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Correlation of environment and root carbohydrate content to picloram translocation in leafy spurge[1]

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Abstract:

^{14}C-picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid) absorption and translocation in leafy spurge (*Euphorbia esula* L.) was evaluated over 2 growing seasons and was compared to selected environmental parameters and root carbohydrate content. ^{14}C-picloram absorption was greatest during the vegetative growth stage (52%) and increased directly with relative humidity but was not affected by the temperature at treatment. ^{14}C-picloram translocation to leafy spurge roots generally was influenced more by the plant growth stage than the environment. The greatest translocation to roots occurred during the true-flower and seed-set growth stages. The water-soluble (monosaccharide and disaccharide) and water insoluble carbohydrate content in leafy spurge roots average across the growing season varied by depth with the lowest amount in the 0- to 8-cm depth, 35 and 53 mg/g, and the most in the 16- to 24-cm depth 84 and 221 mg/g, respectively. ^{14}C-picloram translocation to leafy spurge roots was independent of either carbohydrate fraction when evaluated over the entire growing season. However, ^{14}C-picloram content increased when the water-soluble fraction increased during the true-flower growth stage. ^{14}C-picloram translocation to the roots did not increase in the fall, in contrast to the general hypothesis that herbicides move with photosynthates to the roots.

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Keywords:

Sucrose and herbicide movement, *Euphorbia esula*, temperature, relative humidity, photosynthate flow, herbicide absorption, herbicide translocation.

Picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid) is the most effective and commonly used herbicide for leafy spurge (*Euphorbia esula* L.) control (Lym and Messersmith 1985b). Generally, picloram is most effective when applied during the true-flower growth stage in mid-June or during regrowth in fall from late August until a killing frost occurs in October (Fig. 1).

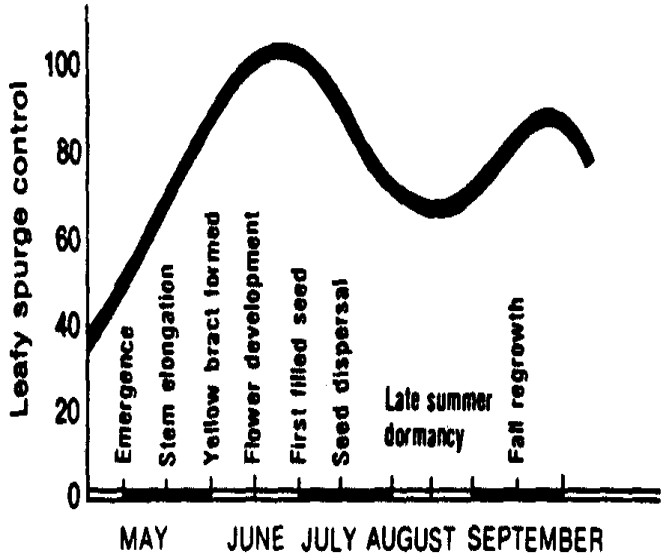

Fig. 1. General leafy spurge control with picloram in North Dakota.

Picloram at 1.1 to 2.2 kg ae/ha normally provides 70 to 90% leafy spurge control for 18 to 24 months in North Dakota (Lym and Messersmith 1985b). Control from picloram can be inconsistent, however, and has given only 5% or less control 2 months after application even when properly applied at 2.2 kg/ha (Lym and Messersmith 1985a).

Relative humidity and air temperature can affect picloram activity in leafy spurge (Lym and Messersmith 1987). ^{14}C-picloram absorption increased from 11 to 34% and translocation to shoot and roots increased from 5 to 21 % when applied at high (90 to 95%) compared to low (20 to 30%) relative humidity, when relative humidity remained high at least 24 hours after treatment (Moxness and Lym 1989). Absorption and translocation were similar when leafy spurge was preconditioned at high or low temperatures (30/18 or 18/10º C day/ night) for 48 hours prior to treatment and was not influenced by post-treatment temperature. However, picloram absorption and translocation increased

when the temperature increased 6 or 12º C 24 hours before treatment but decreased or remained constant when the temperature declined prior to treatment (Lym and Messersmith 1990).

Leafy spurge root water-soluble carbohydrate content varies inversely with changes in the air temperature (Lym and Messersmith 1987). Soluble carbohydrate content increased as the mean temperature decreased and declined as the mean temperature increased, but was independent of a specific temperature. The photosynthate flow in leafy spurge apparently is affected quickly by changes in air temperature, and this relationship may influence herbicide translocation. Thus, the occasional poor control from picloram may be due to application during periods of adverse environmental conditions and/or when low amounts of photosynthates are translocated to leafy spurge roots.

