water, and eluent 'B', 1% acetic acid in acetonitrile (v/v) using a linear gradient of 5 to 80% 'B' in 15 minutes. The detector was a variable wavelength spectrophotometer operated at 254 nm.

⁵ Stuart D. Frear. USDA Biological Sciences Lab., Fargo, ND, Personal communication.

⁶ The film was X-omat AR-5. Eastman Kodak Co., Rochester, NY.

⁷ HPLC = high-pressure liquid chromatography.

⁸ Bio-Sil ODS-5S (0.45 i.d. by 25 cm). Bio-Rad Chem. Div., Richmond, CA.

Statistical analyses

The absorption and translocation experiments were conducted twice and the metabolism studies were conducted four times. The experiments had similar variances so the combined data are presented. These experiments were in a randomized complete block design with four replicates per treatment. The data were analyzed using the protected LSD mean separation technique.

Results and discussion

Absorption and translocation

Approximately 2.5 times more ^{14}C -2,4-D was absorbed and translocated in leafy spurge than ^{14}C -picloram (Table 1); and since translocation expressed as percent of absorbed was similar for both herbicides, total 2,4-D translocation was about 2.5 times greater than picloram. More ^{14}C was absorbed when either herbicide was applied alone than when applied together. Leafy spurge absorbed 14% of the ^{14}C -picloram when applied alone but only 10% when applied with 2,4-D. Similarly, leafy spurge absorbed 34% of the ^{14}C -2,4-D when applied alone but only 24% when applied with picloram.

Table 1. Absorption and translocation of ^{14}C -picloram applied alone and with 2,4-D, and of ^{14}C -2,4-D applied alone and with picloram in leafy spurge 72 hours after treatment.

Treatment	Percent of herbicide, plant section			Total ^{14}C	
	Treated leaf	Shoot	Root	Absorbed	Translocated
	(% of applied)			(% of absorbed)	
^{14}C -picloram	9.8	2.1		14	28
^{14}C -picloram plus 2,4-D	5.2	2.9	1.7	10	48
^{14}C -2, 4-D	25.8	5.3	1.9	34	25
^{14}C -2, 4-D plus picloram	17.5	5.2	3.3	24	28
LSD (0.05)	3.7	1.4	1.5	4	

These data differ from those reported for field bindweed, which absorbed more ^{14}C -picloram than ^{14}C -2,4-D (2). However, 2,4-D caused necrosis to field bindweed leaves, which may have limited 2,4-D absorption. Absorption of ^{14}C -picloram in field bindweed was greater when ^{14}C -picloram was applied with 2,4-D than when applied alone (1). Absorption of ^{14}C -2,4-D in field bindweed was similar when ^{14}C -2,4-D was applied alone and with picloram.

When the herbicides were applied alone more ^{14}C -2,4-D than ^{14}C -picloram was translocated to the shoot and root sections of leafy spurge (Table 1). The ^{14}C translocated to the shoot portion of leafy spurge averaged 2.1 and 5.3% of applied ^{14}C -picloram and ^{14}C -2,4-D, respectively. The amount of ^{14}C in the shoot was similar when either herbicide was applied alone compared to together and averaged 2.5 and 5.2% for ^{14}C -picloram and ^{14}C -2,4-D, respectively. An average of 1.8% of applied ^{14}C -picloram was translocated to

the leafy spurge roots and was not influenced by adding 2,4-D. However, over twice as much ^{14}C was recovered in the leafy spurge roots when ^{14}C -2,4-D was applied alone (3.3%) compared to applying with ^{14}C -picloram (1.5%).

The amount of herbicide translocated to the root system of perennial weeds probably influences long-term control. Separate studies have shown that 5% of the applied ^{14}C -picloram was translocated to leafy spurge roots, but only 2% was retained while the remaining 3% was in the nutrient solution (11). The amount of ^{14}C -picloram recovered from the nutrient solution was similar for plants treated with ^{14}C -picloram alone and with ^{14}C -picloram plus 2,4-D. The authors concluded that the synergistic action of picloram plus 2,4-D on leafy spurge was not due to reduced release of picloram from the roots.

Thus, the synergistic response of picloram plus 2,4-D for leafy spurge control probably is not from increased absorption or translocation of either herbicide, nor from reduced picloram release from leafy spurge roots. It may be due to an effect of picloram on 2,4-D release. Lingle and Suttle (4) reported over 80% of the 2,4-D translocated to leafy spurge roots was leaked from the roots. However, less ^{14}C translocated to the leafy spurge roots when ^{14}C -2,4-D was applied with picloram compared to ^{14}C -2,4-D alone (Table 1). Therefore, even if the amount of 2,4-D lost from the roots was decreased by adding picloram, there still would be less total 2,4-D retained in the roots than when 2,4-D was applied alone and probably would not be the reason for increased leafy spurge control.

