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Phytotoxicity and Transport of Clopyralid from Three Formulations in Honey Mesquite

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Abstract. Foliar sprays of the monoethanolamine salt, oleylamine salt, and 1-decyl ester of clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) were about equally effective in killing greenhouse-grown honey mesquite (*Prosopis glandulosa* Torr.). Treated leaves absorbed more clopyralid within 15 min after pipet application of the oleylamine salt compared to the other formulations. After 24 h, treated leaves absorbed and transported more clopyralid into the plant after application of the salt formulations compared to that of the 1-decyl ester. There were no consistent differences among clopyralid formulations in transport of clopyralid from foliar sprays at 4 h or 1, 3, or 8 days after treatment. Only the acid form of clopyralid was transported from the site of application of either ester or the amine formulation.

The monoethanolamine salt of clopyralid is highly effective for control of honey mesquite at 0.56 kg/ha or when clopyralid is mixed with picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid) or triclopyr ([(3,5,6-trichloro-2-pyridrinyl)oxy]acetic acid) at 0.28 + 0.28 kg/ha (Bovey and Meyer 1985, Jacoby et al. 1981). The search for the most effective chemical formulation of clopyralid against honey mesquite resulted in evaluation of the potassium salt, free acid, 1-decyl ester, and 2-ethylhexyl ester compared to the monoethanolamine salt. All formulations were about equally effective in killing greenhouse-grown honey mesquite at rates of 0.21 or 0.28 kg/ha, except that all were superior to the 2-ethylhexyl ester (Bovey et al. 1989). An oil-soluble formulation of clopyralid (such as the esters) is also desired for aircraft spraying and individual plant treatment alone or in mixtures with other herbicides. However, effectiveness of the 2-ethylhexyl ester of clopyralid is inconsistent, and production of the 1-decyl ester is not economical at this time (Bovey et al. 1989).

The objectives of this study were (a) to compare the phytotoxicity of the oleylamine salt (oil-soluble amine salt) to the monoethanolamine salt and the 1-decyl ester of clopyralid as foliar sprays on greenhouse-grown honey mesquite; (b) to compare the absorption and translocation of the three formulations from pipet application; and (c) to quantify the transport of clopyralid after foliar sprays of the monoethanolamine salt, the 1-decyl ester, and the oleylamine salt.

Materials and Methods

Plant Material

Honey mesquite plants were grown from seed in the greenhouse for 12 weeks in pots (12.7-cm diameter × 12.7-cm deep) containing a mixture of Bleiblerville clay (a member of the fine montmorillonitic Udic Pellusterts), sand, and peat moss (1:1:1, vol/vol/vol) from March to June 1988. Daytime temperature was 35°C and night temperature was 25°C. Two plants per pot were grown and each had single woody stems with an average height of 36 cm and 17 leaves/plant. Pots were watered daily. A commercial fertilizer (13-13-13) was applied at 0.85 g/pot.

Efficacy of Clopyralid Formulations

Foliar sprays of the monoethanolamine salt, the 1-decyl ester,

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and the oleylamine salt of clopyralid and the butoxyethyl ester of triclopyr (prepared by Dow Chemical USA, Midland, MI, USA) were applied in May and June 1988 at acid equivalent rates of 0.07, 0.14, 0.18, 0.21, 0.28, 0.42, and 0.56 kg/ha in 93 L/ha water carrier in a laboratory spray chamber (Bouse and Bovey 1967) to greenhouse-grown honey mesquite plants. Rates of clopyralid selected were based on previous studies with the monoethanolamine salt which at these rates killed an average percentage of stem tissue on each plant below, at, and above 50% (Bovey et al. 1989; Bovey and Meyer 1985). Triclopyr was included for comparison. When sprayed, surfactants or emulsifiers in the formulations were at ≤0.1% (vol/vol), except for the oleylamine salt formulations which were 0.13, 0.16, 0.19, 0.25, 0.38, and 0.50% (vol/vol) for the 0.14, 0.18, 0.21, 0.28, 0.42, and 0.56 kg/ha rates, respectively. No additional adjuvants were used in the treating solutions. The soil was protected from spray by placing 1-cm deep vermiculite in the pot before treatment. The vermiculite was discarded immediately after spraying and plants were returned to the greenhouse. The soil was watered after 24 h and daily thereafter. Care was taken to avoid washing clopyralid from the plants onto the soil.

