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Chlorsulfuron Effects on Shoot Growth and Root Buds of Canada Thistle (Cirsium arvense)1,2

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Abstract. The effect of 67 g ai/ha chlorsulfuron {2-chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-amino] carbonyl] benzenesulfonamide on Canada thistle [Cirsium arvense (L.) Scop. #4 CIRAR] root bud growth was examined in a series of greenhouse trials in which potted plants were treated with foliar sprays. Injury to root buds was assayed by determining their ability to form secondary shoots. Added surfactant, 0.2% (v/v) oxysorbic [oxysorbic (20 POE) polyethylene sorbitan monooleate], did not enhance chlorsulfuron-induced inhibition of parent shoot growth, but it increased root bud injury from foliarly applied chlorsulfuron. Cuttings taken from controls formed more secondary shoots than did chlorsulfuron-treated plants 2 weeks following spraying. However, root fresh weight and final secondary shoot growth from cut roots were unchanged 3 weeks after chlorsulfuron treatment compared to the time of spraying. Foliar treatment or a combination of foliar and soil treatment inhibited root fresh weight accumulation and secondary shoot growth equally 1 month following treatment relative

to harvest controls. Soil treatment alone did not reduce either root fresh weight gains or secondary shoot outgrowth from root buds. Foliar treatment of vegetative Canada thistle with chlorsulfuron inhibited subsequent secondary shoot outgrowth from root buds more than did treatment at flowering.

Additional index words. Herbicide, perennial weed, root buds, CIRAR.

INTRODUCTION

Chlorsulfuron was recently introduced for selective control of broadleaf and some grass weeds in cereals (10, 16). It belongs to a new structural class of herbicides that control many weeds at exceptionally low application rates (10 to 67 g/ha). Chlorsulfuron has both preemergence and postemergence activity, although slightly higher rates are needed for preemergence treatment. It is most effective as a postemergence treatment when applied between the two-leaf and tillering stages of cereals.

Chlorsulfuron is unique in its capacity to provide selective, season-long control of Canada thistle in cereals at rates of 34 to 67 g/ha (1, 2, 7, 11). Rates of 275 to 560 g/ha provided control for 12 to 15 months (12, 13). Chlorsulfuron also controls Canada thistle at several growth stages. Chlorsulfuron treatments (34 to 275 g/ha) at the rosette, prebud, and flower stages provided greater than 90% control in the following fall (7).

Although rates as low as 11 to 67 g/ha chlorsulfuron provided selective weed control in cereals, herbicide residues may persist in the soil from one growing season to the next and damage susceptible rotational crops (15, 16). Sunflower

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⁴WSSA-approved computer code from Important Weeds of the World, 3rd ed., 1983. Available from WSSA, 309 West Clark St., Champaign, IL 61820.

(Helianthus annuus L.), sugarbeets (Beta vulgaris L.), and dry beans (Phaseolus vulgaris L.) are only a few examples of the rotational crops that can be injured by carryover of rates as low as 30 g/ha applied to cereals in the previous growing season (4, 16, 20). Its persistence may limit the use of this herbicide.

Canada thistle is a serious perennial weed because it spreads vegetatively and forms new shoots from root buds. Information on how chlorsulfuron affects Canada thistle shoot growth, root growth, and plant establishment from root buds is needed to improve the use of this herbicide in the field. The objectives of this greenhouse study were to determine: a) how foliarly applied chlorsulfuron influences parent shoot growth and secondary shoot development from root buds; b) whether added surfactant enhances the ability of foliarly applied chlorsulfuron to reduce the outgrowth of root buds; c) whether the length of the root cuttings influences secondary shoot outgrowth in response to chlorsulfuron; d) how much time must elapse after chlorsulfuron treatment before secondary shoot emergence is prevented or delayed; e) whether growth stage at the time of spraying influences secondary shoot emergence from root buds; and f) whether secondary shoot establishment is affected equally by foliar or soil treatment with chlorsulfuron.

MATERIALS AND METHODS

General methods. Canada thistle (var. 'integrifolium' Wimm. and Grab.) (14) plants were started from 4- to 8-cm-long root cuttings which were grown for 1.5 to 2 months in trays (21- by 30- by 8-cm) filled with fine vermiculite. The vermiculite was watered periodically with 1/3 strength nutrient solution or tap water (3). Uniform plants were selected and transplanted to potting soil and allowed to grow for an additional 1.5 to 2 months before treatment. Plants in pots (16.5-cm-diam by 18-cm, 2.8-L) of soil were watered with tap water as needed. At the time of transfer from vermiculite to soil, plants were fertilized with slow-release granular fertilizer (N:P:K, 3:1:1). The potting soil was 73% sand, 19% silt, 8% clay, and 3.6% organic matter, at pH 7.9.

