AN ABSTRACT OF THE DISSERTATION OF

Ryan Daniel Lins for the degree of Doctor of Philosophy in Crop Science presented on April 28, 2005.

Title: Small Broomrape (*Orobanche minor*) Biology and Management in Red Clover (*Trifolium pratense*) Seed Production

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Jed B. Colquhoun

Small broomrape (*Orobanche minor*) is a parasitic weed that attaches to the roots of red clover (*Trifolium pratense*). Small broomrape invasion presents a significant threat to the future of red clover seed production in Oregon. This study was conducted to investigate and develop small broomrape management options for red clover seed production. Experiments were conducted to evaluate herbicide treatments applied after small broomrape emergence in red clover. Imazamox and imazamox plus bentazon treatments were the only herbicide treatments that consistently exhibited a high level of crop safety, reduced small broomrape density, and did not reduce red clover yield. Herbicide treatments did not prevent production of viable small broomrape seed. Small broomrape seed must be stimulated by host plant exudates for germination and attachment to occur. However, false-host plant species can stimulate parasitic seed germination without attachment. Wheat was a false-host of small broomrape; therefore, experiments were conducted to evaluate the effect of wheat on small broomrape

germination. Wheat cultivars induced 20 to 70% of small broomrape seed to germinate. Small broomrape attachment was reduced on red clover plants grown in potting mix previously planted to wheat compared to where none were grown. Red clover plants also had fewer small broomrape attachments when grown in field soil following wheat compared to plants in soil where no wheat was previously grown. The effect of small broomrape parasitism on the biomass partitioning of its primary host, red clover, has not been documented. An experiment was conducted to determine the relationship between small broomrape and red clover biomass accumulation. Total biomass of parasitized red clover plants and their attached small broomrape was 40% less than the total biomass of non-parasitized red clover plants. Small broomrape parasitism reduced the amount of dry matter allocated to red clover inflorescences by 70%. Red clover establishment with winter wheat in a small broomrape management system was investigated. Red clover that was spring interseeded into wheat produced minimal dry matter and ground cover by the following summer. Red clover fall interseeded with wheat produced enough ground cover for stand retention in one of two sites. Wheat yield was not affected by row spacing or red clover competition.

Small Broomrape (*Orobanche minor*) Biology and Management in Red Clover (*Trifolium pratense*) Seed Production

by

Ryan Daniel Lins

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APPROVED:

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Dean of the Graduate School

I understand that my dissertation will become a part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Ryan Daniel Lins, Author

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This project would not have been possible without the contributions of my wife, Katrina, who tirelessly supported me. Her love and confidence in me are truly inspirational. Finally, I would like to thank my family and friends for their unending support and encouragement throughout this endeavor.

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DEDICATION

This project is dedicated to my father, Duane Lins, who instilled in me a love of agriculture. He has always been a shining example of determination and character.

Small Broomrape (*Orobanche minor*) Biology and Management in

Red Clover (*Trifolium pratense*) Seed Production

CHAPTER 1

General Introduction

Holoparasitic angiosperms of the genus *Orobanche* (the broomrapes) have long been problematic weeds in the regions surrounding the Mediterranean (Pieterse 1979). Damage to crops by broomrape species is common in warm, dry climates (ter Borg 1986) and yield loss has been documented to range from zero to complete crop failure, depending on the level of infestation (Barker et al. 1996; Foy et al. 1989; Manschadi et al. 1996; Sauerborn 1991). Broomrape species cause damage by drawing nutrients and water from host plants by way of root attachment. Crenate broomrape (*Orobanche crenata* Forsk.) and Egyptian broomrape (*Orobanche aegyptiaca* Pers.) were reported to act as a resource sink for host plants, accounting for biomass and seed yield loss from host crop species (Manschadi et al. 1996; Hibberd et al. 1996).

Small broomrape (*Orobanche minor* Sm.) is a parasite of red clover (*Trifolium pratense* L.) and several other crop and weed species. Small broomrape lacks chlorophyll and depends solely on the host plants for nutrients and water (Baccarini and Melandri 1967; Saghir et al. 1973). Small broomrape infestations often are difficult to identify in host crop fields until stalk emergence because of the parasite's below-ground growth habit. Additionally, small broomrape is a prolific seed producer of up to 1,000,000 per plant. Its dust-like seeds are spread by wind, water, machinery, contaminated crop seed and forage, animals, and clothing. Seed may remain dormant in the soil for 10 years or more and may still be viable after flowering plants have been hand-pulled.

In Oregon, there had only been six reports of small broomrape from 1923 to 1997. However in 1998, after being identified on a single farm, the number of infestations increased to 15 in 2000 and 22 in 2001. Small broomrape is listed as a federal noxious weed that has quarantine significance to many of Oregon's trading partners. Seed contamination, reduction in seed yield, and host plant death may all be consequences to small broomrape infestation (Colquhoun and Mallory-Smith 2001).

Much research on broomrape species has been conducted with crop hosts in the Mediterranean area. Many of these studies made inferences on the effect of broomrape parasitism on host growth and biomass allocation. However, small broomrape parasitism of red clover in Oregon is unique in that it is first seed crop to be affected by a broomrape species in the United States of America (Colquhoun et al. 2001). No research has been conducted on the effect of small broomrape on red clover seed production. Therefore, opportunities exist to conduct novel research on the biology, interactions, and management of small broomrape in red clover seed production systems.

Small broomrape management in a red clover seed production system may prove to be difficult given the growth habit and reproductive capabilities of small broomrape. However, integrating chemical and cultural practices may lead to effective control options. False-hosts of broomrape species have been identified and can be used to deplete the soil seedbank (Hershenhorn et al. 1996). A false-host stimulates parasitic seed germination, without root attachment, resulting in parasite death. Parker and Riches (1993) reported sorghum (*Sorghum vulgare* Pers.), corn (*Zea mays* L.), mung bean (*Phaseolus aureus* Roxb.), and cucumber (*Cucumis sativus* L.) to be false-hosts for branched broomrape (*Orobanche ramosa* L.), while Bischof and Koch (1974) reported similar results with sweet pepper (*Capsicum anuum* L.) as a false-host for Egyptian broomrape. Research by Ross et al. (2002) indicated that wheat (*Triticum aestivum* L.) was a false-host for small broomrape. Thus, by intercropping wheat with red clover, the small broomrape soil seedbank could be dramatically reduced.

