

Modification of Root Bud Growth in Canada Thistle with Selected **Exact Growth Regulators: Effects on Translocation of Glyphosate**

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Abstract. Root applications of 0.1 μ M 6-benzyladenine (BA) and 10.0 μ M indole-3-butyric acid (IBA) enhanced or inhibited, respectively, root bud growth in hydroponically grown Canada thistle [Cirsium arvense (L.) Scop.], Translocation of ¹⁴C-glyphosate [N-(phosphonomethyl)glycine] into roots was positively correlated with this growth . Foliar applications of ethephon or chlorfluorenol also enhanced root bud growth, but glyphosate translocation was only weakly correlated with such growth in soil-grown Canada thistle. At glyphosate rates above 0.56 kg/ha, root bud growth was not stimulated by plant growth regulators (PGRs) and basipetai translocation was not enhanced. Paradoxically, ethephon and chlorflurenol restrained root bud growth in the field since thistle control steadily improved during the 3 years following treatment.

Canada thistle is a noxious weed that is widespread across North America (Hodgson 1968). Thistle root systems can expand horizontally to over 300 cm per Soon 1906). This cross can capture to the second plants can develop when
A year (Amor and Harris 1975), and independent plants can develop when adventitious buds and their aerial shoots grow from these roots (Moore 1975). M_{diff} root buds occur on a single thistle root, but most buds are suppressed by eQ nelative factors (Hamdoun 1972) or environmental constraints . Root buds have been stimulated to produce shoots by temperature changes (Hoeffer 1981) $\frac{\text{deg}}{\text{deg}}$ well as altered water and nitrogen availability (McIntyre 1979, McIntyre and H_{unit} as altered water and introgen availability (included difficult, since herbi-
 H_{unit} 1975). Reduction of thistle populations is made difficult, since herbicides may not be translocated to the roots and root buds in lethal concentra t_{top} (Parker 1975). Some research has sought to stimulate root buds so that the Charles (Parker 1975). Some research has sought to stimulate root buds so that h_e resulting sink activity might promote basipetal translocation of a foliar-
and resulting sink activity might promote basipetal translocation of a foliarapplied herbicide (Baradari et al. 1980, McIntyre and Hunter 1975, Hoefer 1981 .

Some plant growth regulators (PGRs) have been reported to stimulate bud activity in several weed species, possibly by interfering with a correlative signal (Baradari et al. 1980, Chykaliuk et al. 1982, Tworkoski and Sterrett 1984 , Waldecker and Wyse 1985). Several of these investigations have revealed much variability in response to PGR treatments, particularly in the field. The current research was designed to evaluate more closely the relationship $\frac{dv}{dt}$ tween PGR treatment, herbicide movement, and root bud activity in Canada thistle. thistle .

The objectives were to (1) identify PGRs that stimulate development of root buds in Canada thistle, (2) determine the effect of root bud number and growth on basipetal translocation of a foliar-applied systemic herbicide, and (3) details mine whether a root bud-enhancing PGR can improve efficacy of a systemic herbicide applied to Canada thistle.

Materials and Methods

Root Bud Stimulation by PGRs

Canada thistle was germinated and grown in soil $(2:1:1:1, \text{loam:} \text{peak:} \text{sand:} \text{vec})$ miculite, $v/v/v/v$ for \sim 5 weeks to the rosette stage before treatment. In one experiment, soil was thoroughly rinsed from roots, and each plant was placed in a 1-liter container of aerated, half-strength Hoagland's solution at $25 \pm 1^{\circ}C$ with a 12-h photoperiod and 158 μ E m⁻² s⁻¹ PAR. One week after plant transfer, solutions of benzyladenine (BA), indolebutyric acid (IBA), abscisive acid (ABA), ethephon [(2-chloroethyl)phosphonic acid], or gibberellic acid (GA_3) were added to the hydroponic medium at final concentrations of 10^{0} . 1.0, or 0.1 μ M. Four weeks after PGR treatment, root bud number and total root bud length were measured. root bud length were measured .