Uniform plants were selected for spray treatment. Because the growth of Canada thistle changes throughout the year, uniform plants of comparable height and morphology were selected, rather than plants of the same chronological age. Foliar chlorsulfuron treatments were applied with a greenhouse hood-sprayer equipped with a #80067 Teejet nozzle operated at 177 kPa providing 303 L/ha in one pass at 0.77 km/h. Formulated chlorsulfuron (75% dry flowable) was applied at 67 g ai/ha plus 0.2% (v/v) oxysorbic surfactant⁵. The soil surface was covered with 2.5 cm of vermiculite which was discarded after the spray had dried. Care was taken in watering the sprayed plants to prevent crosscontamination.

Plant height differences were calculated from measurements made at the time of spraying and after 1 month. Likewise,

vegetative phenological stage was measured as the number of leaf nodes (leaves and leaf scars) present at the time of treatment and after 1 month. Leaves less than 3 to 4 mm in length were not measured. Flowering stage was defined as the number of branches per plant with at least one floral bud.

Plants were harvested 1 month after chlorsulfuron treatment, except in time of exposure studies. Roots were washed free of soil, weighed, and divided into 2.0- to 2.5-cm-long segments. Roots were cut into segments to break correlative inhibition of root buds by apical dominance and promote maximal secondary shoot sprouting. Segments were placed 1 cm deep in pots (12.0- by 16.5- by 5.5-cm, 1-L) with fine vermiculite as quickly as possible to prevent drying or bud death and were grown for 5 weeks. All roots were exposed to ambient light during handling. Secondary shoots from root buds were counted at 1, 2, 3, and 5 weeks after the roots were segmented. A 5-week interval was chosen because few additional new secondary shoots emerged between 3 and 5 weeks after cutting the roots. After 5 weeks, secondary shoots were excised, washed free of vermiculite, dried at 70 C for at least 48 h, and weighed. This methodology provides a measure of the vigor of secondary shoot emergence but may not adequately measure total root bud numbers, the proportion of viable buds, or bud death. Currently, there is no generally accepted way to measure bud death or dormancy.

Greenhouse experiments were conducted from September 1981 to June 1983. Sunlight was supplemented with fluorescent lighting to provide at least a 14-h photoperiod throughout the year. Light intensity and photosynthetically active radiation at plant height, 40 to 75 cm below fluorescent bulbs, ranged between 20 and 68 W/m^2 and between 90 and $168 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. The temperature and relative humidity varied seasonally over the course of the experiments. Throughout the winter, when most experiments were conducted, the day temperature ranged between 18 and 28 C and the night temperature ranged between 15 and 20 C. Relative humidity ranged between 28 and 75% depending upon the season.

All experiments were of a completely randomized design with 9 to 11 plants/treatment (21). Data were subjected to analysis of variance (17, 21), regression analysis (9), and means were separated by Duncan's multiple range test (P=0.05) (5), where it was appropriate. Each experiment was repeated on one to two separate occasions and the results of one typical trial are presented.

Effects of surfactants. Treatments included a control, chlor-sulfuron at 67 g/ha, oxysorbic at 0.2% (v/v), and the combination of chlorsulfuron and oxysorbic at the same rates. At the time of treatment, vegetative plants had 26 to 30 leaf nodes/plant and were 64.5 ± 8.8 cm (mean \pm SE) tall. Additional control plants were harvested at the start of the experiment and root bud sprouting was studied. Inclusion of additional controls at the time of spraying provided information on whether chlorsulfuron suppressed the capacity of plants to form new secondary shoots from cut roots to the same extent at harvest as at the start of the experiment.

⁵Tween 80.

Effect of length of root cutting. Plants were treated with 67 g/ha chlorsulfuron plus oxysorbic at 0.2% (v/v) and were harvested 1 month later. Vegetative plants had 20 to 28 leaf nodes/plant at the time of treatment and were 58.1 ± 9.8 cm tall. The harvested root systems of control and herbicide-treated plants were either left intact [90 \pm 23.6 cm (mean \pm SE)] or were cut into 2.5-, 5.0-, 10.0-, or 20.0-cm segments and secondary shoot sprouting was studied.