Two months after spraying, the responses of treated plants to clopyralid formulations were evaluated by estimating the percentage of dead stem tissue on each plant. Plants with 100% dead stem tissue and no resprouts were considered dead. Six replications with two plants/replicate were used in a randomized block design. The experiment was repeated and data were pooled for statistical analysis, since the date by treatment interaction was not significant. Data were subjected to analysis of variance, and means were compared by Tukey's Critical Range Test at the 5% level (Steel and Torrie 1980).

Absorption and Translocation of Clopyralid

Analytical grade clopyralid was applied with a micropipet in 10 µl aqueous solution/leaf to the 4th and 5th fully developed leaf from the apex (four plants/replication). A commercial surfactant, DuPont WK (trimethylnonylpolyethoxyethanol), at 0.025% (vol/vol) was required for uniform distribution of the herbicide solution on the leaf surface. Total micrograms of clopyralid applied/replication was 633, 563, and 504 for the monoethanolamine salt, the oleylamine salt, and the 1-decyl ester, respectively, as determined by gas chromatographic (GC) analysis. Plants were harvested at 0 h (within 15 min of treatment) and at 24 h following treatment and analyzed for clopyralid content in the leaf wash, treated leaf, and the entire portion of the remaining plant, excluding roots.

In a second experiment, foliar sprays of the monoethanolamine salt, the oleylamine salt, and the 1-decyl ester of clopyralid were applied at rates of 0.14 or 0.28 kg/ha in 93 L/ha water carrier in a laboratory spray chamber (Bouse and Bovey 1967). No surfactant was added to the spray solution. The upper canopy was sprayed while the lower 10 cm of leaves and stem were protected by fitting split styrofoam cups and cotton over the lower plant and soil surface to prevent herbicide contact. The cups and cotton were removed after spraying, and the plants returned to the greenhouse. Plants were watered after 24 h and daily thereafter, taking care not to wash herbicide from the treated plants.

Plants were harvested at 0 and 4 h and 1, 3, and 8 days after treatment. Only the upper canopy (all tissue >10 cm above the soil surface) was harvested at 0 h and analyzed for clopyralid immediately after treatment. On another group of plants, leaf and stem tissue of the lower 10 cm of the canopy were harvested at 4 h and 1, 3, and 8 days after treatment and analyzed. The upper canopy and 1-cm stem section at the transition between the upper and lower canopy were discarded.

In both experiments, three replications were used in a randomized complete block design with four plants/replication. The experiments were repeated and data were pooled for statistical analysis. Data were subjected to analysis of variance, and means were compared with Tukey's Critical Range Test at the 5% level.

Upon harvesting, unabsorbed clopyralid (monoethanolamine salt) was washed from the leaves or canopy by shaking them once for 30 s in 50 ml hexane and twice for 30 s each in 50 ml aqueous base (1 ml concentrated NH_4OH/L water). The hexane was allowed to evaporate overnight at room temperature under the hood, and the remaining water fraction was made acidic (pH 2–3) with concentrated HCl, and then extracted three times with 50 ml diethylether. The clopyralid was esterfied by preparing the 1-butyl ester. The residue in the test tube was dissolved in 1 ml of 1-butanol, three drops of concentrated sulfuric acid were added, and the tube stoppered and placed in a boiling water bath for 30 min. After cooling, 20 ml of water and 5 ml of hexane were added, and the tube shaken vigorously. An aliquot of the organic phase was suitably diluted for chromatography (Cotterill 1978).