A second PGR experiment was conducted with 5-week-old Canada this plants growing in 1-liter containers of the soil medium, but otherwise under the same environmental conditions. The foliage of each plant was sprayed to the point of runoff with flurprimidol α -(1-methylethyl)- α -(4-trifluorometho α) phenyl-5-pyrimidine methanol], chlorflurenol (methyl 2-chloro-9-hydroxy fluorene-9-carboxylate :methyl 9-hydroxyfluorene-9-carboxylate:methyl 2,7' dichloro-9-hydroxyfluorene-9-carboxylate, $8.8/2.1/1.6$, $v/v/v$), ethephon, GAF 141 [(2-chloroethyl)phosphonic acid + N-methylpyrrolidone], atrinal [sodium salt of 2,3:4,6 bis- \overline{O} -(1-methylethylidene)- O -L-xylo-2-hexulofuranosonic acid₁, or BA at concentrations of 100, 10, 1, 0.1, or 0.01 mM. or BA at concentrations of 100, 10, 1, 0.1, or 0.01 mm.

were replicated 5 times, and means were separated by D uncan new multiple range test.

Glyphosate Translocation Response to Root Bud Growth

Canada thistle was grown from seed and prepared as described in the previous hydroponic experiment. One week following transfer, each nutrient solution was modified by adding $0.1 \mu M$ BA, $10.0 \mu M$ IBA, or no PGR treatment. GIYPhosate (isopropyl amine salt formulation) was applied concurrently with the PGRs or sequentially 1 week following BA or IBA. Droplets containing a total of 1200 µg glyphosate were applied to leaves of each plant. Two leaves per plant (donor leaves) received droplets of ¹⁴C-glyphosate [N-(phosphono-¹⁴C-methyl)glycine; 1.97 mCi/mM]. Plants treated concurrently received 0.8 μ Ci/plant and those treated sequentially received 0.6 μ Ci/plant. All plants were harvested 2 weeks after BA or IBA was added to roots. A randomized complete block design with S replications was used.
At harvest, plants from 4 replications were each dissected into 4 shoot and 4

 \sim α vest, plants from 4 replications were each dissected into 4 shoot and 4 root segments . All plant parts were lyophilized and weighed . Number and length of buds from each root segment were also measured. Subsamples of each dried plant part were then assayed for radioactivity by combustion (Peterson 1969), followed by liquid scintillation spectrometry. Since previous studies have demonstrated little metabolism of glyphosate (Gottrup et al. 1976 , we assumed that the ¹⁴C activity was closely correlated with 14 C-glyphosate. Means were separated by Waller-Duncan k-ratio t test. Relationships between herbicide translocation and root bud number, length, and weight were the settle transfer and two correlation analysis and by multiple linear regression. One plant from each treatment was autoradiogrammed (Crafts and Yamaguchi 1964) .