The objectives of the projects described herein were to investigate small broomrape control with chemical and cultural methods, to gain a better understanding of small broomrape-red clover interactions, and to assess the agronomic feasibility of a red clover-wheat intercropping system. Red clover growers are commonly unaware of small broomrape infestations until parasitic shoots emerge; therefore, chemical control options for this project have been focused on applications made to emerged small broomrape. Cultural control of small broomrape was evaluated using typical Pacific Northwest winter wheat cultivars. All experiments were managed to provide meaningful results that could be effectively integrated into crop management systems by Oregon red clover seed producers.

CHAPTER 2

Postemergence Small Broomrape (*Orobanche minor*) Control in Red Clover (*Trifolium pratense*)

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ABSTRACT

Small broomrape is an annual, parasitic weed that was recently discovered in Oregon's red clover seed production system. Field experiments were conducted in 2002 and 2003 at two locations to evaluate 10 herbicide treatments applied after small broomrape emergence in red clover. Bentazon, bromoxynil, glyphosate, imazamox, imazamox plus bentazon, imazethapyr, MCPA, and pendimethalin were evaluated. Small broomrape density, small broomrape seed viability after treatment, and clover injury and seed yield were quantified. Small broomrape control with imazamox, glyphosate, and imazamox plus bentazon treatments was greater than the nontreated check in both years. However, imazamox and imazamox plus bentazon treatments were the only herbicide treatments that consistently exhibited a high level of crop safety, reduced small broomrape density, and did not reduce red clover yield. Herbicide treatments did not prevent production of viable small broomrape seeds. Future research is needed to develop control options that will prevent red clover yield loss and viable small broomrape seed production when applied prior to small broomrape emergence.

Nomenclature: Bentazon; bromoxynil; glyphosate; imazamox; imazethapyr; MCPA; pendimethalin; small broomrape, *Orobanche minor* Sm. #[1] ORAMI; red clover, *Trifolium pratense* L. # TRFRE.

Additional index words: Parasitic weed.

Abbreviations: DAT, days after treatment.

INTRODUCTION

Holoparasitic angiosperms of the genus *Orobanche* (the broomrapes) have long been problematic weeds in the regions surrounding the Mediterranean (Pieterse 1979). Damage to crops by *Orobanche* spp. is common in these warm and dry areas (ter Borg 1986) with yield loss ranging from zero to complete crop failure, depending on the level of infestation (Barker et al. 1996; Foy et al. 1989; Manschadi et al. 1996). *Orobanche* spp. cause damage by drawing nutrients and water from host plants through root attachment (Baccarini and Melandri 1967; Parker and Riches 1993; Saghir et al. 1973). Small broomrape is a parasite of red clover and several other crop and weed species. Small broomrape is a prolific seed producer with fecundity of over 1,000,000 seeds per plant (Pieterse 1979). Dust-like small broomrape seeds can be spread by wind, water, machinery, contaminated crop seed, animals, and clothing. Seed may remain dormant in the soil for long periods of time until induced to germinate by host exudates (ter Borg 1986).

In Oregon, small broomrape germination and attachment occurs in red clover from January to February. The parasite then stays beneath the soil surface for approximately 4 to 5 months drawing nutrients and water from its host. Small broomrape emerges and flowers from June to July, producing viable seed within 3 weeks after stalk emergence. Viable seed can be produced even after flowering plants have been hand pulled.

Seed contamination, reduction in seed yield, and host plant death all may be consequences of small broomrape infestation. There were six reported infestations of small broomrape from 1923 to 1997 in Oregon. However, in 1998 after identification in a single red clover seed production field, the number of reported infestations increased to 15 in 2000 and 22 in 2001 (Colquhoun, unpublished data). Small broomrape is listed as a federal noxious weed and may be prohibited as a seed contaminant by many of Oregon's trading partners, potentially eliminating Oregon red clover seed export.

To date, small broomrape control options are limited and there are no registered herbicides that control small broomrape in red clover. Additionally, growers are often unaware of an infestation until small broomrape shoots emerge. Recent research has shown that *Orobanche* spp. can be controlled in various crops with glyphosate, sulfonylurea, and imidazolinone herbicides (Aly et al. 2001; Garcia-Torres et al. 1987; Hershenhorn et al. 1998; Kleifeld et al. 1998). However, herbicides have not been evaluated for postemergence control of small broomrape in red clover seed production. Control options for emerged small broomrape are needed as "rescue treatments" given the rapid spread to previously uninfested fields in recent years. Therefore, our objectives were to evaluate several postemergence herbicide treatments for crop safety, crop yield, control of small broomrape, and effect on small broomrape seed viability in red clover production systems in western Oregon.

MATERIALS AND METHODS

Studies were conducted in small broomrape-infested red clover seed production fields in Washington and Clackamas Counties, OR, in 2002 and 2003, respectively. Prior to planting, fields were disked and harrowed to prepare a suitable seed bed. 'Kenland' medium red clover was planted into a Hillsboro loam soil (fine-silty, mixed, mesic Ultic Argixerolls) at the Washington County location in October, 2000, and a

Quantama loam soil (fine-loamy, mixed, mesic Aquultic Haploxerolls) at the Clackamas County location in October, 2001. Red clover fields were managed with common grower practices, consisting of a forage harvest in the first year of production, and herbicide plots were established in the second year of red clover production. The experimental design was a randomized complete block with four replications and plot size was 2.4 by 9.1 m.