Time of exposure to chlorsulfuron. Plants were treated with 67 g/ha chlorsulfuron plus oxysorbic at 0.2% (v/v). Roots were harvested and segmented at the time of spraying and 7, 14, and 21 days later before observation of secondary shoot emergence. Vegetative plants had 24 to 32 leaf nodes/ plant at the time of treatment and were 60.0 ± 19.5 cm tall. Effect of growth stage. Experimental treatments consisted of 67 g/ha chlorsulfuron plus oxysorbic at 0.2% (v/v) or controls and three plant growth stages, small vegetative (22 to 30 leaf nodes), large vegetative (35 to 45 leaf nodes), and flowering (35 to 45 leaf nodes). Vegetative and flowering plants were roughly comparable in height and foliar display at spraying. No additional control plants were started at the time of treatment due to a lack of suitable plant material.

Effect of site of treatment. Plant treatments included a control, a 10-ml soil drench application of 67 g/ha chlorsulfuron plus 0.2% (v/v) oxysorbic, foliar treatment at the same rate, and a combination of foliar and soil treatment at the same rate. Vegetative plants were 65.7 \pm 5.9 cm tall at spraying and had 26 to 31 leaf nodes. Additional control plants were assayed for root bud outgrowth at the time of spraying.

RESULTS AND DISCUSSION

Shoot injury symptoms. Foliar application of 67 g/ha chlorsulfuron plus 0.2% (v/v) oxysorbic to Canada thistle stopped parent shoot growth (cv = 45 to 50%) (Figure 1). Although young leaves remained turgid, they became chlorotic within 5 days and failed to expand normally, forming a rosette. Control plants continued normal leaf addition and expansion. whereas treated plants did not (Figure 2). The lower, older leaves became chlorotic between 1 and 2 weeks after treatment, and yellowing progressed acropetally. Stem and petiole discoloration and browning did not follow a pattern. After the petioles became discolored and weakened, the lower leaves collapsed along the side of the stem, yet maintained their turgidity for several weeks following treatment. Like chlorosis, necrosis of the older leaves progressed acropetally. Larger vegetative plants were damaged more slowly than were younger plants.

Effects of surfactant. Adding oxysorbic surfactant to chlorsulfuron hastened foliar chlorosis of Canada thistle, but did not reduce shoot elongation any more than chlorsulfuron alone (Table 1). Fresh weight of roots from control and oxysorbic-treated plants increased similarly after 1 month. Final root fresh weight following treatment with chlorsulfuron plus surfactant was less than for either the harvest controls or chlorsulfuron alone, but no different than the controls started at the time of treatment. The roots from plants exposed to chlorsulfuron plus surfactant were light brown

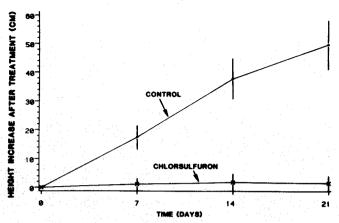


Figure 1. The effect of foliar-applied chlorsulfuron at 67 g/ha with oxysorbic (0.2%, v/v) on shoot elongation of Canada thistle. Controls were 71.5 cm tall at the start of the experiment. Treatment means and standard deviations for each time were different by an F-test (P<0.05).

and had fewer lateral roots than the controls.

One month following treatment with chlorsulfuron alone, plants formed the same number of secondary shoots five weeks after root segmentation as controls started at the time of spraying (cv 22 to 57%) (Table 1). Fewer secondary shoots formed when surfactant was added to chlorsulfuron than did in controls at harvest, or in the chlorsulfuron treatment alone. The final number was the same as the controls at the start. In addition, the number of secondary shoots was reduced relatively more than was root fresh weight compared to the harvest controls (Table 1). Secondary shoot number and root fresh weight were 15 and 53% of these controls, respectively. However, both parameters were no different than the respective controls at the start. Perhaps the herbicide plus surfactant may have stopped root growth and further bud formation without irreversibly damaging root buds that were present. This might explain the temporary control of Canada thistle in the field for 12 to 15 months following treatment with 60 to 125 g/ha chlorsulfuron (12, 13).