GIYphosate Translocation Following Foliar Applications of Ethephon

Canada thistle was grown from seed for 10 weeks in I-liter containers of soil at 25 \pm 3°C with a 14-h photoperiod and 350 μ E m⁻² s⁻¹ PAR. At treatment, all plants were \sim 30 cm tall at the flower bud stage. A 3×6 factorial experiment was well \approx 50 cm can at the Howei but stage. At \approx 100 mM concentral to concentrate conducted with ethephon as one main effect (0, 10, and 100 mM concentration). $t_{\text{rad}(0) \text{ns}}^{q}$ and glyphosate as the other $(0, 0.56, 1.12, 1.68, 2.24, \text{and } 3.36 \text{ kg/ha}).$ Each of the 18 treatment combinations was applied as an over-the-top spray ¹⁰ plants/treatment). Three of the 10 plants in each treatment also received He plains/ireatment). Three of the 10 plains in case of the mid-
C-glyphosate by treating two adjacent leaves (donor leaves) near the mid- $\frac{\mu_{\text{out}}}{\mu_{\text{out}}}$ of a stem with 0.1 μ Ci ¹⁴C-glyphosate. Four weeks after treatment, plants were of a stem with our post-o-gryphosale. Then were also and shoot weights, area of living leaves, number and weight of root buds that had emerged (root shoots) or had not emerged from the soil surface (root buds) (terminology of \mathbf{A} , McJay or had not emerged from the son surface (for each on the average of the average of three visual estimates. Radioisotope distribution was evaluated by detaching donor risual estimates, Katholsonope ulstribution was extended stream of methanol (unabsorbed glyphosate) and by separating apical shoot (above donor leaves), $b_{\text{asal}}^{\text{recoon}}$ shoot (below donor leaves), and roots. Plant parts were quantified for radioactivity as described previously.
 $\frac{I_{\text{R}}}{I_{\text{N}}}$ a parallel experiment, similarly treated plants were used to quantify gly-

It, a parallel experiment, similarly treated plants were used to quantify gly p_{on} accumulation in roots with time and to correlate glyphosate accumula- $\lim_{n \to \infty}$ with root bud growth. Plants were grown as above and treated with 10 mM ethephon and 1.68kg/ha glyphosate. Ten replications each were harvested 1, 2, $\frac{3}{16}$, and 5 weeks after treatment. Three of the 10 replications for each harvest interval initially received ¹⁴C-glyphosate. In both experiments, main effects were tested using orthogonal contrasts (Ostle and Mensing 1975). Where appropriate, effects were examined for linear and quadratic relationships .

Response to PGRs and Glyphosate in the Field

Based on our greenhouse results and previously reported data (Peterson 1983) Baradari et al. 1980, Beasley 1969), chlorflurenol and ethephon were selected as potential stimulators of root buds in the field. Glyphosate (4.5 kg/ha) w^{as} applied alone, tank mixed with 1 mM ethephon or with 0.1 mM chlorflurenol, or applied sequentially 1 week after the two PGRs . Spray applications were made in June 1984 (561 $1/ha$) to 2-m² plots. Prevalent weeds were foxtail $[5e_6]$ taria glauca (L.) Beauv. J, bindweed (*Convolvulus arvensis* L.), and velvetleat (Abutilon theophrasti Medic). During the growing seasons of 1984, 1985, and 1986, the number of thistle stems and percent area covered by thistle (visual estimate of two people) were measured. The experimental design was a rad^{th} domized complete block with 8 treatments and 4 replications. Means were separated by Waller-Duncan k -ratio t test.

Results and Discussion

Root Bud Stimulation by PGRs

In our controlled environment experiments, foliar applications of chlorflur. enol, and ethephon and root applications of BA, stimulated development of root buds. Untreated hydroponically grown thistle produced 7 root buds with a total length of 346 cm (Table 1). Stimulation of root bud formation occurred only in BA-treated plants (Table 1). The 0.1 - μ M BA-treated plants produced 1^6 buds with a total length of 1164 cm. In contrast, IBA (at 1 and 10 μ M) sup pressed growth (30 cm). These results suggest that a cytokinin/auxin balance which is thought to regulate the correlative inhibition of buds in shoots (Phillips 1975), may also be regulating shoots that arise adventitiously from roots. Other investigators have also reported that bud growth is stimulated by direct cytokinin application to subterranean parts of milkweed (Asclepias syl', iaca L.) (Waldecker and Wyse 1985) and Johnsongrass (Sorghum halpense)
(Beasley 1969). $(Beasley 1969)$.

