Weed Technology. 2006. Volume 20:356–360

Herbicide Placement Site Affects Small Broomrape (*Orobanche minor*) Control in Red Clover[1]

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Abstract: Small broomrape is an annual holoparasitic weed that was recently discovered in red clover production fields in Oregon. Imidazolinone herbicides such as imazamox control small broomrape; however, the mechanism of uptake by the parasite is largely unknown. Studies were conducted to determine the imazamox route of uptake by small broomrape in red clover, and to determine the potential for imazamox to be exuded from red clover and the subsequent effect on small broomrape. Small broomrape control was best at 90% when imazamox was foliar-applied, and worst at 42% or less when imazamox was soil-applied. The presence of activated charcoal to adsorb imazamox at the soil surface did not affect efficacy of broadcast foliar treatment. Small broomrape control was also evaluated when a foliar-treated red clover plant was grown in the same pot as a nontreated, parasitized red clover plant that was bagged during herbicide application. Activated charcoal was spread on the soil surface to adsorb imazamox, thus limiting herbicide uptake routes to the foliage of one of two red clover plants in the pot. Small broomrape attachment decreased on nontreated red clover when the other red clover plant in the pot was treated, suggesting roots exuded the herbicide or an active metabolite.

Nomenclature: Imazamox; small broomrape, *Orobanche minor* J. E. Smith. # ORAMI; red clover, *Trifolium pratense* L. # TRFPR.
Additional index words: Parasitic plant, imazamox.
Abbreviations: DAT, days after treatment.

INTRODUCTION

Small broomrape is a parasitic weed that attaches to the roots of its host plant. Once attached, it receives nutrients and water from the host plant. In Oregon, small broomrape has been identified as a parasite on red clover. It attaches to red clover roots early in the growing season but does not emerge for 4 to 5 mo after attachment. While individual small broomrape plants typically attach to a single host plant, multiple parasites often attach to an individual host. Small broomrape damage to the host plant is greatest prior to parasite emergence, thus making control difficult because its presence may not be evident. Small broomrape infestations can reduce crop yield and in some cases cause death of the host plant (Colquhoun and Mallory-Smith 2001). If red clover seed is contaminated with small broomrape seed, the red clover seed may be quarantined, thereby preventing its sale.

Chemical control of other *Orobanche* spp. has been achieved through soil fumigation and herbicide soil and foliar applications (Foy et al. 1989). Although soil fumigation with methyl-bromide has been reported to be effective (Musselman et al. 1989), it has not been considered for small broomrape control in Oregon because (1) the rocky soils are not conducive to the smooth seedbed required for methyl-bromide application, (2) methyl-bromide application is costly relative to low-value crops, and (3) methyl bromide has been targeted for phase-out because of environmental concerns (Schneider et al. 2003). Soil fumigation with metam-sodium was not effective for small broomrape control in Oregon (Colquhoun and Mallory-Smith 2001).

Multiple studies have shown sulfonylurea and imidazolinone herbicides to be effective for the control of *Orobanche* spp. (Aly et al. 2001; Eizenberg et al. 2001, 2004; Garcia-Torres and Lopez-Granados 1991; Goldwasser et al. 2001). Herbicide effectiveness and application timing varied depending on the crop species present and the particular herbicide used. It was not clear in all cases whether foliar or root uptake (or both) were

[1] Received for publication December 15, 2004, and in revised form October 10, 2005.

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responsible for *Orobanche* spp. control. Egyptian broomrape (*O. aegyptiaca*) was controlled when the herbicide was soil-applied, and not when it was applied to foliage only (Eizenberg et al. 2004; Hershenhorn et al. 1998). Foliar applications of rimsulfuron, a sulfonylurea herbicide, controlled Egyptian broomrape and branched broomrape (*O. ramosa* L.) in potatoes (*Solanum tuberosum* L.) (Goldwasser et al. 2001). Imazapic, an imidazolinone herbicide, provided effective control of sunflower broomrape (*O. cumana* Wallr.) when applied postemergence to sunflowers (*Helianthus annuus* L.) (Aly et al. 2001). Imidazolinone herbicides applied preplant or preemergence controlled bean broomrape (*O. crenata* Forsk.) in broad bean (*Vicia faba* L.) when infestations were low, but required a combination of preemergence and postemergence treatments for heavy infestations (Garcia-Torres and Lopez-Granados 1991). However, in many of the studies that included postemergence treatments, soil activity still may have been important for control of the parasite because the soil was not covered. From these studies, it also cannot be determined whether any of the parasites were killed by herbicides before attachment to the host plant or whether the herbicide must be absorbed by the host plant and then translocated to the attached parasite. Jurado-Exposito et al. (1999) reported that attached bean broomrape was a strong sink for imazethapyr applied to peas (*Pisum sativum* L.). Concentration in the parasite was 10 times higher than in the pea root and greatest when applied as a seed treatment. Otherwise, preemergence applications resulted in greater concentrations in the parasite than postemergence applications, indicating that soil application and soil activity may be important for *Orobanche* spp. control.

