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The influence of glyphosate on bud dormancy in leafy spurge[1]

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Abstract:

Glyphosate [*N*- (phosphonomethyl)glycine] was applied in the spring and fall to leafy spurge (*Euphorbia esula* L. #[2] EPHES) in the field at rates of 0.14 to 4.48 kg ai/ha. Fall applications of glyphosate at rates of 0.56 to 4.48 kg/ha stimulated axillary branching and caused an increase in the number of stems/m^2 by the end of the following summer in a dense population. This was a result of shoot growth from buds on the crown region of the root system. The absorption and translocation of ^{14}C-glyphosate applied to leaves of mature leafy spurge plants were evaluated at pre-bloom, full-bloom, and senescence phenological stages. Approximately 81% of the ^{14}C-glyphosate applied to the leaves of senescing plants was absorbed. There was a decrease in the proportion of ^{14}C translocated out of the treated leaf when applications were made after full bloom. Translocation of ^{14}C to the treated stem, non-treated stems, root crown, and roots did not differ with phenological stage in 1983. Translocation and concentration of ^{14}C in most plant parts in 1985 differed with phenological stage. Translocation to the crown buds as a percentage of the ^{14}C absorbed was highest at the senescence stage. At senescence and before soil freezing, leafy spurge crown buds demonstrate transient but active elongation toward the soil surface. This active development may account for the enhanced translocation of ^{14}C into the crown buds.

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[2] Letters following the # symbol are the WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 309 West Clark Street, Champaign, IL 61820

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Absorption, translocation, "witches' broom," developmental arrest, EPHES.

Introduction

Leafy spurge is a perennial noxious weed of pastures and rangeland in the north-central United States and Prairie Provinces of Canada (2). It is difficult to control with conventional chemical, cultural, and biological measures due, in part, to the massive crown and root system (16) which possess numerous vegetative buds (6), each capable of producing a new shoot.

A small number of vegetative buds on the crown and lateral root system emerge early in the spring to produce new shoots. The development of shoots from remaining buds is largely arrested until the shoot growth is disturbed, removed, or senesces (16). How the emerged shoots inhibit the development of this remaining root and crown buds is unclear. One possible explanation is that the auxin produced in the shoot apex exerts an inhibitory effect on root and crown bud development in a manner similar to the apical dominance phenomena characterized by inhibition of axillary buds (9).

To provide long-term control of a perennial weed such as leafy spurge, a means must be developed to manipulate the shoot growth from all dormant buds on the crown and root system. The discovery and development of a growth regulator that could induce or inhibit shoot growth from buds would represent a significant step toward long-term control of many perennial weed species.

Glyphosate at sublethal concentrations causes tillering, axillary bud, and root bud growth in other species (5, 7, 18, 19). These results indicate that glyphosate can temporarily overcome apical dominance either directly or indirectly and cause uncharacteristic stem and shoot growth. The objective of this study was to determine the effect of sublethal and lethal rates of glyphosate on stimulation of shoot growth from leafy spurge buds under field conditions. An additional objective was to determine whether a relationship exists between the pattern of glyphosate movement in leafy spurge and the release of buds from dormancy.

Materials and methods

Field experiments

Each experiment was conducted on mature leafy spurge stands at two separate locations in south-central Montana. Site one had a relatively sparse population of leafy spurge (108 stems/m^2) and site two was dense (650 stems/m^2). The commercially formulated isopropylamine salt of glyphosate was applied to 16.3-m^2 plots at 0.14, 0.28, 0.56, 1.12, and 4.48 kg ai/ha on September 11, 1982, to plants in the senescence phenological

growth stage. Applications were made with a CO_2 -pressurized backpack sprayer delivering 92 L/ha of water at 276 kPa pressure with a flat-fan nozzle. The experimental design was a randomized complete block with three replications. The numbers of leafy spurge stems/m^2 at the soil surface were counted 8 months after application on May 23 and 24, 1983, and 11 months after application on August 8 and 9, 1983. The data from both sites were combined in a factorial analysis and sites were significantly different. Therefore, the data for each site were analyzed separately. The average number of stems/m^2 in each herbicide treatment was compared using a protected LSD mean separation technique. In addition, regression analysis was conducted to determine if the apparent growth-regulating properties of glyphosate followed a dose-response relationship.