Among the foliar-applied PGRs, 0.1 mM chlorflurenol caused the greates and most consistent stimulation of root bud growth (20 buds, 104 cm long) compared to control (12 buds, 53 cm long) (data not shown). Of the two ethylene-releasing PGRs, 1 mM ethephon caused greater root bud growth (15) buds, 85 cm long). Based on these and other data (Baradari et al. 1980, Beasley 1969), chlorflurenol and ethephon were used to stimulate root bud grow $\mathfrak{m}^{\mu\nu}$ subsequent experiments.

Herbicide Translocation and Root Bud Growth of Hydroponically Grown Thistle

Basipetal movement of foliar-applied glyphosate was correlated with PGR' promoted increases in root bud growth . If BA was applied before glyphosate, it

Modification of Bud Root Growth Modification of Bud

PGR type	PGR concentration (μM)	No. of buds	Total bud growth (cm)	
C_{ontrol}		7a ^a	346 bc	
BA	10	10ab	72ab	
	1	16 _b	311 bc	
IBA	0.1	16 _b	1164 d	
	10	3a	30a	
	1	4a	39a	
ABA	0.1	6 a	241 bc	
	10	7a	176 _{bc}	
	1	5a	316 bc	
Ethephon	0.1	6 a	516c	
	10	7a	376 bc	
	$\mathbf{1}$	7a	434 bc	
G _A	0.1	5 a	382 bc	
	10	10ab	818 c	
	I	6 a	490 bc	
	0.1	6 a	588 c	

Table 1. Root bud growth of Canada thistle resulting from plant growth regulator applications to hydroponic media.

^a Within each column, means followed by the same letter do not differ at the 0.05 level.

Produced significantly more root bud growth than other PGR treatments (Table 2). This growth was concentrated in the 10-cm section immediately below the root collar (Fig. 1). More glyphosate moved to below-ground parts in this treatment than in any other (70% compared to 51% or less). Waldecker and Wyse (1985) were able to stimulate root bud accumulation of lethal quantities of glyphosate in milkweed root buds by applications of BA. Increased root bud activity in Canada thistle also enhanced basipetal movement of dicamba (Baradari et al. 1980) and glyphosate (Hoefer 1981). Our BA treatment enhanced movement of subsequently applied glyphosate, but not to lethal concentrations.

Glyphosate movement was more closely associated with sink strength than with sink number. For example, glyphosate content in roots was more strongly ^{correl}ated with average root bud length ($r = 0.71$) than with number of buds $r_s = 0.15$) (Table 3). Although glyphosate content decreased with increasing distance from the root collar (Table 2), glyphosate concentration remained uniform along the root (\sim 20,000 dpm/g). The sink activity within 10 cm of the root collar did not suppress glyphosate distribution to root apices. Autoradiograms $\frac{\text{coll}}{\text{in}}$

illustrate a rather uniform ¹⁴C-glyphosate distribution in roots (Fig. 1).
Bud growth and basipetal translocation were stimulated when roots were $\frac{34u}{2}$ growth and basipetal translocation were stimulated when roots were t_{block} at equal treated hydroponically with BA prior to herbicides (Table 2). Shaw et al. (1983) also found that pretreating a root bud-producing weed with a PGR (GAF 141) stimulated basipetal movement of glyphosate whereas near-simultaneous ⁴ calment inhibited it. In contrast, Baradari et al. (1980) reported greater dicamba movement to Canada thistle roots when it was applied simultaneously With chlorflurenol. Our results with hydroponic studies demonstrate that root bud stimulation by root applications of selected PGRs can be counteracted by

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 $\binom{8}{\text{A}}$. ¹⁴C distribution in roots and root shoots of Canada thistle treated with ²⁴C-glyphosate and \sim \sim \sim , (b) IBA, or (C) water alone. Plants are on left and autoradiographs are on right.

Table 3. Correlations and regression equations of ^{14}C -glyphosate distribution with components of thistle growth .