The purpose of this research was to evaluate picloram absorption and translocation in leafy spurge as affected by growth stage, temperature, relative humidity, and root carbohydrate content in the field. The root carbohydrate content at various depths during the growing season also was evaluated.

Materials and methods

^{14}C-picloram

Roots of leafy spurge (accession 1984 ND001)[2] were taken from a natural infestation near Fargo, N. Dak., and were divided into 15-cm segments. The segments were planted into 22-cm diameter by 34-cm deep pots containing a mixture of peat, perlite, and vermiculite. The pots were buried 28 cm deep in a field on the North Dakota State University Experiment Station, Fargo, in July of 1983 and 1985. The plants were watered as needed and were fertilized using a water-soluble fertilizer with a ratio of 23:19:17 N:P:K at 110 kg N/ ha. Pots were left in place and plants were grown until the following growing season.

^{14}C-picloram was applied weekly from mid-May until mid-October in 1984 or 1986. One leaf, midway on the stem of each plant, received 5 µl of 0.15% (v/v) nonionic surfactant[3] in H_2O applied to 1 cm^2 of leaf followed immediately by 850 Bq of uniformly pyridyl-ring-labeled picloram (specific activity 624 MBq/mmole) in 10 µl of 70% (v/v) ethanol followed by an additional 5 µl of surfactant to maximize absorption. A plastic-covered cage was placed over the plant during treatment until the surfactant had dried. The cage was left in place if the sky was overcast and rain was forecast within the next 24 hours. Plants were not protected from rain thereafter.

Plants were harvested 72 hours after treatment and were sectioned into the treated leaf, stem, and leaves both above and below the treated leaf, and roots. The roots were subdivided by depth from 0-to 8-, 8-to 16-, 16-to 24-, and 24-to 32-cm and were washed and frozen immediately. The treated leaf was rapidly dipped 10 times into 15 ml of scin-

[2] Registry of leafy spurge accessions maintained by David G. Davis, USDA Biosciences Research Laboratory, Fargo, N.D. 58105.

[3] Surfactant WK (dodecyl ether of polyethylene glycol), E.I. du Pont de Nemours and Co., Wilmington, Del.

tillation 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-5-phenyloxazolyl]benzene} to remove unabsorbed ^{14}C-herbicide.

Plant sections were dried at 60º C for 24 hours, and root and shoot sections were ground in a Wiley mill (No. 10 mesh) and weighed. Root samples were divided equally for either ^{14}C or carbohydrate analyses. The treated leaf and 2 or more 120 to 150-mg root or shoot subsamples equaling at least 10% of the sample weight were each combusted in a biological materials oxidizer (BMO). The $^{14}CO_2$ 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.

BMO efficiency was determined using methyl-^{14}C-methacrylate and liquid scintillation counting efficiency was determined using an external standard ratio. Root buds were initially analyzed separately but the data have been combined with the appropriate root section for this report. The experiment was a randomized complete block design with 4 replications.

Carbohydrate analysis

Water-soluble carbohydrates were extracted from 200-mg root samples in water at 27º C for 1 hour. Samples were filtered, and residue was saved for insoluble carbohydrate content determination. The water extract was diluted to 100 ml, and a 1.5-ml aliquot was centrifuged at 16,000 rpm for 3 min to precipitate proteins. Water-soluble carbohydrates were determined colorimetrically by adding 1-ml of 20% (v/v) phenol and 5-ml concentrated sulfuric acid to a 0.1-ml aliquot of extract and incubating 20 minutes at 27º C as described by Dubois *et al*. (1956).

Insoluble carbohydrates were extracted by placing the residue in 15-ml distilled water and boiling for 5 min followed by adding 10-ml 0.5% amyloglucosidase buffered to pH 4.9, and incubating at 38º C for 36 hours (Smith 1981). Insoluble carbohydrates were determined colorimetrially as previously described. Total nonstructural carbohydrates (TNC) were calculated by adding the amount of soluble and insoluble carbohydrate for each sampling date. Carbohydrate analyses were repeated, but too little root material remained from the 24- to 32-cm depth for accurate analysis.