Herbicides tested were bentazon at 1120 g ae/ha, bromoxynil at 280 g ai/ha, glyphosate at 26, 53, and 105 g ae/ha, imazamox at 45 g ae/ha, imazamox and bentazon at 45 g ae/ha and 1120 g ae/ha, respectively, imazethapyr at 105 g ae/ha, MCPA at 680 g ae/ha, and pendimethalin at 2780 g ai/ha. Herbicides were chosen based on demonstrated *Orobanche* spp. efficacy (Goldwasser et al. 2003; Qasem 1998) and/or registration for use on *Fabaceae* spp. crops in Oregon or elsewhere. All bentazon, bromoxynil, glyphosate, imazamox, imazethapyr, and MCPA treatments were applied with nonionic surfactant at 0.25% (v/v). A nontreated check treatment was included in both experiments. Treatments were broadcast with a bicycle wheel sprayer calibrated to deliver a solution volume of 187 L/ha. Application dates were June 20, 2002 for the Washington County site, and May 29, 2003 for the Clackamas County site and coincided with small broomrape emergence. The 2003 application date occurred earlier in the calendar year due to more rapid accumulation of growing degree days, and thus, greater small broomrape development and an earlier emergence date (Eizenberg et al. 2005). Red clover was at the four trifoliate growth stage at the time of application in both years.

Small broomrape stalk number per m^2 was counted at herbicide application to quantify the density of emerged plants that were treated. Small broomrape density was quantified 10 and 20 days after treatment (DAT). Small broomrape distribution was variable across experimental sites. Therefore, small broomrape survival and emergence after treatment was assessed as a percent of the pre-treatment small broomrape density. Treatments that reduced pretreatment density below 100% were considered to have postemergence activity on small broomrape.

Red clover herbicide injury was visually evaluated 10 and 20 DAT on a scale of 1 to 100% injury; with 100% injury equal to crop death. Small broomrape injury to red clover did not cause symptoms resembling herbicide injury. Red clover seed yield was quantified from hand-harvested 1 m^2 quadrats in each plot on September 5, 2002 and September 4, 2003.

In a related small broomrape seed viability study, five randomly selected, mature small broomrape plants with dry seed capsules were harvested from each plot of the field experiments on August 9, 2002 and August 13, 2003. On August 16, 2002 and September 10, 2003, the five small broomrape stalks taken from each field plot were mixed with potting media[1] in individual 600 cm^2 pots and one 'Kenland' red clover plant was planted in each pot. Pots were placed in a greenhouse where temperature was approximately 22 C and lights provided 12 h of light per day. The experimental design was a randomized complete block with four replications. Red clover plants were clipped once before initial flowering to simulate a forage harvest commonly practiced by growers. Experiments were harvested at red clover full bloom on December 18, 2002 and January 5, 2004. The number of small broomrape attachments per red clover plant was quantified. Small broomrape seed viability was tested with host plants due the low efficacy of artificial germination stimulants, like GR24 (R. D. Lins, unpublished data), and to mimic field germination conditions.

ANOVA was used for all experiments and treatment means were separated using Fisher's protected LSD test ($P = 0.05$). Data were analyzed with PROC GLM and PROC TTEST using SAS.[2] Red clover injury and small broomrape density data were analyzed as percentages. Red clover yield per year was compared using a T-test.

RESULTS AND DISCUSSION

Red Clover Injury

Red clover injury was generally greater and more variable in 2003 than 2002 (Table 2.1). In 2002, MCPA and imazethapyr caused the most injury at 10 DAT, while glyphosate at 105 g ae/ha and imazethapyr had the highest injury ratings 20 DAT. In 2003, injury at 10 DAT was greatest where MCPA, glyphosate at 105 g/ha, or pendimethalin were applied, but at 20 DAT bromoxynil, glyphosate at 53 g/ha, MCPA, and pendimethalin treatments caused the greatest injury. However, only the MCPA treatment differed from the check at 20 DAT because of the highly variable response in 2003. In both years, red clover injury was lower when bentazon, glyphosate at 26 g/ha, imazamox, and imazamox plus bentazon were applied to control small broomrape. The addition of bentazon to imazamox minimized red clover injury when compared to imazamox applied alone. This is consistent with bentazon antagonism of imazamox reported in other studies (Zollinger and Fitterer 1998).

Red clover seed yield differed between years (T-test; $p < 0.001$). The 2002 seed yield varied among treatments and was greater, on average, than the 2003 seed yield. In 2002, seed yield was lower with glyphosate at 105 g/ha and imazethapyr, than where bentazon, bromoxynil, MCPA, and pendimethalin were applied. In 2003, herbicide treatment did not affect red clover yield. Red clover seed production was less in 2003 compared to 2002, presumably because of differences in small broomrape density and available moisture between years. Prior to herbicide application, mean small broomrape density across each experiment was 14.4 plants per m^2 in 2002 and 29.5 plants per m^2 in 2003.

Table 2.1. Visual estimate of red clover injury, small broomrape density, and red clover seed yield after herbicide application.

		Red clover injury				Small broomrape density[b]				Red clover seed yield	
		2002		2003		2002		2003			
Treatment[a]	Rate	10 DAT[c]	20 DAT	10 DAT	20 DAT	10 DAT	20 DAT	10 DAT	20 DAT	2002	2003
	g ae or ai/ha[d]	--------------------- % ---------------------				----------- % of initial density ------------				----- kg/ha ----	
Untreated check	--	0	0	0	0	130	139	183	264	252	78
Bentazon	1120	0	0	3	5	130	140	228	369	323	47
Bromoxynil	280	3	5	11	19	137	104	121	227	330	61
Glyphosate	26	3	3	8	10	116	104	52	61	280	100
Glyphosate	53	0	14	5	18	110	94	102	80	189	49
Glyphosate	105	10	24	20	14	106	84	160	26	53	60
Imazamox	45	3	3	13	16	93	42	114	77	285	95
Imazamox + bentazon	45 + 1120	0	0	0	3	89	52	100	25	272	73
Imazethapyr	105	15	24	8	14	88	37	148	174	172	66
MCPA	680	18	20	26	38	172	155	153	163	344	73
Pendimethalin	2780	5	3	15	20	132	98	183	236	336	57
LSD (0.05)		3	3	12	20	18	24	48	62	135	NS

[a] All treatments except pendimethalin were applied with nonionic surfactant at 0.25% (v/v).