Translocation experiments

The leafy spurge plants used in this experiment were located at the Post Research Farm, Bozeman, MT. The plants were grown in metal canisters (17.8 cm in diameter by 90 cm deep) that were placed inside 20-cm-diameter by 90-cm-deep concrete cylinders buried in the ground every 1.2 m. The bottoms of the canisters were punctured with small holes to allow drainage. Periodically, canisters were raised slightly to eliminate root growth into the surrounding soil. Herbicide treatments were applied at the pre-bloom (June 2, 1983, and May 29, 1985), full bloom (July 25, 1983, and July 8, 1985), and senescence (September 5, 1983, and August 29, 1985) phenological stages.

Methyl-labeled ^{14}C-glyphosate (specific activity 1.97 mCi/mmol) was mixed with an equimolar amount of ethylamine and isopropylamine in 1983 and 1985, respectively, and 0.25% (v/v) of a polyethoxylated tallow amine surfactant (MONO818). Commercial formulation of glyphosate mixed for a field application rate of 92 L/ha at 1.1 kg ai/ha was added to the ^{14}C-glyphosate to obtain a radioactivity level of 0.02 µCi/µl.

Commercially formulated glyphosate, applied as in the field studies, was sprayed on randomly selected plants at 1.1 kg ai/ha. Immediately after application, one mature leaf on the upper one-third of each plant was selected and held in the horizontal position with a stiff support wire inserted into the ground. ^{14}C-glyphosate (0.2 µCi) was applied in ten 1-µl drops to the adaxial surface of the horizontal leaf. White plastic tents sheltered the plants throughout the duration of the experiment to prevent loss of ^{14}C-glyphosate by rain or wind.

Each treatment was replicated four and five times in 1983 and 1985, respectively. Plant material was collected 120 hours after application. The treated leaf was removed and placed in a capped test tube that contained 20 ml of water. The ^{14}C remaining on the leaf surface was removed by vigorous washing for 1 minute. The wash was repeated twice and a 1-ml aliquot removed from the combined wash to determine radioactivity. The metal canisters were removed from the field and cut longitudinally with tin shears. Soil was gently washed from the root system and the treated plant was carefully separated from other plants growing in the same canister. The treated plant was divided into zones 1 through 6 from further sampling. Zone 1, the above-ground portion of the plant, consisted of the treated leaf, the stem and other leaves, additional stems originating from the treated plant, and the leaf wash collected from the treated leaf. Zones 2 through 6 consisted of pink-colored dormant buds and the roots of the treated stem. Zone 2 was the root crown.

The root material below the crown was divided into equal 18- to 20-cm sections, which represent zones 3 to 6.

The samples were oven-dried at 55ºC, weighed, and ground with a mortar and pestle. Subsamples of ground material were oxidized in a biological material oxidizer[3]. The ^{14}C content was measured by liquid scintillation spectroscopy. These data were analyzed using a completely randomized design with the protected LSD mean separation technique.

Results and discussion

Field experiments

Glyphosate applied in the spring to plants in the pre-bloom growth stage retarded shoot growth and caused a proliferation of branching from axillary buds on stems 2 months after application, but there was no effect on the number of stems/m^2 at the soil surface (data not shown). Fall applications of glyphosate caused a linear dose-response relationship (r^2 = .7199, P = 0.033), and a twofold increase (P = 0.000) in the number of stems at the soil surface at site two; however, this effect was not observed until late in the following growing season (Table 1). These stems originated from bud growth initiation on the root crown and upper portions of the root system. Earlier in the season, the number of stems/m^2 was decreased significantly (P = 0.001) by glyphosate at site two, but there was no significantly linear dose-response relationship. The delay in stimulation of stem growth may be a result of prolonged herbicidal activity of glyphosate within the plant, since glyphosate is resistant to degradation in leafy spurge and other higher plants (8). The less consistent effect of glyphosate on stems/m^2 observed at site one (Table 1) was attributed to midsummer drought and poor growing conditions.