Statistical analysis

The relationship between ^{14}C-picloram absorption and translocation and the temperature and relative humidity at time of treatment, maximum and minimum air temperature, and mean air temperature observed for 6 consecutive days (sampling day and the previous days, e.g., Day 0 + 1, 0 + 1 + 2, etc.) were estimated by computing correlation coefficients. The relationship between the root carbohydrate and picloram content were also estimated.

Environmental data were obtained either on site during treatment or from the National Weather Service in Fargo, N.D., located about 1 km from the research site (temperature

and relative humidity data prior to treatment). The data were analyzed using correlation and stepwise regression procedures (SAS/ STAT User's Guide 1990) to determine the relationship of environment and root carbohydrate content to picloram absorption and translocation.

Results and discussion

^{14}C-picloram

^{14}C-picloram absorption was similar throughout most of the growing season and averaged 36% except during summer dormancy when absorption declined to 14% (Fig. 2). Maximum absorption averaged 52% and occurred during the vegetative growth stage when the plant began growth in the spring. Generally, ^{14}C-picloram remains as the parent acid in leafy spurge (>85%) (Lym and Moxness 1989) so the ^{14}C recovered will be referred to as picloram.

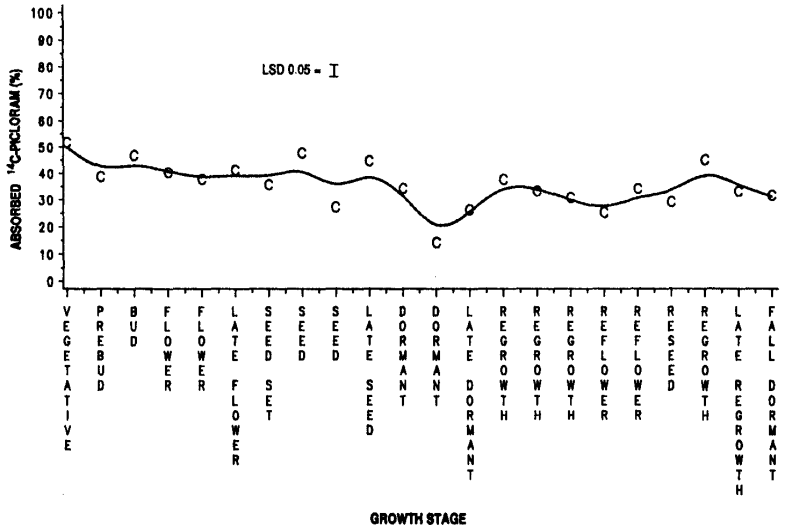

Fig. 2. ^{14}C-picloram absorption by leafy spurge averaged over 2 growing seasons.

Absorption of picloram increased directly with the relative humidity with a partial correlation coefficient of 0.59 and 0.40 in 1984 and 1986, respectively (Table 1), which agrees with previous research results from growth chamber experiments (Moxness and Lym 1989). However, picloram absorption was not affected by the temperature at time of treatment or the maximum temperature 24 and 96 hours before treatment in the field. This finding contrasts with previous research conducted in the growth chamber in which absorption increased or decreased directly by a 1:1 ratio with each 1º C change in temperature 24 hours before treatment (Lym and Messersmith 1990).

Table 1. Partial correlation of ^{14}C-picloram absorption and translocation with various environmental parameter in the field.

	Temperature when treated		Relative humidity when treated		High temperature before treatment: 24 hours before		High temperature before treatment: 96 hours before	
^{14}C-picloram	1984	1986	1984	1986	1984	1986	1984	1986
	r[a]							
Absorbed (%)	0.16	-0.07	0.59	0.40	0.13	0.05	0.14	0.08
Probability	0.53	0.76	0.01	0.07	0.59	0.83	0.56	0.73
Above treated leaf (dpm/gm)	-0.08	-0.29	0.26	0.19	0.02	-0.37	0	-0.51
Probability	0.77	0.19	0.35	0.42	0.94	0.10	0.99	0.02
Below treated leaf (dpm/gm)	0.03	0.15	0.20	-0.50	-0.04	0.01	0.26	-0.24
Probability	0.92	0.51	0.46	0.12	0.88	0.95	0.29	0.30
Root 0 to 8 cm depth (dpm/gm)	0.08	0.04	0.15	-0.20	0.12	0.23	0.34	0.24
Probability	0.76	0.87	0.56	0.40	0.64	0.31	0.15	0.31
Root 8 to 16 cm depth (dpm/gm)	0.05	0.12	0.32	-0.17	0.16	0.36	0.41	0.38
Probability	0.83	0.59	0.20	0.46	0.50	0.11	0.08	0.09
Root 16 to 24 cm depth (dpm/gm)	0.17	0.32	0.20	0.11	0.26	0.44	0.53	0.50
Probability	0.51	0.16	0.42	0.64	0.29	0.05	0.02	0.02
Root 24 to 32 cm depth (dpm/gm)	0.20	0.41	0.09	0.04	0.27	0.58	0.54	0.59
Probability	0.42	0.07	0.74	0.85	0.27	0.01	0.02	0.01
Root 32 to 40 cm depth (dpm/gm)	0.32	0.45	0.13	-0.11	0.41	0.68	0.63	0.60
Probability	0.20	0.04	0.62	0.63	0.08	0.01	0.01	0.01