Translocation of ^{14}C -picloram in leafy spurge was 48% of that absorbed when ^{14}C -picloram was applied with 2,4-D but only 28% when applied alone (Table 1). The percent of absorbed ^{14}C -2,4-D translocated in leafy spurge, however, was not influenced by adding picloram to ^{14}C -2,4-D. This suggests that picloram and 2,4-D do not compete for translocation in leafy spurge and that 2,4-D may increase the amount of ^{14}C -picloram translocated despite less absorption when picloram is applied with 2,4-D than when applied alone.

Metabolism

Generally, ^{14}C -picloram and ^{14}C -2,4-D remained as the parent acid in leafy spurge regardless of whether applied alone or together (Table 2). Many researchers have found that most ^{14}C recovered following treatment with ^{14}C -2,4-D or ^{14}C -picloram is the parent compound in susceptible species while water-soluble metabolites are detected in resistant species (7, 8, 9, 21, 22, 23, 25).

A second ^{14}C compound was detected in ^{14}C -picloram-treated leafy spurge leaves and was designated picloram-metabolite I. Picloram-metabolite I had an R_f and HPLC retention time similar to the glucose ester-conjugate of picloram, the major metabolite of picloram in leafy spurge. *N*-glucoside and gentiobiose esters of picloram also occur in leafy spurge but were not detected in these studies (4). In general, picloram is unaltered in highly susceptible plants but forms sugar conjugates in more resistant plants such as Canada thistle [*Cirsium arvense* (L.) Scop.], rapeseed (*Brassica napus* L.), and field bindweed (6, 13, 25, 26).

Table 2. R_f values relative amounts, and HPLC retention times of ¹⁴C compounds recovered from various leafy spurge plant sections % 96 hours following treatment with ¹⁴C-picloram or ¹⁴C-2, 4-D applied alone and together.

Herbicide	¹⁴ C source	¹⁴ C Compound ^a	Two-way TLC ^b (R _{f1} x R _{f2})	¹⁴ C amount (% recovered)	HPLC retention time (min)	
¹⁴ C-picloram	Standard	Picloram	0.31 x 0.44	...	6.6	
		Picloram	0.31 x 0.41	72	6.6	
	Treated leaves	Picloram-MI	0 x 0.32	4.3	7.9	
		Picloram	0.20 x 0.44	8.9	...	
	Above treated leaves	Picloram-MI	0 x 0.35	10.5	...	
		Picloram	0.29 x 0.48	2.5	...	
	Below treated leaves	Picloram-MI	0 x 0.35	1.1	...	
		Picloram	0.26 x 0.45	1	6.4	
	Roots	Picloram-MI	0 x 0.32	0.2	8.0	
		Picloram	0.58 x 0.8	...	12.1	
¹⁴ C-2,4-D	Standard	2,4-D	0.58 x 0.8	...	12.1	
		2,4-D	0.57 x 0.78	85	12.2	
	Treated leaves	2,4-D-MI	0 x 0.68	4.3	...	
		2,4-D-MII	0 x 0.27	3	...	
		2,4-D	0.56 x 0.71	0.9	...	
	Above treated leaves	2,4-D-MI	0 x 0.68	0.2	...	
		2,4-D	0.57 x 0.79	1.3	...	
	Below treated leaves	2,4-D-MI	0 x 0.68	0.5	...	
		2,4-D	0.53 x 0.72	2.9	12.0	
	Roots	2,4-D-MI	0 x 0.65	0.8	...	
		2,4-D	0.30 x 0.46	12	...	
	¹⁴ C-picloram + ¹⁴ C-2,4-D	Treated leaves	Picloram	0.30 x 0.46	12	...
			2,4-D	0.56 x 0.82	74	...
			Picloram-MI	0 x 0.32	0.5	...
			2,4-D-MIU	0 x 0.68	1	...
2,4-D-MII			0 x 0.27	> 0.1	...	
Above treated leaves		Picloram	0.28 x 0.46	0.8	...	
		2,4-D	0.54 x 0.75	2.5	...	
		Picloram-MI	0 x 0.37	1.4	...	
		2,4-D-hg	0 x 0.65	1.2	...	
Below treated leaves		Picloram	0.26 x 0.48	0.5	...	
		2,4-D	0.54 x 0.77	2.7	...	
		Picloram-MI	0 x 0.35	0.1	...	
		2,4-D-MI	0 x 0.65	0.3	...	
Roots		Picloram	0.28 x 0.47	2	6.5	
		2,4-D	0.52 x 0.78	1.1	12.1	
	Picloram-MI	0 x 0.36	< 0.1	...		
	2,4-D-MI	0 x 0.66	< 0.1	...		