[b] Values greater than 100 indicate that emerged small broomrape was not controlled and additional flowering stalks emerged after herbicide treatment. Values less than 100 indicate that small broomrape density at time of application was reduced by postemergence activity of the herbicide treatment.

[c] Abbreviation: DAT, days after treatment on June 20 and May 29, for 2002 and 2003, respectively.

[d] Bentazon, glyphosate, imazamox, imazethapyr, and MCPA are expressed as g ae/ha.

Small Broomrape Control

Herbicide effects on small broomrape density differed between years (Table 2.1). In 2002, none of the treatments reduced small broomrape density at 10 DAT compared to small broomrape density prior to herbicide application. By 20 DAT, the imazamox, imazamox plus bentazon, and imazethapyr treatments reduced pre-treatment small broomrape densities by as much as 63%. Similarly in 2003, herbicides did not decrease small broomrape density 10 DAT compared to pre-treatment density. Only the high rate of glyphosate and imazamox plus bentazon treatments reduced pre-treatment densities at 20 DAT; however, these treatments did not differ significantly from the imazamox or the other glyphosate treatments.

Plots treated with MCPA at 10 DAT in 2000 and bentazon at 20 DAT in 2003, had greater small broomrape densities than the untreated check. These herbicides do not appear to have activity on small broomrape. However, red clover plants damaged by these herbicides may have triggered an increase in small broomrape plant density. Small broomrape emergence appears to increase when red clover is injured or wounded (R. D. Lins, unpublished data). This may be the result of ethylene production, which Zehhar et al. (2002) has shown to be capable of inducing germination in branched broomrape (*Orobanche ramosa* L.).

Small Broomrape Seed Viability

In the small broomrape seed viability study, the effect of herbicide treatment on small broomrape attachment per red clover plant differed in 2002, but not in 2003 (Table 2.2). However, even though treatments differed in 2002, no herbicide reduced the number of small broomrape attachments per red clover plant as compared to the untreated check. Therefore, herbicide treatment effects may be insignificant given the copious seed production of small broomrape.

Table 2.2. Parasitism of red clover plants by small broomrape seed produced from herbicide treated plants. [a]

Treatment [b]	Rate	Small broomrape parasitism 2002	2003
	g ae or ai/ha[c]	Attachments/clover plant	
Untreated	--	3.8	1.8
Bentazon	1120	5.3	5.3
Bromoxynil	280	4.5	4.0
Glyphosate	26	2.3	0.8
Glyphosate	53	2.8	2.5
Glyphosate	105	0.3	1.0
Imazamox	45	0.3	8.3
Imazamox + bentazon	45 + 1120	1.3	6.3
Imazethapyr	105	0.3	2.8
MCPA	680	1.0	9.0
Pendimethalin	2780	1.5	4.3
LSD (0.05)		3.6	NS

[a] Abbreviation: NS, not significant
[b] Treatments applied to red clover in the field from which small broomrape seed stalks were harvested and seed viability tested. All treatments except pendimethalin were applied with nonionic surfactant at 0.25% (v/v).
[b] Bentazon, glyphosate, imazamox, imazethapyr, and MCPA are expressed as g ae/ha.

Implications for Small Broomrape Management

This study demonstrated that small broomrape emergence and density in red clover can be reduced through the use of postemergent herbicide treatments. However, in this study imazamox and imazamox plus bentazon were the only herbicide treatments that consistently exhibited a high level of crop safety, reduced small broomrape density, and did not reduce red clover yield. Glyphosate at 105 g/ha also controlled small broomrape, but crop safety and subsequent crop yield were poor. Although these herbicides reduced small broomrape density, seed production and viability of seed from treated plants were not eliminated. Given the ability of small broomrape to produce large amounts of seed, the weed seed bank will likely increase even when herbicides are applied. With this in mind, integrated management of small broomrape must include an approach to reduce emergence of small broomrape shoots and subsequent contributions to soil seed banks. Strategies such as rotation or intercropping with small broomrape false host crops are recommended for an integrated small broomrape control program (Ross et al. 2004).

Herbicides were evaluated with the intention that they could serve as rescue treatments for emerged small broomrape populations in the growing season in which they are discovered in red clover production fields. Preliminary results of subsequent research suggest that imazamox applied prior to small broomrape emergence prevented both emergence and small broomrape seed production. Given that small broomrape is a holoparasite that utilizes water and nutrient resources that would otherwise be available to red clover, early management is critical to red clover seed yield. Further research has been conducted to develop a predictive temperature-based model for imazamox application after small broomrape attachment, but prior to emergence (Eizenberg et al. 2005). The use of this model will require frequent field inspections or knowledge of infested fields for proper herbicide application timing.

SOURCES OF MATERIALS

[1] Sunshine Mix #1 potting mix, SunGro Horticultural Inc., 110th Avenue NE, Suite 490, Bellevue, WA 98008.

[2] SAS Institute Inc., 1996, Box 8000, SAS Circle, Cary, NC 25711-8000.

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CHAPTER 3

Control of Small Broomrape (*Orobanche minor*) with Wheat as a False-host Crop

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ABSTRACT

Small broomrape is a parasitic weed that attaches to the roots of red clover and a number of other broadleaf plant species in the Pacific Northwest. Small broomrape seed must be stimulated by host plant exudates for germination and attachment to occur. However, plant species called false-hosts can stimulate parasitic seed germination without attachment. These species could be utilized to reduce the amount of parasitic seed in infested soil. Wheat was a false-host of small broomrape; therefore, growth chamber, greenhouse, and field experiments were conducted to evaluate the effect of 7 winter wheat cultivars and 1 triticale cultivar on small broomrape germination. In growth chamber experiments, wheat/triticale cultivars induced 20 to 70% of small broomrape seed to germinate. In greenhouse studies, small broomrape attachment was minimal on red clover plants grown in pots previously planted to a wheat/triticale cultivar. In pots that did not receive a false-host treatment, red clover plants averaged 4.2 small broomrape attachments per plant. Red clover plants also had fewer small broomrape attachments when grown in field soil following wheat/triticale compared to plants in soil where no wheat or triticale was previously grown. Our results demonstrate that wheat can be effectively integrated into a small broomrape management system.