Table 1. Effect of glyphosate applied at the senescence phenological stage on stem production by leafy spurge.

	Months after application[a]			
	8		11	
Glyphosate rate	Site one	Site two	Site one	Site two
(kg/ha)	(stems/m^2)			
0	32	222	90	567
0.14	43	158	83	466
0.28	49	115	47	825
0.56	29	43	39	858
1.12	25	72	115	1170
4.48	37	43	82	1399
LSD	...	67	57	273
P - value	NS	0.001	0.104	0.000

[3] Harvey Oxidizer, Ox 300, Harvey Instrument Co., 123 Patterson St., Hillsdale, NJ 07642.

[a]Mean separation tests which resulted in P-values above 0.150 were reported as nonsignificant (NS).

Stimulation of shoot growth from buds by glyphosate prompted an investigation into the nature of glyphosate translocation when the herbicide was applied at the different phenological stages.

Translocation experiments

This study was designed to provide a detailed analysis of the accumulation pattern of glyphosate in roots of mature, field-grown leafy spurge plants. Previous studies of glyphosate movement in leafy spurge were conducted with immature plants[4] (8).

The average recovery of applied ^{14}C was 80%. The ^{14}C-glyphosate was readily absorbed by leafy spurge plants when applied at all phenological stages (Table 2). There were significant differences (P = 0.000) in absorption at different plant growth stages in 1985 but not in 1983. Absorption of ^{14}C-glyphosate after 5 days by field-grown leafy spurge plants averaged 78%. This compares favorably with 89% absorption of ^{14}C-glyphosate by greenhouse-grown plants cultured for 7 days at high relative humidity (8).

Table 2. The percent of applied ^{14}C-glyphosate that was absorbed by leafy spurge plants five days after application.

	^{14}C-glyphosate absorbed[a]	
Plant stage at application	1983	1985
	(%)	
Prebloom	62	95
Full bloom	79	67
Senescent	86	76
Average	76	79
LSD	...	8
P-value	NS	0.000

[a]Mean separation tests which resulted in P-values above 0.000 were reported as nonsignificant (NS).

Absorbed ^{14}C-glyphosate translocated throughout the plant at all phenological stages (Table 3). In 1983 and 1985, 53 and 41%, respectively, of the absorbed ^{14}C accumulated in the mature treated shoot, which includes the treated leaf. The treated leaves accounted for a large share of the ^{14}C in the shoot. The percentage translocated out of the treated leaf was greater when glyphosate was applied to leafy spurge plants in the prebloom than the senescence stage. This pattern of movement may be due to early-season growth of shoots and secondary roots since glyphosate moves to areas of high metabolic activity in both annual and perennial plant species (17). The accumulation of ^{14}C in the treated stem and

[4] Bybee, T. A. 1979. Factors affecting leafy spurge control including leafy spurge reestablishment, herbicide application dates, herbicide translocation, and root carbohydrates. Ph.D. Thesis, North Dakota State Univ., Fargo, ND.

leaves was not significantly different between growth stages in 1983, but significant differences (P = 0.000) existed both in terms of percent accumulation and concentration in 1985. More than one stem can arise from the root system of a leafy spurge plant. Low levels of radioactivity were found in untreated stems.

Phenological growth stage did not influence ^{14}C distribution in the root crown in 1983 (Table 3). In 1985, the percentage and concentration of ^{14}C in the root crown were higher at the pre-bloom stage than at the senescence stage of application.