Imazamox applied postemergence to red clover and preemergence to small broomrape provided excellent control in recent Oregon field studies (Lins et al. 2005). However, it was not determined whether foliar or soil activity of imazamox was more important for small broomrape control, and absorption of imazamox by small broomrape was not studied.

Imazamox is absorbed rapidly by foliage while root absorption is slower (Vencill 2002). Once absorbed, imazamox is translocated in both phloem and xylem. Imazamox, with a reported half-life of 20 to 30 d, can provide residual control of germinating weeds, especially if soil moisture is adequate. Plants have been shown to exude imidazolinone herbicides from their roots (Little and Shaner 1991; Pester et al. 2001). Imazamox was detected in root leachates during translocation studies with feral rye (*Secale cereale* L.) and jointed goatgrass (*Aegilops cylindrica* Host) (Pester et al. 2001). Feral rye exuded 22% of the applied imazamox, which was twice as much as jointed goatgrass exuded. In another study, imazapyr was applied foliarly to two of three corn plants in a pot (Little and Shaner 1991). Six days after treatment, 37% of the total imazapyr absorbed was found in the sand media, and 5% was found in the nontreated plant. Small broomrape damage to host plants could seemingly be prevented if red clover exuded imazamox, thus resulting in small broomrape death before attachment to red clover.

The objectives of this research were to (1) determine the imazamox route of uptake for small broomrape in red clover (Study 1), and (2) determine the potential for root exudation of imazamox or an active metabolite from red clover as well as the subsequent effect on small broomrape (Study 2).

MATERIALS AND METHODS

Greenhouse studies were conducted using commercial potting mix[3] artificially infested with small broomrape seed (10 mg seed/L of soil) collected from a red clover field naturally infested with small broomrape. The small broomrape infested potting mix was placed in 0.5-L pots and four red clover ('Kenland'[4]) seeds per pot were planted. Plants were grown in 25/15 C day/night temperatures with 12 h of supplemental light. Each study was repeated. In Study 1, after red clover emergence, plants were thinned to one plant per pot. Imazamox treatments were applied 30 d after red clover emergence. Treatments were placed in a 5 $\times$ 3 factorial arrangement with four replications. Factors were imazamox rate (0, 10, 20, 30, or 40 g ai/ha), and herbicide placement (foliar-applied with soil exposed, foliar-applied with soil covered, or soil-applied with no foliar contact). Foliar applications were made with a single nozzle cabinet sprayer delivering 187 L/ha and included 0.25% (v/v) nonionic surfactant. Soil applications were made by placing the appropriate imazamox concentration in 0.1 L of water and uniformly dispersing the solution on the soil surface. Activated charcoal (50 g) was homogenized with 0.5 L of water and applied uniformly to the soil surface of each pot before herbicide application of the foliar-applied with soil-covered treatments.

The number of small broomrape attachments emerged from the soil was quantified at 10, 20, and 30 d after

[3] Sunshine Mix #1 potting mix, Sun Gro Horticulture, Inc., 110th Avenue NE, Suite 490, Bellevue, WA 98008.

[4] Tangent Seed Lab International, 33731 Highway 99E, P.O. Box 331, Tangent, OR 97389.

treatment (DAT). At 30 DAT, red clover plants and attached small broomrape were harvested and washed, and the number of herbicide-affected small broomrape attachments were counted and expressed as a percentage of total attachment number per pot. Herbicide-affected attachments appeared desiccated and blackened. Small broomrape biomass per pot was determined after oven-drying for 48 h at 72 C.