Nomenclature: Small broomrape, *Orobanche minor* Sm. ORAMI; red clover, *Trifolium pratense* L.; triticale, *Triticale hexaploide* Lart.; winter wheat, *Triticum aestivum* L.

Key words: Parasitic plant, integrated weed management, red clover.

INTRODUCTION

Broomrapes (*Orobanche* spp.) are parasitic angiosperms whose host range includes members of the Apiaceae, Asteraceae, Fabaceae, and Solanaceae plant families (Goldwasser et al. 1997, Parker and Riches 1993, Romanova et al. 2001, Ross et al. 2004). Broomrape species lack chlorophyll and depend solely on host plants for photosynthate and water accumulation (Baccarini and Melandri 1967, Saghir et al. 1973). Crop injury caused by parasitic plants is common in the warm and dry regions surrounding the Mediterranean (ter Borg 1986) and crop yield loss has been documented to range from zero to complete crop failure (Barker et al. 1996, Foy et al. 1989, Manschadi et al. 1996).

The interactions of plant species with broomrapes can be classified in three categories. Hosts plants stimulate parasitic seed germination, tubercle development, and seed production, false-host plants stimulate parasitic seed germination without tubercle formation, and non-host plants do not stimulate parasitic seed germination or attachment. Host plant species release exudates that stimulate both parasitic seed germination and attachment, while false-host species release exudates that only promote germination (Joel et al. 1995). Upon receiving a signal for germination, a radicle emerges from the seed coat of a broomrape species. However, an additional chemical signal is needed for the radicle to penetrate host roots and form a haustorium, which is the vascular bundle between the parasite and host. This enables the parasite to become a resource sink for host plants. Once attached to a host plant, development of a tubercle (below-ground vegetative growth form) signifies the connection and functionality of the haustorium.

Small broomrape, a parasite of red clover and several other plant species, has recently invaded the world's primary red clover seed production region in the Willamette Valley of Oregon. There were six reports of small broomrape in Oregon from 1923 to 1997. However, since 1998 after identification in a single red clover seed production field, the number of infestations has increased to 15 in 2000 and 22 in 2001. Small broomrape is federally listed as noxious weed and contaminated red clover seed is quarantined and restricted from export. Pacific Northwest growers risk losing the ability to produce red clover seed from a combination of crop seed contamination with small broomrape seed, reduction in seed yield, and host plant death.

Control and prevention of small broomrape infestations in Oregon have proven to be difficult. This is a widespread phenomenon as management strategies targeting *Orobanche* spp. worldwide have been greatly unsuccessful (Goldwasser et al. 1997). There are several reasons that small broomrape control operations have been ineffective. Early detection of an infestation is difficult due to the growth habit of a root parasite and significant crop injury can occur prior to small broomrape stalk emergence. Control options after small broomrape emergence are limited and do not prevent parasite seed production (Lins et al. 2005). This lack of control can lead to a significant build-up in the weed seedbank, as *Orobanche* spp. can produce up to 1,000,000 seeds per flowering stalk (Pieterse 1979) and dust-like seeds can remain viable for 50 years or more when receiving no host signal to germinate (H. Eizenberg, personal communication). Crop rotation is also not a complete solution because small broomrape can parasitize weeds common to the Pacific Northwest, such as prickly lettuce (*Lactuca serriola* L.), wild carrot (*Daucus carota* L.), and spotted catsear (*Hypochaeris radicata*

L.) (Ross et al. 2004) which can lead to weed seed production even when non-host crops are grown in infested soils.

The use of host catch crops (Acharya et al. 2002, Linke et al. 1993) and false-host cover crops (Ross et al. 2004) have been suggested as tools to reduce broomrape in infested soil. Linke et al. (1993) reported a 30% reduction in the crenate broomrape (*Orobanche crenata* Forsk.) seedbank after one catch crop cycle. In Oregon, wheat was identified as a false-host of small broomrape (Ross et al. 2004) and therefore, has the potential to be implemented into an integrated small broomrape management system. It is critical to know if small broomrape germination stimulation varies by wheat cultivar to optimize the effectiveness of wheat as a false-host. Therefore, growth chamber, greenhouse, and field experiments were conducted to evaluate the effect of 7 wheat cultivars and 1 triticale cultivar on small broomrape germination.

MATERIALS AND METHODS

Growth Chamber Experiments

Two growth chamber experiments were conducted in 2003. Common Pacific Northwest soft white winter wheat cultivars of 'Foote,' 'Gene,' 'Madsen,' 'Stephens,' 'Weatherford,' 'Yamhill,' the winter Durum cultivar 'Connie,' and the triticale cultivar 'Bogo' were tested for small broomrape germination response. Winter cereal cultivars, as opposed to spring cultivars, were evaluated as false-host treatments because winter cereal growth coincides with soil conditions most favorable for small broomrape germination in Oregon's climate (Eizenberg et al. 2004). The experiments were arranged in a completely randomized design with three replications.

Seeds of each cultivar were germinated in germination boxes with moistened filter paper for 7 d, after which one healthy seedling was randomly selected and placed in the hydroponic polyethylene bag system described by Ross et al. (2004). Whatman®[1] glass microfiber filter paper was moistened with distilled water and evenly inoculated with 1 mg (approximately 350 seeds) of small broomrape seeds that were collected in 2002 from an infested Clackamas County, Oregon red clover field. The polyethylene bags with their associated crop plants were set upright and placed in file folders. The file folders from each experiment were then positioned in individual file crates and placed in separate growth chambers that maintained 12 hours of light at 20 C and 12 hours of dark at 10 C. Plants were watered as needed and supplemented with 10 ml of half strength Hoagland solution (Hoagland and Arnon 1950) every 14 d for 77 d. After a 14 d preconditioning period, small broomrape germination was quantified every 7 d for 63 d. Small broomrape germination was determined by emergence of a radicle from the seed coat. Total non-germinated small broomrape seed was quantified at the termination of the experiments to determine germination percentage. Preliminary experiments found that the synthetic germination stimulant GR24 (Mangnus et al. 1992) stimulated less small broomrape seed germination than wheat and therefore was not used as a control treatment.