[a]Partial correlation coefficient for ^{14}C-picloram and environmental parameter with time held statistically constant.

The amount of ^{14}C-picloram in leafy spurge topgrowth was greatest when the herbicide was applied during the vegetative growth stage, averaging 26,000 dpm/g (Fig. 3). The amount of ^{14}C-picloram translocated to the topgrowth declined rapidly (4,000 dpm/g) when leafy spurge began to form flower buds. Less ^{14}C-picloram (775 dpm/g) translocated to the topgrowth during summer dormancy than any other growth stage. There was a small increase when picloram was applied during fall regrowth before the amount again declined to its lowest level.

The amount of picloram in the above and below treated leaf sections generally was not influenced by the temperature or relative humidity at time of treatment, or the maximum temperature or 96 hours before treatment (Table 1). The only exception occurred in

1986 when the amount of picloram in the above treated leaf section was inversely correlated with the temperature 96 hours before treatment.

The pattern for picloram translocation to leafy spurge roots was similar for all 4 depths regardless of growth stage so that data were combined (Fig. 3). In general, the amount of picloram translocated to the root system was influenced more by the leafy spurge growth stage than the short-term environmental changes and was similar to the expected pattern based on long-term control in the field (Fig. 1 and 3). The maximum picloram translocation to the roots (14,500 dpm/g), occurred during the late-flowering and seed set growth stages in late June. The amount declined steadily (1,300 dpm/g) during summer dormancy before increasing during fall regrowth.

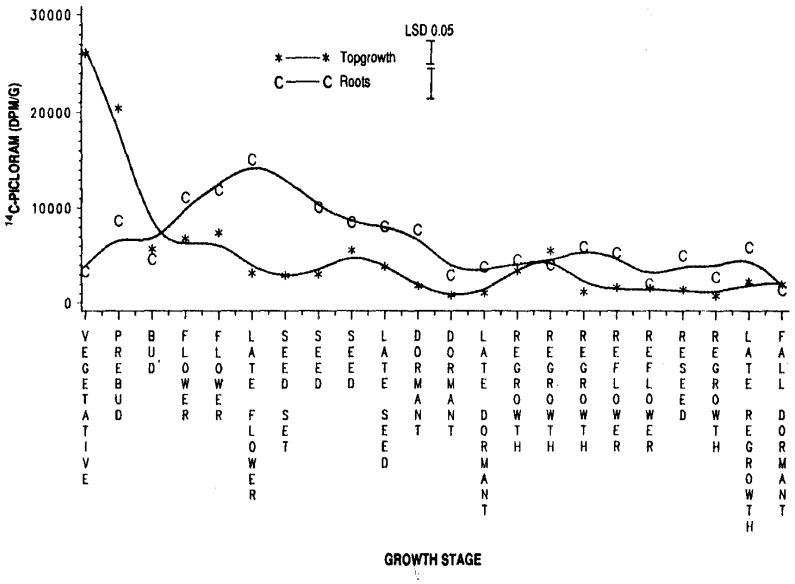

Fig. 3. ^{14}C-picloram translocation to leafy spurge top growth and roots averaged over 2 growing seasons.

No consistent correlation was shown between temperature or relative humidity and the amount of picloram translocated to the roots regardless of depth (Table 1). However, there was a positive correlation (>0.10) both years between the high temperature 24 hours before treatment and picloram in the roots at a depth of 32- to 40-cm and between the high temperature 96 hours before treatment and all depths except 0 to 8 cm. Temperature prior to treatment appears to directly influence the depth of ^{14}C-picloram translocation through the root system even though it affects neither absorption nor the total amount of herbicide translocated.