^a Pearson correlation coefficients; *designates significance at the 0.05 level. NM indicates varia^{ple} not measured .

b Best-fit multiple linear regression equations based on the stepwise procedure.

simultaneous foliar application of glyphosate. In concurrent treatments, g/y' phosate may have moved to areas of potential meristem activity and prevented BA stimulation of growth. Alternatively, glyphosate may have altered the carbon metabolism of leaves, as reported by Gougler and Geiger (1984), and disrupted phloem transport.

Glyphosate Translocation Following Foliar Applications of Ethephon to Canada Thistle Growing in Soil

Four weeks following treatment, injury to shoots and roots increased quadratically with increasing glyphosate rates (Table 4). Glyphosate effects reached ^a maximum of 1.68 kg/ha (91% injury of shoots and 56% of roots). Ethephon had an interactive effect with glyphosate on shoot injury: at low glyphosate rates, ethephon markedly increased injury (visual shoot injury ratings of 53, 65, and 81% when 0.56 kg glyphosate was applied with 0, 10, and 100 mM ethephon, respectively); at glyphosate rates of 1.68 kg/ha or greater, ethephon had n^0 effect on injury ratings. Root injury was not affected by ethephon at any dosage of glyphosate. Greatest shoot injury (98%) and root injury (69%) $\frac{W^{25}}{M}$ obtained with combined applications of 3.36 kg glyphosate/ha and 100 mM ethephon.

 $\frac{1}{6}$ $\frac{1}{4}$ at $\frac{1}{2}$ ratings of 0 indicate no damage and 100 represents a dead plant. N . Injury ratings of 0 indicate no damage and 100 represents a dead plant.

Any probability value greater than 0 but less than 0.005 was rounded to 0.01.

Glyphosate movement, as measured by ¹⁴C activity, was linearly related to Pplication rates of glyphosate, but not ethephon (data not shown). $T_{\text{C-gly}}$ Phosate concentration was high following applications of 1 .68 kg/ha compared $^{16}_{2}$ 0.56 kg/ha (4093, 4058, and 6782 dpm/g compared to 2001, 2249, and 3356 d_{pnn}/g in the shoot apex, shoot base, and root, respectively). ¹⁴C content in $r_{\rm tot}$ of these soil-grown thistles was not significantly correlated with root bud $\frac{14}{14}$ uber or dry weight (Table 3). In general, root bud growth had less effect of χ^2 movement in soil-grown than in hydroponically grown thistle.
C movement in soil-grown than in hydroponically grown thistle.
 χ^2 Glyphosate translocation apparently occurs for a limited time following ap-

 \mathbb{F}^3 phosate translocation apparently occurs for a limited time following ap- $\sum_{n=1}^{\infty}$ atton. Growth of root shoots and root buds increased with time (Table 5) but glyphosate accumulation in roots did not increase. After 1 week, ¹⁴C con- $\text{[eq:1]}\xspace$ entrations remained unchanged (\sim 4000 dpm each week). Most foliar absorption of glyphosate occurred by 3 days after application in Canada thistle, and S_{an} derg et al. (1980) found that basipetal translocation may continue between and 14 days following treatment. Our work indicates that little translocation

Table 5. Growth and injury of soil-grown Canada thistle and distribution of ¹⁴C-glyphosate during 5 weeks following foliar treatments with ethephon (10 mM) and glyphosate (1.68 kg/ha) in the greenhouse .

^a Growth and injury measurements are based on 10 replications harvested each week. ¹⁴C contenu-(dpm) is derived from 3 of those replications. Injury values of 0 indicate no damage and 100 indicate a dead plant. indicate a dead plant .

 \degree Orthogonal contrasts were used to determine nonsignificant (NS) or significant linear (LIN) and quadratic (QUAD) changes in measured variables with time. Contrasts were considered signific^{an} at the 0 .05 level .

occurred after 7 days following treatment. It is possible that at rates of 1.68 kg/ha and above, shoots were too badly injured to support transport. Hunter and Smith (1972) found delayed topkill of Canada thistle may provide better control, due to greater translocation to roots. However, results from our study indicate that lower glyphosate doses did not kill roots satisfactorily. Although α basipetal translocation can be enhanced when low glyphosate rates are used such rates will likely be too low to control thistle effectively. Five weeks after treatment, thistle treated with ethephon (10 mM) and glyphosate (1.68 kg/ha) appeared to be recovering by means of vigorous root buds/shoots .