Data from experiments were analyzed using ANOVA and means were separated using Fisher's protected LSD test ($P = 0.05$). Experiments were tested for combined analysis using Levene's test ($P > 0.05$) to confirm homogeneity of variance between experiments.

Greenhouse Experiments

Two greenhouse experiments were conducted in 2003. Each previously mentioned wheat/triticale cultivar was planted (one plant per pot) in 1 L pots filled with potting media[2] that was thoroughly mixed with 10 mg (approximately 3,500 seeds) of small broomrape seed. The small broomrape seed source was the same as what was used for the growth chamber experiments. Pots were placed in a greenhouse where temperature was approximately 22 C and light[3] was provided 12 h per day. Experimental design was completely randomized with six replications of nine treatments (8 cultivars plus a no false-host check). Pots were watered as needed and the check treatment pots were kept moist. When wheat/triticale plants reached the boot stage (72 d), they were cut at the soil surface and three 'Kenland' red clover seeds were sown into each pot and into the check treatment pots. Following emergence of the first trifoliate leaf, red clover was thinned to one plant per pot. After 53 d of growth, red clover plants were cut approximately 5 cm from soil surface, simulating a forage harvest commonly practiced by red clover growers. Pots were watered as needed and fertilized with 15 ml of Miracle Gro®[4] all-purpose plant food (15:30:15 with micro nutrients) once prior and once subsequent to the red clover forage cutting. Experiments were harvested 26 d after red clover forage harvest to coincide with red clover floral initiation. Small broomrape attachment per red clover plant was quantified through the examination of red clover root material. Data from experiments were analyzed using ANOVA and means were separated using Fisher's protected LSD test ($P = 0.05$). A Levene's test ($P > 0.05$) was used to confirm homogeneity of variance between experiments as to assess the validity of combining the data for analysis.

Field Experiment

A field experiment was initiated on October 9, 2002 in a Clackamas County, Oregon red clover field that had a relatively uniform infestation of small broomrape. Experimental design was a randomized complete block with four replications of nine treatments (treatments as in above greenhouse experiments) and a plot size of 2.4 m by 9.1 m. Wheat/triticale cultivars were sown in 15.2 cm rows at a depth of 2.5 cm. Seeding rate was 140 kg ha^{-1} for all cultivars. Plots were located on a Quantama loam soil (fine-silty, mixed, mesic Aquultic Haploxerolls) and were undisturbed for 280 d, until crop harvest. After crop harvest (28 d), three 3 L soil subsamples were taken from each plot through stratified random sampling. On November 17, 2003 1 L pots were filled with soil from each subsample and one 'Kenland' red clover plant was grown in each pot. Pots were placed in a greenhouse where temperature was approximately 22 C and lights provided 12 h of light per day. Pots were watered as needed and fertilized with 15 ml of Miracle Gro® all-purpose plant food three times after red clover establishment. After 104 d, red clover plants were cut approximately 5 cm from soil surface, simulating a forage harvest. Experiments were harvested 28 d later, after red clover floral initiation. On March 26, 2004 the remaining soil from the subsamples was prepared in the same fashion as the previous subsamples. For the second set of subsamples, a red clover forage harvest was conducted 60 d after planting and was harvested 25 d later. Small broomrape attachment per red clover plant was quantified and averaged across subsamples for each plot. Data from the field study were analyzed using ANOVA. A contrast was used to test for a difference in small broomrape attachment to red clover between false-host treatments and the no false-host check.

RESULTS AND DISCUSSION

Growth Chamber Experiments

The two growth chamber experiments exhibited homogeneous variance in the germination response of small broomrape to wheat/triticale (Levene's test $P = 0.92$) and were combined for analysis. Differences existed among wheat/triticale cultivars ($P < 0.0001$, Figure 3.1) and small broomrape germination response due to false-host cultivar ranged from 20% to 70%. Most of the wheat cultivars stimulated a large proportion of small broomrape germination; however the cultivar 'Connie' stimulated the lowest germination response of any cultivar tested (LSD = 18.0). In addition, the triticale cultivar 'Bogo' germinated a smaller percentage of small broomrape seed than did the wheat cultivars 'Weatherford' and 'Gene'.

Results obtained from the growth chamber experiments indicate that common Pacific Northwest wheat/triticale cultivars can stimulate germination in a greater proportion of small broomrape (up to 70%) than previously reported by wheat (up to 40% by Ross et al. 2004) or GR24 (Rodriguez-Conde et al. 2004). Most of the tested soft white winter wheat cultivars and the triticale cultivar worked well for small broomrape seedbank reduction, but the winter Durum wheat cultivar 'Connie' only germinated 20%. The genetic lineage of the tested wheat cultivars may be responsible for the differential response of small broomrape germination that was observed and could indicate that germination response of small broomrape may be a selectable trait for wheat breeding purposes. In preliminary polyethylene bag experiments, there appeared to be differences in the level of small broomrape germination when seed was placed in the root zone of diploid, tetraploid, and hexaploid genetic wheat stocks (unpublished data). Further investigations will utilize Quantitative Trait Loci (QTL) analysis to confirm and locate which wheat genome and which genes are potentially responsible for the production of small broomrape germination stimulants.