The 1-decyl ester of clopyralid and the oleylamine salt were washed from the leaves or canopy by shaking them twice for 30 s each in 50 ml hexane and once for 30 s in 50 ml basic water. The hexane fraction containing the 1-decyl ester was analyzed directly by GC, while the hexane fraction containing the oleylamine salt was evaporated to dryness. The water fractions of the washes from both the ester and amine salt formulations were made acidic and extracted with diethylether. All nonester fractions of clopyralid were converted to the 1-butyl ester for GC analysis.

Clopyralid Analysis

Herbicide analysis has been described previously (Bovey et al. 1989, Cotterill 1978). Plant tissue was subdivided into 2- to 3-cm sections, and the salt forms of clopyralid were extracted by blending them twice in acidified acetone [2 ml concentrated HCl/3.8 L acetone and water (7:1, vol/vol)]. Each sample was evaporated to 30 ml, and the pH was adjusted to 12 with concentrated NH_4OH. Interfering compounds were removed by partitioning three times with 50 ml diethylether. The aqueous solutions were adjusted to pH 3 with concentrated HCl and extracted three times with 50 ml diethylether. Clopyralid was esterfied by preparing the 1-butyl ester as indicated (Cotterill 1978).

To extract the 1-decyl ester of clopyralid, the tissue was blended twice in 225 ml acetone and water (5:1, vol/vol). The solution was made basic with concentrated NH_4OH (pH 10) and extracted twice with 50 ml hexane. The hexane fraction was extracted once with 50 ml water, and the hexane fraction was analyzed by GC. The acid fraction was cleaned, extracted, and determined as indicated for the salt forms.

Clopyralid was analyzed in a gas chromatograph equipped with an electron capture detector (^{63}Ni). The injector port was operated at 280°C. The column and detector temperatures were 150 and 300°C, respectively, to determine the 1-butyl ester. To determine the unaltered 1-decyl ester, column and detector temperatures were 195 and 350°C, respectively.

The 2-m column was packed with 3% OV210 on 80-100 mesh Supelcorport. Herbicide concentrations were determined by comparing peak areas with those of prepared standards. Clopy-

Table 1. Percent dead stem tissue of greenhouse-grown honey mesquite 2 months after application of three clopyralid formulations and triclopyr at eight rates.

Herbicide and formulation	Rate (kg/ha)								Tukey's critical range (5%)
	0.00	0.07	0.14	0.18	0.21	0.28	0.42	0.56	
Clopyralid									
Monoethanolamine salt	1	11	35	19	25	66	83	83	29
Oleylamine salt	1	11	31	41	52	59	75	87	28
1-decyl ester	1	19	53	43	50	81	82	82	27
Triclopyr									
Butoxyethyl ester	1	31	63	79	84	97	98	97	21
Tukey's critical range (5%)	<1	19	28	25	30	28	19	19	

ralid recovery from spiked samples averaged 80% and was easily detected down to 0.05 μg/g.

Results and Discussion

Efficacy of Clopyralid Formulations

For each respective herbicide rate, there were no differences in percent dead stem tissue for the monoethanolamine salt, the oleylamine salt, or the 1-decyl ester of clopyralid (Table 1). The oleylamine salt and the 1-decyl ester at 0.21 kg/ha killed about 50% of the stem tissue. Increasing herbicide rates from 0.21 to 0.28 kg/ha increased kill of stem tissue using the monoethanolamine and 1-decyl ester formulation, but rates of 0.56 kg/ha were required before significant increases were obtained with the oleylamine salt. Triclopyr was more effective on greenhouse-grown honey mesquite than the monoethanolamine salt of clopyralid at rates of 0.07 through 0.28 kg/ha; the oleylamine salt at rates of 0.07 through 0.42 kg/ha; and the 1-decyl ester at rates of 0.18 and 0.21 kg/ha. Triclopyr is typically highly effective on greenhouse-grown honey mesquite, but less effective than clopyralid on honey mesquite in the field (Bovey and Meyer 1985, Jacoby et al. 1981).