The secondary shoots that emerged following treatment were capable of normal growth (Table 1). Chlorsulfuron plus surfactant did not affect the dry weight of individual secondary shoots. This trend was consistent from experiment to experiment, although the absolute dry weight per secondary shoot varied and depended upon the growth stage of Canada thistle at the time of spraying and the size of root segments. More mature plants formed more and larger secondary shoots.

The addition of surfactants to postemergence herbicides to enhance their phytotoxicity has a long history (8). Without oxysorbic, chlorsulfuron had little effect on secondary shoot growth from buds on root segments (Table 1). The results were unusual in that addition of surfactant to the herbicide brought about an "all-or-none" response to chlorsulfuron in Canada thistle.

Effect of length of root cutting. Secondary shoot numbers and outgrowth depended on root segment length and chlor-sulfuron treatment (Figure 3). At all cutting lengths, chlor-sulfuron delayed secondary shoot emergence. There was no emergence for 2 weeks after cutting roots of treated plants to 2.5- or 5.0-segments, although there was limited emergence from intact roots or segments cut into longer lengths. In contrast, secondary shoots of controls emerged 1 week following cutting at all root segment lengths. Differences in secondary shoot numbers due to cutting lengths were first observed in controls 2 weeks after harvest. Cutting roots of controls into 5.0-cm segments promoted secondary shoot growth most. Three and 5 weeks following cutting, secondary shoot outgrowth from intact roots of chlorsulfuron-treated plants equaled the controls, in contrast to the response

of roots cut to shorter lengths. Three-week data are not presented.

Dry weight per secondary shoot was a quadratic function of cutting length 5 weeks after harvest for controls (Figure 4). Shorter root segments formed more, but smaller, secondary shoots than did longer segments or intact roots (Figures 3 and 4). The effect of cutting length on secondary shoot numbers per plant may reflect suppression of bud outgrowth in longer root segments due to stronger apical dominance. Less dry weight per individual secondary shoot may reflect slower initial shoot emergence and slower attainment of autotrophic growth due to less available carbohydrate reserves in shorter root segments. These untested hypotheses require further research for verification.

Time of exposure to chlorsulfuron. Root fresh weight of

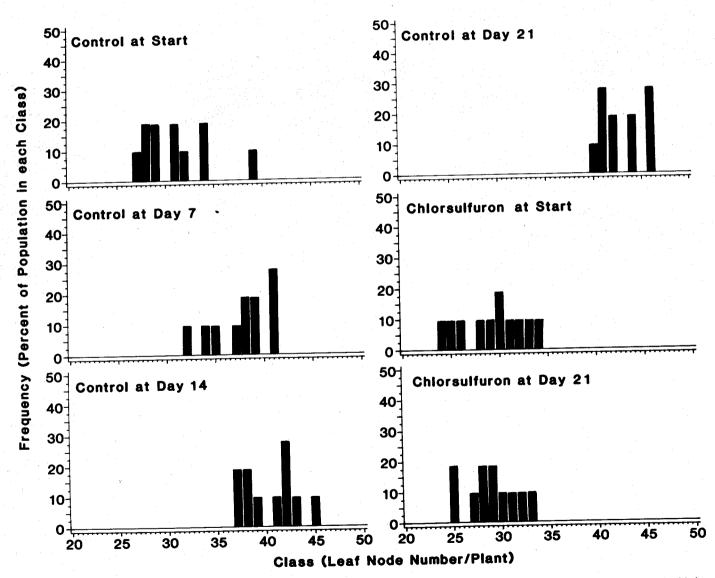


Figure 2. The effect of foliar-applied chlorsulfuron at 67 g/ha with oxysorbic (0.2%, v/v) on vegetative phenological development of Canada thistle.

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Table 1. The effects of oxysorbic surfactant (0.2% v/v) and chlorsulfuron (67 g/ha) on root growth, shoot growth, and secondary shoot growth from root buds of segmented Canada thistle roots 1 month after foliar treatment^a.

Treatment	Increase in height from start		Root fresh weight per plant		Secondary shoo following roo	Secondary shoot dry weight ^b	
	(cm)	(%)	(g)	(%)	(g)	(%)	(g/shoot)
Control at start			38.3 cd		15.2 b		0.02 a
Control at harvest	20.7 a	100	60.6 a	100	53.0 a	100	0.03 a
Oxysorbic	25.4 a	123	52.5 ab	85	42.4 a	80	0.02 a
Chlorsulfuron	9.5 b	46	46.8 bc	77	39.8 a	75	0.03 a
Chlorsulfuron + oxysorbic	3.6 b	18	32.2 d	53	8.0 b	15	0.02 a

^aMeans in a column followed by the same letter do not differ at P<0.05 by Duncan's multiple range test.

the controls increased over the course of the experiment (cv = 20 to 30%) (Figure 5). In contrast, the fresh weight of chlorsulfuron-treated roots did not change, as in the first experiment (Figure 5 and Table 1).