In Study 2, after red clover emergence, plants were thinned to two plants per pot. Before foliar application of imazamox at the rates stated in Study 1 at 30 d after red clover emergence, soil in all pots was covered with activated charcoal as described in Study 1. Treatments were arranged in a 5 $\times$ 2 factorial design with four replications. Factors were imazamox rate (the same rates as used in Study 1), and foliar application to (1) both plants per pot exposed or (2) only to one plant per pot by bagging the other plant in the pot before application. At 30 DAT, the red clover plants bagged before application and one randomly selected plant from each pot with both plants exposed at application were harvested. Root washing, controlled attachment assessment, and biomass collection and determination were conducted as described in Study 1.

There were no experiment time–by-treatment interactions in either study; therefore, the data for each study were combined over repeated experiments and analyzed as eight replications of each treatment. ANOVA was conducted using JMP® software (version 4.0.3; SAS Institute Inc., Cary, NC). Significant interactions were observed between herbicide rate and place of action in both studies; therefore, means were separated for all combinations of the effects by LSD on the basis of the Tukey-Kramer honestly significant difference test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Study 1. Imazamox Route of Uptake for Small Broomrape in Red Clover. Ten and 20 DAT, small broomrape emergence was reduced compared with that of the nontreated check by all imazamox applications, regardless of placement site or herbicide rate (Table 1). Small broomrape emergence was prevented 20 DAT by all imazamox applications at the 40 g/ha rate. Twenty DAT, emergence was greater when imazamox was soil-applied at 10 or 20 g/ha compared with emergence when similar rates were foliar-applied. By 30 DAT, emergence was similar when the nontreated check was compared with imazamox soil-applied at 10 or 20 g/ha. When imazamox was applied at 30 or 40 g/ha, emergence 30 DAT was greater in the soil application than in the foliar treatments.

Table 1. Small broomrape emergence 10, 20, and 30 d after treatment relative to imazamox foliar application with soil exposed, foliar application with soil covered, and soil application at five herbicide rates.

Treatment	Rate	Small broomrape attachments 10	20	30
	g ai/ha	Number/plant		
Foliar application/soil exposed	0	2.00[a]	4.52	8.21
	10	0.11	0.72	3.54
	20	0	0	1.11
	30	0.10	0.12	0.12
	40	0	0	0
Foliar application/soil covered	0	2.21	5.20	7.82
	10	0.10	1.12	4.22
	20	0	0	1.21
	30	0	0.11	0.22
	40	0	0	0.13
Soil application	0	1.92	6.14	9.01
	10	0.23	4.02	8.04
	20	0.21	2.74	7.89
	30	0	0.30	4.22
	40	0	0	2.85
LSD (0.05)		0.86	1.18	1.86

[a] Means were separated with LSD on the basis of the Tukey-Kramer honestly significant difference test ($P = 0.05$).

Activated charcoal on the soil surface did not affect small broomrape emergence at any imazamox rate or evaluation timing. Small broomrape emergence when imazamox was soil-applied was generally greater than when imazamox was foliar-applied. Small broomrape emerged earlier and in greater numbers when 10 and 20 g/ha were applied to the soil rather than to the foliage. These observations support the hypothesis that imazamox soil activity on small broomrape is minimal and short-lived compared with imazamox applied to red clover foliage.

Small broomrape biomass observations 30 DAT reflected small broomrape emergence among treatments (Table 2). Small broomrape biomass of all attachments to a single red clover plant in each pot was minimal when imazamox was foliar-applied. All foliar-applied imazamox rates reduced small broomrape biomass compared with that of the nontreated check. Although there was a rate response in small broomrape emergence number in foliar imazamox applications, small broomrape biomass was similar among rates. This observation suggests that small broomrape biomass per attachment was less when imazamox was applied at the low rate compared with the higher rates, and that all imazamox rates limited small broomrape resource uptake from the host red clover. There were no differences in small broomrape

Table 2. Small broomrape biomass and controlled attachments 30 d after treatment relative to imazamox foliar application with soil exposed, foliar application with soil covered, and soil application at five herbicide rates.