Thistle Response to Glyphosate and PGRs in the Field

Neither sequentially applied nor tank-mixed PGRs consistently improved thistle control by glyphosate in the field. During 1984, only ethephon enhanced the effects of glyphosate in reducing weed density (from 28 to 17 plant m^{-1})

	1984		1985		1986	
Treatment ^a	No.	Area (%)	No.	Area (%)	No.	Area (%)
Alone Water E C G Sequential E/G C/G Concurrent $E + G$ $C + G$	28 ab ^b 24 bc 33a 28ab 23 bc 28ab 17c 33a	21cd 23 bc $26a - c$ $27a-c$ 23 bc 28ab 16 d 29 а	43 a 26 bc 19 _c 31 _b 27 _b 27 _b 30 _b 30 _b	38 a 20de 19 e 26 _{bc} $23c-e$ 25cd 26 bc 31 _b	56 a 16e 12 _e 45 bc 54 ab 31 d $52a-c$ 44 c	33ab 13 d 11 d 27 _{bc} 38a 23c 27 bc 23c

Table 6. Number of thistle plants per square meter and percent ground area covered by thistle after applications of glyphosate and/or PGRs in 1984.

The following symbols were used: $E =$ ethephon, $C =$ chlorilurenol, and $G =$ glyphosate. Applications of PGRs and glyphosate were separate (alone), 1 week apart (sequential), or tank m ixed together (concurrent)

maged (concurrent).
Means within each column followed by the same letter do not differ at the 0.05 level.

and area covered (from 27 to 16%). In 1985, percent thistle coverage decreased in response to all PGR or herbicide treatments, compared to control (Table 6). However, during 1986, PGRs reduced glyphosate's effects on thistle number and coverage. These results underscore the variability of plant responses to PGRs. As in investigations by others (Peterson 1983, Shaw et al. 1983), we found PGRs could stimulate root bud activity and enhance basipetal herbicide translocation under laboratory or greenhouse conditions, but, in the field, enhanced control was not obtained. It seems likely that environmental variation, as well as herbicide counteraction of PGR-induced root bud activity, was responsible for lack of PGR effects.
By the third posttreatment growing season, the number of thistles in the field

 $\frac{\text{Euler}}{\text{E}}$ reduces internode elongation (Abeles 1973), and chlorinuitien in- \sim y the third positiveatment growing season, the number of thistles in the field was reduced most by chlortlurenol or ethephon. Besides summating root bud outgrowth, both of these PGRs may have inhibited subsequent elongation. hibits development of growing tips (Weed Science Society of America 1983). Following their initial stimulatory effect on bud outgrowth, both PGRs, or a metabolite, may have slowly accumulated in root meristems to inhibitory levels, and eventually reduced topgrowth.

Based on our investigation, PGR promotion of root bud development (perhaps by release from correlative inhibition) is possible, although it may be a transient phenomenon with a new dominance hierarchy developing. Although basipetal transport of foliar-applied glyphosate is positively correlated with root bud growth, the impact of the activity on glyphosate transport is less In soil-grown than in hydroponically grown thistle. These factors, together with the uncertainty of timing for a foliar herbicide application to take advantage of unseen root meristem activity, present a formidable task for enhancing here of unseen root mension activity, process a competition experience translocation with PGRs. A direct tactic for reducing competition from weeds that reproduce adventitiously from roots may entail suppressing growth and development of root shoots. In our field experiment, we obtained the greatest long-term thistle control by applications of either chlorflurenol or ethephon.

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