There were usually no differences at each respective herbicide rate among the clopyralid formulations in killing greenhouse-grown honey mesquite (Table 2). At 0.28 kg/ha, the monoethanolamine salt, the oleylamine salt, and the 1-decyl ester of clopyralid killed 50, 42, and 71% of the plants, respectively. At 0.14 and 0.28 kg/ha, the 1-decyl ester was more effective than the oleylamine salt. The monoethanolamine salt was the only clopyralid formulation that resulted in a higher mortality rate when applied at 0.42 kg/ha compared to 0.28 kg/ha. Rates of 0.56 kg/ha were not more effective than 0.42 kg/ha for all formations. Triclopyr killed more plants than clopyralid at most rates of application.

Absorption and Translocation of Clopyralid

At 0 h (within 15 min), most of the clopyralid applied by pipet as the monoethanolamine salt was recovered from the leaf surface (Table 3). About 12% or 78 μg of the total clopyralid applied was absorbed by the treated leaves as the acid. After 24 h, little clopyralid was recovered in the leaf wash (29 μg), with most (330 μg) recovered in the treated leaves. About 15% (63 μg) of the total recovered at 24 h was transported from the treated leaves into the plant minus the treated leaf. These data agree with a previous investigation using the monoethanolamine salt formulation (Bovey et al. 1989). Absorption of clopyralid at 0 h after application of the oleylamine salt was even more rapid than the monoethanolamine salt with about half of the total recovered detected in the treated leaves (Table 3). Similar to the monoethanolamine salt, little clopyralid (20 μg) was recovered from the leaf wash after 24 h, and the 86 μg was transported into the plant minus the treated leaf.

In contrast to the amine salts, most of the clopyralid from application of the 1-decyl ester remained on the treated leaf as the ester even after 24 h (Table 3). After 24 h only 31 and 15 μg of clopyralid were recovered from the treated leaves and plant minus treated leaves, respectively. Evidently, only a limited amount of the ester was converted to acid and transported within the plant. These data agree with another investigation in which clopyralid applied as the 2-ethylhexyl ester was limited in transport in honey mesquite compared to the monoethanolamine salt formulation (Bovey et al. 1989).

At least twice as much clopyralid applied in a spray chamber was recovered at 0 h from the upper

Table 2. Percent mortality of greenhouse-grown honey mesquite 2 months after application of three clopyralid formulations and triclopyr at seven rates.[a]

Herbicide and formulation	Rate (kg/ha)							Tukey's critical range (5%)
	0.07	0.14	0.18	0.21	0.28	0.42	0.56	
Clopyralid								
Monoethanolamine salt	8	29	13	13	50	75	75	25
Oleylamine salt	4	8	21	33	42	58	79	23
1-decyl ester	4	42	25	38	71	67	83	19
Triclopyr								
Butoxyethyl ester	8	46	67	71	96	96	96	22
Tukey's critical range (5%)	8	17	24	27	24	20	16	

[a] Untreated plants had 0 mortality.

Table 3. Amount of clopyralid (μg) in the leaf wash, treated leaf and plant minus treated leaf and root at 0 and 24 h after application of the monoethanolamine salt, the oleylamine salt, and the 1-decyl ester to greenhouse-grown honey mesquite.[a]

Formulation	Hours after treatment	Plant part			Tukey's critical range (5%)
		Leaf wash	Treated leaf	Plant minus treated leaf and root	
Monoethanolamine salt	0	325	78		74
	24	29	330	63	52
Oleylamine salt	0	215	213		49
	24	20	263	86	58
1-decyl ester (acid fraction)	0	<1	<1		<1
	24	<1	31	15	7
1-decyl ester (ester fraction)	0	442	37		61
	24	376	70	<1	59
Tukey's critical range (5%)		83	70	16	