There were no significant differences in the accumulation and concentration of ^{14}C between application stages in root zones 3 through 6 in 1983. However, in 1985 on a percentage basis generally more ^{14}C remained in the proximal portion of the roots (zone 3 and 4) when applied at the pre-bloom stage than the other two growth stages of application (Table 3). In 1985, the concentration of ^{14}C in all root zones except zone 5 was higher at the prebloom stage than at the other two stages of application.

The amount of ^{14}C in the crown buds on the root crown in 1983 and 1985 varied significantly with growth stage at the time of application (Table 3). The percentage of ^{14}C in the crown buds was highest when ^{14}C-glyphosate was applied at senescence. This accumulation coincided with high concentrations of ^{14}C in the buds in 1983 but not in 1985. The phenological status at the time of glyphosate application had little influence on the accumulation of ^{14}C in root buds in zones 3 and 4 in 1983. In 1985, the root buds in zone 3 accumulated significantly (P = 0.020) more ^{14}C at the pre-bloom and senescence stages than the full-bloom stage of application.

Comparison of field observations with the pattern of ^{14}C accumulation indicates there may be a relationship between glyphosate translocation and shoot growth from buds in leafy spurge. in early fall when leafy spurge plants are senescing, the crown and root buds are actively elongating and become sinks for glyphosate accumulation. Root bud elongation occurs over a relatively short period of time, 2 to 4 weeks, but ceases before the new shoots emerge from the soil (13). These buds then remain dormant over the winter until conditions suitable for growth occur the following spring. When bud growth resumes in the spring, growth of the elongated buds is inhibited by glyphosate so they do not emerge until the middle of July. Growth inhibition was measured by stem counts (Table 1). When new shoots did appear midway through the growing season, shoot growth and branching from buds were observed, a characteristic of sublethal rates of glyphosate observed on other species (5, 7, 18, 19). These observations indicate that glyphosate may have the ability to interfere with correlative inhibition, which is dormancy enforced by the main shoot, and facilitate prolific shoot bud growth from previously inhibited buds on leafy spurge plants. The loss of correlative inhibition may indicate either a direct effect of glyphosate on the growth of basal buds (3) or an indirect effect caused by a blockage of shoot apical meristem activity.

Glyphosate may have the potential to release lateral buds from dormancy by inhibiting the synthesis of aromatic amino acids, which are precursors to indoleacetic acid (IAA) (1, 10).

Table 3. The percent of total absorbed ^{14} C -radioactivity and the dpm/g dry weight in each plant part five days after the application of ^{14}C-glyphosate to leafy spurge plants that were at three different stages of growth[a].

Growth stage at application	Plant part	1983		1985	
		(%)	(dpm/g dw)	(%)	(dpm/g dw)
Pre-bloom	Treated leaf	38	9.9×10^6	11	1.4×10^6
Full bloom		40	5.9×10^6	37	2.9×10^6
Senescence		59	1.4×10^7	36	1.3×10^7
	LSD	11	6.1×10^6	9	6.7×10^6
	P-value	0.000	0.050	0.000	0.009
Pre-bloom	Treated stem	7	3.5×10^4	10	2.0×10^4
Full bloom	and leaves	8	19.6×10^3	18	7.4×10^3
Senescence		8	1.4×10^4	10	3.7×10^3
	LSD	...	...	6	2.5×10^3
	P-value	NS	0.010	0.010	0.000
Pre-bloom	Nontreated stems	2	1.8×10^3	1	5.6×10^3
Full bloom	and other leaves	0.5	4.6×10^2	b	
Senescence		1	5.6×10^2	b	
	LSD	...	...	...	...
	P-value	NS	NS	...	
Pre-bloom	Crown buds	0.2	5.0×10^4	0.1	9.8×10^3
Full bloom		0.1	3.0×10^4	0.3	3.0×10^4
Senescence		1.0	1.2×10^5	3	2.0×10^4
	LSD	0.6	9.4×10^4	2.5	2.0×10^4
	P-value	0.010	0.100	0.030	0.130
Pre-bloom	Root crown	3	1.4×10^4	6	1.6×10^4
Full bloom		6	1.5×10^4	5	6.1×10^3
Senescence		4	1.2×10^4	2	1.9×10^3
	LSD	...	...	3	4.4×10^3
	P-value	NS	NS	0.050	0.000
Pre-bloom	Buds on the root	0.2	2.1 k 10^4	1	3.2×10^4
Full bloom	in zone 3	0.1	2.7×10^4	0.2	4.0×10^3
Senescence		0.3	2.4×10^4	4	2.6×10^4
	LSD	...	...	2.9	1.9×10^4
	P-value	NS	NS	0.07	0.020
Pre-bloom	Roots in zone 3	17	9.6×10^3	24	2.2×10^4
Full bloom		10	3.6×10^3	12	7.1×10^3
Senescence		11	6.0×10^3	8	3.9×10^3
	LSD	...			3.5×10^3
	P-value	NS	NS	NS	0.000
Pre-bloom	Buds on the root	0.008	9.9×10^3	0.03	7.2×10^3
Full bloom	in zone 4	0.01	8.3×10^3	0.01	4.4×10^2
Senescence		0.01	5.5×10^3	0.03	6.7×10^3
	LSD	...	...	...	...
	P-value	NS	NS	NS	NS