A 3-week exposure to chlorsulfuron delayed secondary shoot emergence from root buds following root segmentation at harvest by at least 2 weeks relative to the controls (Figure 6). The number of secondary shoots per plant 5 weeks after

cutting was no different than at the start (P = 0.05) (Figure 6). Three weeks after exposure to chlorsulfuron, secondary shoot number (20.8% of harvest controls) was relatively more affected than was root fresh weight (53.1% of harvest controls) (Figures 5 and 6). Conceivably, the effect of chlorsulfuron on secondary shoot number could be an indirect

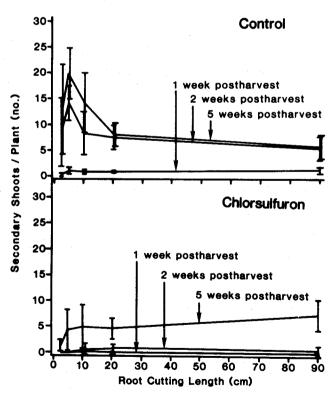


Figure 3. The effect of chlorsulfuron at 67 g/ha with oxysorbic (0.2%, v/v) and root cutting length on secondary shoot number per Canada thistle plant at various times after harvest. Means and standard deviations of 10 plants per treatment are presented. Intact roots were 90 \pm 23.6 cm (mean \pm SE) long.

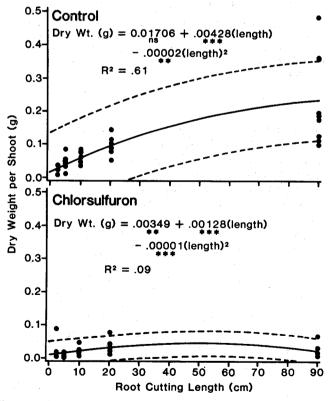


Figure 4. The effect of root cutting length and chlorsulfuron at 67 g/ha with oxysorbic (0.2%, v/v) on dry weight per secondary shoot of Canada thistle 5 weeks after harvest. The best fitting regression equation and 95% confidence interval are presented. Intact roots were 90 ± 23.6 cm (mean \pm SE) long.

bFive weeks after segmenting roots.

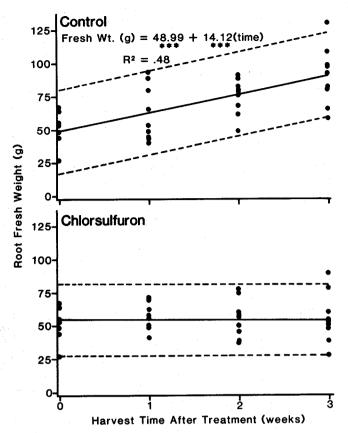


Figure 5. The effect of chlorsulfuron at 67 g/ha with oxysorbic (0.2%, v/v) on root fresh weight accumulation of Canada thistle. The best fitting regression equation and 95% confidence interval are presented. Overall regression analysis for the chlorsulfuron treated plants was nonsignificant.

effect of inhibited root growth. However, sprouting was delayed from herbicide-treated plants but not from controls started at the time of spraying (Figure 6). In addition, direct exposure of root buds on small excised root segments to chlorsulfuron concentrations between 10 nM and 1 μ M reduced subsequent bud growth (6).

Effect of growth stage. The response of Canada thistle to chlorsulfuron depended on growth stage at application. Chlorsulfuron stopped shoot growth and flowering of small vegetative plants shortly after treatment. However, large plants developed to a limited extent following spraying.

At harvest, the fresh weights of roots of large vegetative and flowering controls were the same, whereas small vegetative controls weighed less (Table 2). In contrast, the root fresh weights of the small and large vegetative plants were equal 1 month following chlorsulfuron treatment, but less than their respective controls. Root fresh weight was 30 and 23% of the harvest controls for plants treated at the small and large vegetative stages, respectively, and 77% for plants treated at flowering.