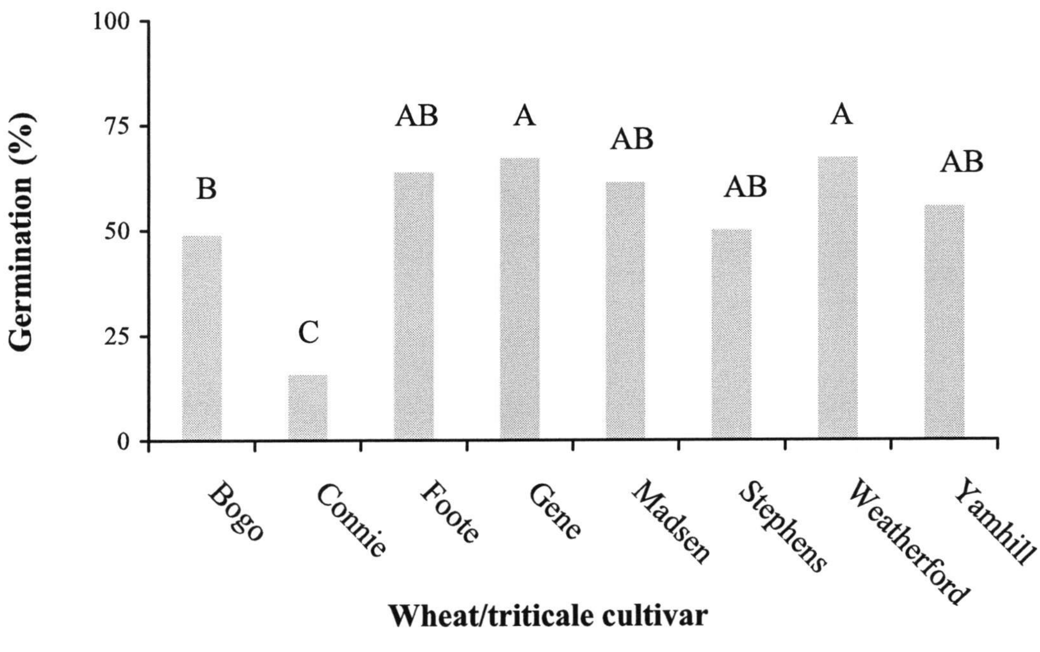

Figure 3.1. Small broomrape germination response (%) to common Pacific Northwest wheat/triticale cultivars in polyethylene bag experiments. Means with same letter are not significantly different at the $\alpha = 0.05$ level.

Greenhouse Experiments

Combined analysis was also used for the greenhouse experiments, as variance was homogeneous between experiments (P = 0.61). The number of small broomrape attachments per red clover plant differed among treatments (P < 0.0001, Figure 3.2). Red clover grown in soil where there was no previous wheat/triticale cultivar averaged 4.17 parasitic attachments per plant. All other false-host treatments were similar (LSD = 1.11) and red clover grown after the wheat cultivars of 'Connie,' 'Foote,' 'Gene,' 'Madsen,' 'Stephens,' and 'Weatherford' did not have any parasitic attachments.

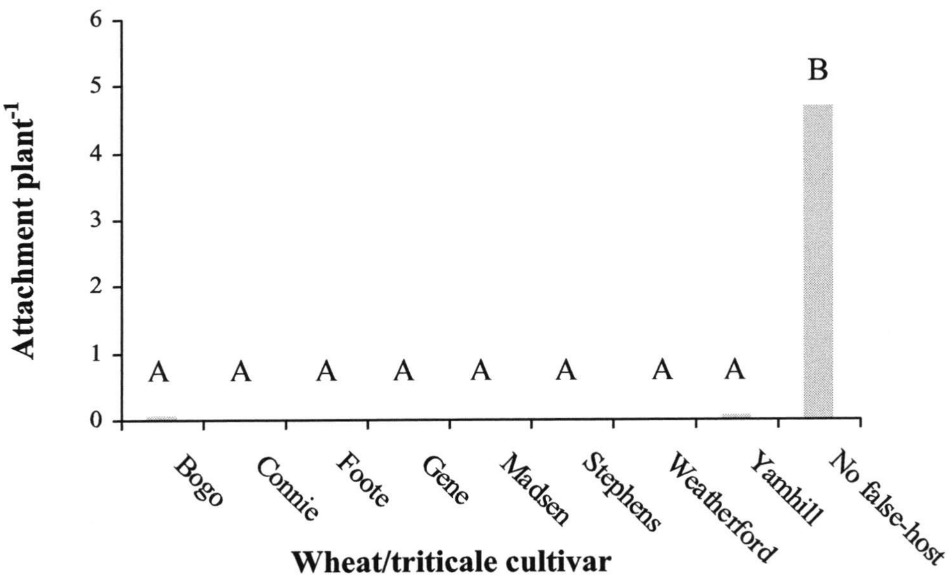

Figure 3.2. Small broomrape attachment per red clover plant grown in potting media after wheat/triticale treatments. Means with same letter are not significantly different at the α = 0.05 level.

Greenhouse experiment results indicated that wheat growth in small broomrape infested soil can greatly reduce the number of parasitic attachments on subsequently grown red clover. All tested false-host cultivars greatly reduced or completely inhibited parasitic attachment to red clover. This result suggested that false-hosts, such as wheat, could be used to deplete small broomrape in the soil seedbank. In the case of wheat, the benefit of the small broomrape soil seedbank depletion has an added economic benefit in the form of marketable wheat grain.

Small broomrape attachment per red clover plant did not differ among wheat/triticale cultivars in soil sampled from a small broomrape infested field (Figure 3.3). However, red clover plants grown in soil following a false-host treatment had fewer parasitic attachments than red clover plants in soil where no wheat or triticale were previously grown (P = 0.074).