[a] Total micrograms as clopyralid applied/replication was 633, 563, and 504 for the monoethanolamine salt, the oleylamine salt, and 1-decyl ester, respectively, as determined by GC analysis. Clopyralid was applied in 10 μl aqueous solution/leaf to the 4th and 5th fully developed leaf from the apex (four plants/replication) with 0.025% trimethylnonylpolyethoxyethanol surfactant (vol/vol) using a micropipet.

canopy of honey mesquite after application of 0.28 kg/ha versus 0.14 kg/ha (Table 4). GC analysis indicated treatment solutions were accurate within 5% error (data not shown). At 4 h, concentration of clopyralid in the lower canopy was greater from application of the oleylamine salt and the monoethanolamine salts than the 1-decyl ester at an application rate of 0.28 kg/ha. At a rate of 0.14 kg/ha, clopyralid content was greater from application of the oleylamine salt than from the 1-decyl ester. After 1 day, the concentration of clopyralid from application of the oleylamine salt was greater than from the monoethanolamine salt and the 1-decyl ester at 0.28 kg/ha, but there were no differences among formulations applied at 0.14 kg/ha. After 3 or 8 days, clopyralid content of all formulations were usually no different with the exception of the lower concentration of clopyralid from application of the monoethanolamine salt at 0.14 kg/ha.

These data indicate that the oleylamine salt, the monoethanolamine salt, and the 1-decyl ester of clopyralid were equally effective, with few exceptions, on greenhouse-grown honey mesquite. Triclopyr usually killed more stem tissue and plants than the clopyralid formulations at the comparable rates in the greenhouse. However, uptake and transport studies indicated significantly less triclopyr was absorbed and transported in field or greenhouse-grown honey mesquite compared to clopyralid (Bovey et al. 1983) and was not as effective in the field (Bovey and Meyer 1985, Jacoby et al. 1981). Absorption and transport of clopyralid applied as the oleylamine salt was equal to the monoethanolamine salt and superior to the 1-decyl es-

Table 4. Concentration of clopyralid (μg/g fresh wt) in the upper canopy of greenhouse-grown honey mesquite at 0 h and in the lower canopy at 4 h and 1, 3, and 8 days after application of the monoethanolamine salt, the oleylamine salt, and the 1-decyl ester at 0.14 and 0.28 kg/ha.

Formulation	Rate (kg/ha)	Time after treatment					Tukey's critical range (5%)
		Upper canopy	Lower canopy				
		0 h	4 h	1 day	3 days	8 days	
Monoethanolamine salt	0.14	9.1	0.2	0.9	1.1	0.6	1.6
	0.28	29.6	0.7	3.2	2.3	1.5	3.9
Oleylamine salt	0.14	18.4	0.5	2.6	2.2	1.6	2.5
	0.28	45.6	0.7	5.1	3.4	1.9	4.4
1-decyl ester[a]	0.14	0.5	0.1	1.5	1.1	1.3	0.6
	0.28	1.2	0.1	1.8	2.3	1.6	1.0
Tukey's critical range (5%)		8.5	0.3	1.7	1.6	0.7	

[a] Initial concentration of 0 h of 1-decyl ester at 0.14 and 0.28 kg/ha in the upper canopy was 15 and 43 μg/g fresh wt, respectively, but could not be detected in the lower canopy at any time.

ter from pipet application to leaves. When applied as foliar sprays, the oleylamine salt was greater than or equal to the monoethanolamine salt and 1-decyl ester formulations in clopyralid concentrations transported to the lower canopy of honey mesquite at equal rates of application. Only the acid form of clopyralid was transported from the treated leaves into the plant from application of either the ester or amine formulations. Based on these data, the oleylamine salt of clopyralid should be as effective as the monoethanolamine salt or the 1-decyl ester of clopyralid in the field on honey mesquite.

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