Correlation with carbohydrates

The carbohydrate concentration in leafy spurge roots varied by depth with the lowest amount in the 0- to 8-cm (35 and 53 mg/g) depth and the most in the 16- to 24-cm (84

and 220 mg/g) depth for the water-soluble and -insoluble carbohydrates, respectively (Fig. 4A and 4B). In early spring during vegetative regrowth, nearly all the TNC content within 1 depth of leafy spurge roots was equally distributed between soluble and insoluble carbohydrates, but insoluble carbohydrates predominated after flowering. These concentrations are similar to samples collected in the field when the 4-year average TNC concentration in the 0- to 15-cm depth reached maximum levels of 300 mg/g but declined to 190 mg/g or less late in the growing season (Lym and Messersmith 1987).

Picloram translocation to the leafy spurge root system was independent of the water-soluble and -insoluble root carbohydrate content when evaluated over the entire growing season, regardless of depth (Table 2). Auxin herbicides often are considered to flow with photosynthate (Crafts and Robbins 1962) but this does not appear to be true with leafy spurge, at least over the entire growing season. However, it may be that picloram translocation is aided by photosynthate flow during the peak flow of picloram to the root system during flowering.

Table 2. Correlation of the water-soluble and water-insoluble nonstructural carbohydrate contents and ^{14}C-picloram in leafy spurge roots 72 hours after treatment averaged over 2 years.

Growth stage and root depth	Carbohydrate type	
	Water-soluble	Water-insoluble
	---------- (r^a) ----------	
All season		
0 to 8 cm	-0.15	-0.32
Probability	0.50	0.14
8 to 16 cm	0.03	-0.19
Probability	0.89	0.40
16 to 24 cm	-0.28	-0.33
Probability	0.20	0.14
True-flower		
0 to 8 cm	0.78	0.60
Probability	0.07	0.21
8 to 16 cm	0.95	0.56
Probability	0.01	0.24
16 to 24 cm	0.45	-0.34
Probability	0.37	0.59
Fall regrowth		
0 to 8 cm	-0.37	0.30
Probability	0.37	0.47
8 to 16 cm	-0.16	-0.03
Probability	0.71	0.94
16 to 24 cm	-0.63	-0.59
Probability	0.10	0.12

[a]Partial correlation coefficient for carbohydrate and environmental parameter with time held statistically constant.

Picloram and carbohydrate content in the 3 sections of roots were analyzed separately during the true-flower and fall regrowth stages only. The picloram content increased at the same time the water-soluble fraction increased during the true-flower growth stage with a correlation coefficient of 0.78 and 0.95 at the 0- to 8-and 8- to 16-cm depths, respectively (Table 2). However, picloram translocation was independent of the soluble carbohydrate content at the 16- to 24-cm depth and with the insoluble carbohydrate fraction at all depths.

Despite a large increase in the movement of soluble carbohydrates to the roots in the fall, there was no increase in ^{14}C-picloram translocation (Fig. 3 and 4A, Table 2). This is in contrast to the general hypothesis that herbicides move with the photosynthates as they are stored for overwintering in the fall (Crafts and Robbins 1962, Mitchell and Brown 1946). Although some herbicides such as glyphosate a [*N*-(phosphonomethyl)glycine] translocate at rates, direction, and with distribution pattern similar to sucrose, others, especially phenoxy-acids such as 2,4-D, differ from sucrose in both distribution rate and pattern (Martin and Edgington 1981).

Physiological factors other than carbohydrate movement and air temperature influence picloram movement in leafy spurge. Phenoxy-acid herbicides appear to move in a free state, i.e., not chemically bound to sugars, and may reduce their own movement by inhibiting energy-requiring processes associated with translocation, by initiating sinks in mature leaves thereby altering the direction of movement, or from blockage by physiological barriers not present in early summer growth (Robertson and Kirkwood 1970). Translocation of picloram may be affected by similar physiological processes since it is an acid herbicide with auxin activity similar to the phenoxy-acid herbicides.

The optimum timing of picloram application for maximum translocation to the roots is during the true-flower growth stage and to a lesser extent during fall regrowth. Within these application periods, treatment should be made during periods of high humidity (Table 1 and Moxness and Lym 1989). Air temperatures seems to be less important than relative humidity although application during a cool period immediately following a few days of hot weather may increase picloram translocation to the roots and thus increase control slightly (Table 1 and Lym and Messersmith 1990).

Acknowledgements

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