Growth stage at application	Plant part	1983		1985	
		(%)	(dpm/g dw)	(%)	(dpm/g dw)
Pre-bloom	Roots in zone 4	8	1.1×10^4	16	1.9×10^4
Full bloom		13	1.2×10^4	10	6.6×10^3
Senescence		5	4.3×10^3	10	5.5×10^3
	LSD	...	...	4	4.9×10^3
	P-value	NS	NS	0.000	0.000
Pre-bloom	Roots in zone 5	6	1.0×10^4	12	1.4×10^4
Full bloom		6	4.0×10^3	10	5.9×10^3
Senescence		3	2.5×10^3	10	5.6×10^3
	LSD	...			
	P-value	NS	NS	NS	NS
Pre-bloom	Roots in zone 6	3	1.8×10^3	6	9.9×10^3
Full bloom		5	1.0×10^4	4	3.9×10^3
Senescence		5	1.4×10^3	11	3.4×10^3
	LSD			5	4.8×10^3
	P-value	NS	NS	0.040	0.020

[a]Mean separation tests which resulted in P - values above 0.150 were reported as nonsignificant (NS).
[b]There were no nontreated stems at these growth stages.

In studies measuring the effect of glyphosate on auxin transport, glyphosate inhibited IAA transport and consequently released lateral buds from apical dominance (4). More recently, Lee (11) reported that glyphosate induced changes in the metabolism of exogenously applied IAA. He measured a reduction in free IAA and increased breakdown and conjugation of IAA. Changes in IAA metabolism induced by glyphosate may provide the link between glyphosate application and the release of lateral buds from apical dominance (12). The data and observations from our study could be taken as support for any of the three modes of action theories regarding the effect of glyphosate on IAA. This is because the effect of glyphosate on the endogenous levels of IAA in leafy spurge root and crown buds has not been determined. Nevertheless, the prolific shoot growth and branching may be the result of an initial accumulation of glyphosate in apical meri-stems. At stimulatory levels, stimulation of IAA metabolism or inhibition of IAA synthesis or transport may occur, causing shoot growth and branching from buds immediately below the shoot apex. The branching continues because glyphosate remains intact and mobile and is able to move to the new active apical meristem areas where it accumulates again to stimulatory levels causing the process of branching to reoccur. Shoot growth and branching may eventually stop because of dilution of glyphosate during branch production.

The growth-regulating effect of glyphosate has important ramifications on leafy spurge control. Root buds are critical structures, which play a major role in the persistence and spread of leafy spurge. They are under the control of an efficient endogenous growth regulation system that permits release of only one or two buds from dormancy following a major disturbance to the shoot (14, 15). We have shown that glyphosate altered the bud dormancy regulation system of an important perennial weed and therefore could be a possible key to providing long-term perennial weed control.

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