^a MI and MII refer to metabolite I or metabolite II of the respective parent acids.

^b The plates were developed in benzene:acetone:acetic acid:methanol (80:40:10:5, v/v/v/v), allowed to dry (R_{f1}), rotated 90° and developed in chloroform:methanol:water (65:25:4, v/v/v) R_{f2}).

Picloram-metabolite I also was found in the plant sections above and below the treated leaves and in the roots and was 54% of the ¹⁴C recovered in the above treated leaves section (Table 2). The ¹⁴C in the roots, however, was 83% picloram and 17% picloram-metabolite I. Thus, ¹⁴C-picloram and picloram-metabolite I are translocated to the

above treated leaves section in similar proportions, while more ^{14}C -picloram is translocated below the treated leaves than picloram-metabolite I or more picloram metabolism occurs in the upper portion of the leafy spurge plant than in the roots.

Most of the ^{14}C recovered from ^{14}C -2,4-D-treated leafy spurge plants remained as the parent acid, but there were eight metabolites detected in the treated leaves section by autoradiography (Table 2). Only two of these metabolites constituted more than 0.1% of the total ^{14}C recovered from the plants and were designated as 2,4-D-metabolites I and II. These compounds were likely sugar conjugates of 2,4-D and possibly amino acids (7, 9), but no attempt was made to identify these compounds. Only 2,4-D-metabolite I and ^{14}C -2,4-D were detected in the other plant sections and nearly 80% of the ^{14}C detected was unmetabolized 2,4-D.

When ^{14}C -2,4-D and ^{14}C -picloram were applied together, most of ^{14}C recovered was ^{14}C -2,4-D in all plant sections except in the root. The percentage of parent herbicide to metabolite in the treated leaves and above treated leaves sections was similar when the herbicides were applied together compared to alone. However, there was a trend for decreased metabolism of both herbicides in the below treated leaves and root sections when applied together. For example, 69% (2.5/3.6% recovered) and 72% (1.3/1.8% recovered) of the recovered ^{14}C in the below treated leaves section was ^{14}C -picloram and ^{14}C -2,4-D, respectively, when applied alone, but 83 (0.5/0.6% recovered) and 90% (2.7/3% recovered) were ^{14}C -picloram and ^{14}C -2,4-D in the same plant section when applied together (Table 2).

The exact pathway of picloram and 2,4-D metabolism in leafy spurge has not been elucidated completely (4). These two herbicides may be metabolized by similar metabolic pathways in leafy spurge. Competition for similar enzymes may result in less metabolism when 2,4-D and picloram are applied together than alone.

An increase in unmetabolized picloram present in the root system when 2,4-D is applied with picloram compared to picloram alone may be the reason for increased leafy spurge control. Picloram-metabolite I and 2,4-D-metabolite I were less than 0.1 % of the total ^{14}C recovered in the roots when ^{14}C -picloram and ^{14}C -2,4-D were applied together (Table 2). Even though only 0.2 and 0.8% of the recovered ^{14}C in the roots was picloram-metabolite I and 2,4-D-metabolite I, respectively, when the herbicides were applied alone, it was approximately 20% of the total ^{14}C recovered in the roots compared to less than 3% when the herbicides were applied together. Also, the roots were the only plant section where more ^{14}C -picloram than ^{14}C -2,4-D was present. It is unlikely the increased control is due to increased 2,4-D activity since there was less total ^{14}C -2,4-D in the root when applied with picloram than alone (Table 1) and 2,4-D is less toxic to leafy spurge than picloram.

Similar results have been found in horsenettle (*Solanum carolinense* L.) (5). Slightly more ^{14}C was found in the roots from foliar treatments of dicamba and triclopyr (3.8 and 3.6%) than picloram (3%) but picloram was more toxic. The authors concluded that since there was little metabolism of the herbicides, picloram had greater inherent toxicity than dicamba or triclopyr to horsenettle.

Picloram is the most effective herbicide used for leafy spurge control (15). Since only 2 to 5% of the ^{14}C -picloram applied to leafy spurge reaches the root [(11) and Table 1],

even a small increase in unmetabolized picloram at the site of action could increase long-term control (24). Generally, the increase in control in the field ranges from 20 to 30% when picloram is applied with 2,4-D compared to picloram alone (17), which is similar to the increase in unmetabolized ^{14}C -picloram present in the root.

Since so little of the picloram applied to leafy spurge reaches the root, the best opportunity for increased cost-effective control is by increasing picloram absorption and subsequent translocation. This may be possible by applying picloram with spray additives to increase absorption alone or by combining with 2,4-D to also decrease metabolism.

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