Treatment	Rate	Small broomrape attachments	
		Biomass	Control
	g ai/ha	g/plant	%
Foliar application/soil exposed	0	3.61[a]	0
	10	0.19	95
	20	0.11	95
	30	0.23	95
	40	0.09	95
Foliar application/soil covered	0	4.01	0
	10	0.14	94
	20	0.15	97
	30	0.07	95
	40	0.04	94
Soil application	0	3.73	0
	10	2.53	16
	20	1.85	20
	30	1.35	31
	40	1.01	42
LSD (0.05)		1.05	12

[a] Means were separated with LSD on the basis of the Tukey-Kramer honestly significant difference test (P = 0.05).

biomass with or without activated charcoal on the soil surface. Small broomrape biomass was greater when imazamox was applied to soil than when it was applied to foliage.

Controlled parasitic attachments were visually evaluated and expressed as a percentage of the total number of attachments on each red clover plant (Table 2). Foliar imazamox applications controlled more than 90% of attachments, whether initial or subsequent attachments, at all application rates. Small broomrape control was not affected by the activated charcoal. In soil applications, control increased with increasing imazamox rate and reached a maximum of 42%.

Study 2. Potential Root Exudation of Imazamox from Red Clover. In the second study, the soil was covered by activated charcoal, limiting transport of imazamox across the activated charcoal barrier to movement through the treated red clover plant. Biomass of small broomrape attached to the bagged, nontreated red clover when imazamox was applied at 30 or 40 g/ha was less than when imazamox was applied at 10 or 20 g/ha or the nontreated check (Table 3). Desiccated attachments on nearby nontreated, bagged plants in the same pot were also observed 30 DAT, particularly when imazamox was applied at 30 or 40 g/ha (Table 3). These results were similar to observations of small broomrape biomass, for which killed parasitic attachments stop accumulating resources from the host plant. These observations suggest that imazamox or an active metabolite was exuded from the treated red clover roots or from attached small broomrape (or both), and the exudate resulted in partial control of the small broomrape attached to an adjacent, nontreated red clover plant.

Table 3. Small broomrape biomass and controlled attachments 30 d after treatment relative to imazamox foliar application with soil covered and either (1) two treated (foliage exposed) red clover plants in each pot, or (2) a treated (foliage exposed) and nontreated (foliage bagged) red clover plant in each pot at five herbicide rates.

Treatment	Rate	Small broomrape attachments	
		Biomass	Control
	g ai/ha	g/plant	%
Treated red clover (foliage exposed)	0	4.27[a]	0
	10	0.05	97
	20	0.10	91
	30	0.12	96
	40	0.11	94
Nontreated red clover (foliage bagged)	0	3.88	1
	10	3.73	1
	20	3.86	4
	30	2.10	21
	40	1.80	19
LSD (0.05)		0.85	16

[a] Means were separated with LSD on the basis of the Tukey-Kramer honestly significant difference test (P = 0.05).

Small broomrape control with imazamox was greatest when the herbicide was applied to red clover foliage. Small broomrape control from soil-applied imazamox was minimal, given that there were no differences in small broomrape emergence, biomass, or control when imazamox was foliar-applied with soil exposed compared with foliar-applied with soil covered. Additionally, imazamox applied to the soil with no foliar contact resulted in poor small broomrape control compared with control by foliar-applied imazamox. However, small broomrape attachments to nontreated red clover plants were controlled somewhat, and small but significant biomass reductions in these attachments occurred when an adjacent red clover plant was treated with imazamox. Because the soil was covered at application time, the only route to nontreated plant attachments that the imazamox or an active metabolite could have taken was through the treated plant roots as an exudate. This route represents a novel control mechanism. Further research should be conducted to identify and determine the amount of exuded herbicidal compound, whether the compound is exuded from the treated plant roots or the small broomrape attachments to the treated plant (or both); as well as whether the exuded compound is absorbed by the nontreated red clover plant and moves into attached small broomrape or whether small broomrape directly absorbs the exuded compound from the soil (or both).

These results stress the importance of gaining a better understanding of the parasite-host relationship relative to control measures. In practical terms, imazamox applied for small broomrape control in red clover should be timed soon after the majority of the parasites are attached to the host plant but not emerged from the soil, and when the red clover is actively growing or able to translocate and exude the herbicide. Further research is necessary to determine the most efficient herbicide application timing for imazamox on red clover such that the amount of herbicide or metabolite translocated to the parasite and exuded into the nearby rhizosphere is maximized.

ACKNOWLEDGMENT

We appreciate USDA-APHIS for its partial support for this project.

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