Large vegetative and flowering controls formed the same number of secondary shoots (Table 2). In contrast, fewer

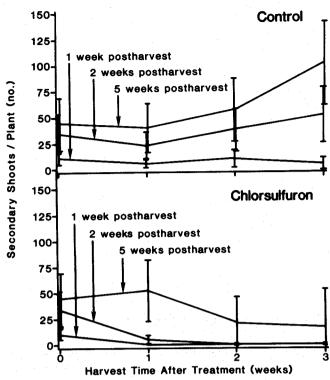


Figure 6. The effect of harvest time after treatment with chlorsulfuron at 67 g/ha with oxysorbic (0.2%, v/v) on secondary shoot number per Canada thistle plant. Means and standard deviations are presented.

secondary shoots formed from small vegetative controls. Stage of growth at the time of treatment modified secondary shoot sprouting in response to chlorsulfuron (Table 2). Secondary shoot numbers were only 10 or 6% of their respective

Table 2. The effect of chlorsulfuron (67 g/ha) plus oxysorbic (0.2%, v/v) and Canada thistle growth stage at the time of spraying on root fresh weight and secondary shoot emergence from buds of segmented Canada thistle roots 1 month after foliar treatment^a.

Treatment	Root fres	-	Secondary shoots per plant following root segmentation		
	(g)	(%)	(no.)	(%) ^b	
Control					
Small vegetative plants	59.9 c		41.1 b		
Large vegetative plants	88.0 ab		78.4 a		
Flowering plants	98.6 a		72.0 a		
Chlorsulfuron + oxysorbic					
Small vegetative plants	18.2 d	30	4.3 c	10	
Large vegetative plants	19.9 d	23	4.4 c	6	
Flowering plants	75.7 bc	77	36.8 b	51	

^aMeans in a column followed by the same letter do not differ at P<0.05 by Duncan's multiple range test.

^bFive weeks after segmenting roots.

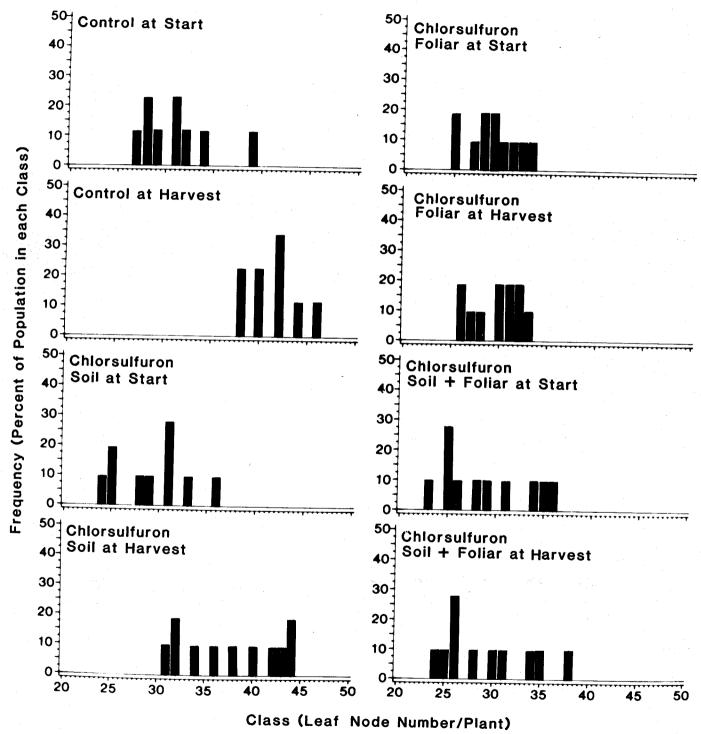


Figure 7. The effect of site of application of chlorsulfuron at 67 g/ha with oxysorbic (0.2%, v/v) on vegetative phenological development of Canada thistle. The percentage of the population in each phenological class is graphed for the controls and chlorsulfuron-treated plants prior to treatment and 1 month later for soil application alone, foliar treatment alone, and the combination of soil and foliar treatment.

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Table 3. The effect of site of application of chlorsulfuron (67 g/ha) plus oxysorbic (0.2% v/v) on Canada thistle root and shoot growth, and secondary shoot emergence from root buds 1 month after treatment².

Treatment	Shoot height increase after treatment			Root fresh weight per plant			Secondary shoots per plant following root segmentation	
	(cm)	(%)		(g)	:	(%)	(no.)	(%) ^b
Control At start At harvest	0.0 d 48.6 a	0 100		22.64 b 50.57 a		100	17.8 bc 24.0 ab	100
Chlorsulfuron + oxysorbic Root-treated Shoot-treated Root + shoot-treated	6.4 b 3.8 bc 3.1 cd	13 8 6		32.11 b 23.98 b 28.32 b		63 47 56	26.0 a 8.7 d 12.1 cd	108 36 50

^aMeans in a column followed by the same letter are not significantly different at P<0.05 by Duncan's multiple range test.

controls for plants treated with chlorsulfuron at the small and large vegetative stages, respectively. Plants treated with chlorsulfuron at flowering were much less affected than were vegetative plants, despite similar foliar display to the large vegetative plants. Differences in spray coverage between flowering and large vegetative plants are unlikely to be totally responsible for the extreme differences in response to this phloem-mobile herbicide (22). Besides, chlorsulfuron delayed secondary shoot emergence following harvest irrespective of growth stage at spraying. Secondary shoots from treated, small vegetative plants formed more slowly following treatment than older plants and more slowly than their respective controls.

Effect of site of treatment. As in previous experiments, foliar treatment stopped shoot development (Figure 7). At harvest, the plants from the treated population had the same number of leaf nodes as at the start. The population response to combined shoot and soil treatment was equivalent to foliar treatment alone. Following soil treatment some plants added new nodes with only a small increase in height (Figure 7 and Table 3). All three types of chlorsulfuron application prevented flowering and severely reduced shoot elongation (Table 3). The response to soil treatment may reflect apoplastic movement from the roots to the shoots, as reported by Ray (18, 19). However, soil-applied chlorsulfuron caused apical chlorosis and inhibited shoot growth without the extensive chlorosis of older leaves or the stem damage that was characteristic of foliar treatment. Soil treatment caused chlorosis of the shoot apex and young leaves, but mature leaves remained green and turgid for at least 1 month. In contrast, all foliar treatments caused significant foliar yellowing and necrosis.

Soil-applied chlorsulfuron stimulated initial elongation of root buds, but bud growth appeared to stop prematurely. This effect deserves further study because of its implications for extended control of Canada thistle. In addition, soil treatment was not nearly as toxic to the lateral roots as was foliar treatment. A month after application, root fresh weight doubled for the controls, but remained the same for all chlorsulfuron treatments (Table 3). Soil-applied

chlorsulfuron did not influence either secondary shoot numbers/plant or their rate of emergence, in contrast to the foliar treatments.

The different effects of foliar and soil treatment with chlorsulfuron on secondary shoot sprouting needs to be explained. The injury symptoms observed on parent shoots showed that chlorsulfuron was available to Canada thistle roots in the soil solution and was absorbed following soil treatment (Table 3 and Figure 7). The sodium salt of chlorsulfuron is soluble in water at concentrations of 5 to 10% (v/v) (10). Moreover, chlorsulfuron is highly mobile in soil immediately after application (10). In contrast, secondary shoot outgrowth was stopped or delayed following foliar treatment. Perhaps, different paths of chlorsulfuron uptake and movement in Canada thistle following either foliar or soil treatment may have influenced phytotoxic concentrations of the herbicide at sensitive root buds. Foliarly applied chlorsulfuron moves in the symplast and follows source-to-sink pathways to sensitive meristematic areas, such as root buds (19). Chlorsulfuron absorbed from the soil might move apoplastically and bypass the symplast and sensitive root buds. This untested possibility might explain the difference between the response of root buds on intact plants exposed directly to chlorsulfuron in soil and excised buds exposed to the herbicide in petri dishes (6).

The drastic reduction in phytotoxicity of foliarly applied chlorsulfuron without surfactant suggests that herbicide uptake may be limited. The data also suggest, but do not prove, that transport of phytotoxic doses of chlorsulfuron to the root system of Canada thistle may be limited. Thus, land infested with Canada thistle should probably not be plowed within 2 to 3 weeks following chlorsulfuron treatment. Higher application rates of chlorsulfuron in the field cannot be used to increase long-term control of Canada thistle because of the herbicide's persistence in the soil and the potential for carryover injury to susceptible rotational crops (4, 16, 20). The data also suggest that root buds present at the time of spraying may be relatively unaffected following foliar treatment with chlorsulfuron. These possibilities are under investigation.

bFive weeks after segmenting roots.

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