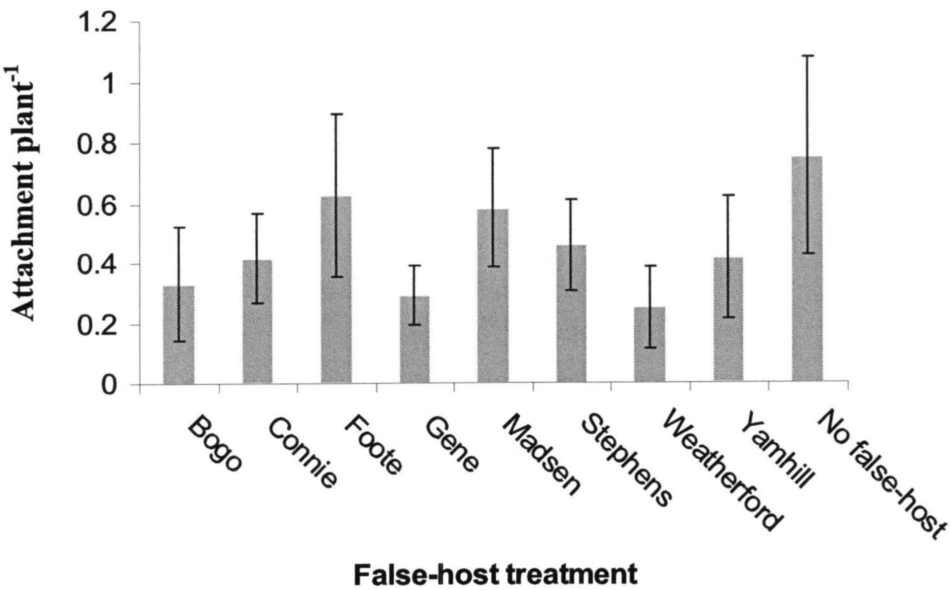

Figure 3.3. Small broomrape attachment per red clover plant grown in soil sampled from false-host field plots in 2003. Bars represent SE of the mean (n = 4).

Implications for Small Broomrape Management

This study demonstrated that the small broomrape soil seedbank can be reduced through the growth of common Pacific Northwest wheat/triticale cultivars and that false-host cultivars have varying degrees of parasitic seed germination potential. However, false-hosts crops cannot entirely deplete the small broomrape soil seedbank and potential weedy hosts may exist within non-host crop fields. With this in mind, integrated management of small broomrape must be utilized to broaden the focus of control strategies. These strategies must reduce the small broomrape soil seedbank and limit the growth of parasites on host plants. Recently, the herbicide imazamox was found to prevent both emergence and seed production when applied to parasitized red clover before small broomrape soil emergence (Eizenberg et al. 2004a). Additionally, imazamox controlled up to 75% of emerged small broomrape plants with minimal red clover injury when applied postemergence (Lins et al. 2004). With the introduction of imazamox-tolerant wheat, potential exists for rotation or intercropping of wheat with red clover. In this system weeds could be controlled in both crops (including small broomrape), while reducing the small broomrape soil seedbank. Imazamox could be applied most effectively to known small broomrape infestations in red clover fields using the predictive temperature-based small broomrape growth model developed by Eizenberg et al. (2004b). Management of small broomrape early in its lifecycle is critical for the prevention of small broomrape seed production and red clover seed yield reduction. The use of this model for imazamox application timing, in combination with wheat as a false host, would limit the early stages of parasite attachment and growth.

SOURCES OF MATERIALS

[1] Whatman® glass microfiber filter, grade 934-AH, Whatman Inc., 9 Bridewell Place, Clifton, NJ 07014.

[2] Sunshine mix #1 potting mix, Sun Gro Horticulture, Inc., 110th Avenue NE, Suite 490, Bellevue, WA 98004.

[3] Sun System III lighting systems with 430-watt metal halide bulb, Sunlight Supply, 5408 Northeast 88th Street, Vancouver, WA 98665.

[4] Miracle Gro® water soluble all-purpose plant food (15:30:15), Miracle Gro®, P.O. Box 606, Maysville, OH 43040.

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CHAPTER 4

Effect of Small Broomrape (*Orobanche minor*) on Dry Matter Production and Partitioning in Red Clover (*Trifolium pratense*) Seed Production

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ABSTRACT

Small broomrape is a parasite of several broadleaf plant species. Consequences of small broomrape infestation in host cropping systems include seed contamination, reduction in crop seed yield, and host plant death. The effect of small broomrape parasitism on the biomass partitioning of its primary host, red clover, has not been previously documented. In 2003 and 2004, a greenhouse experiment was conducted to determine the relationship between small broomrape and red clover biomass accumulation. Total biomass of parasitized red clover plants and their attached small broomrape was 40% less than the total biomass of non-parasitized red clover plants. Small broomrape parasitism reduced the amount of dry matter allocated to red clover inflorescences by 70%. Small broomrape dry matter accumulation was strongly related to total red clover-small broomrape dry matter accumulation. Small broomrape emergence date was a poor indicator of relative small broomrape dry weight accumulation per red clover plant. Results of this study, such as the quantification of small broomrape damage to red clover following forage harvest, have implications for small broomrape management in red clover seed production.

Nomenclature: Red clover, *Trifolium pratense* L. TRFPR; Small broomrape, *Orobanche minor* Sm. ORAMI.

Key words: Biomass partitioning, parasitic plant.

INTRODUCTION

Plants of the genus *Orobanche* (the broomrapes) are obligate, chlorophyll-lacking root parasites that rely completely on host plants for nutrients and water (Parker and Riches 1993). Several broomrape species are important agricultural weeds and can parasitize crops from the Apiaceae, Asteraceae, Brassicaceae, Cucubitaceae, Fabaceae, and Solanaceae plant families (Goldwasser et al. 1997; Parker and Riches 1993; Romanova et al. 2001; Ross et al. 2004). Broomrape species are responsible for substantial yield loss and greatly limit agricultural production in the Mediterranean and West Asia because of their wide host range and mode of resource acquisition (Linke 1992; Oerke et al. 1994; Parker and Riches 1993; Sauerborn 1991).

Several studies have investigated dry matter partitioning and solute fluxes within host-parasite associations. Among these studies there is general agreement that broomrape species compete strongly with resource sinks within host plants and can cause significant changes in host plant allometry (Barker et al. 1996; Hibberd et al. 1998). Changes in allometry can drastically reduce yield in seed crops, particularly when parasitic and reproductive sinks are in direct competition for host plant resources. Manschadi et al. (1996), for example, reported complete seed yield loss when crenate broomrape (*Orobanche crenata* Forsk.) bud stage coincided with the onset of flowering in faba bean (*Vicia faba* L.).

Recently, small broomrape has become a concern for agricultural producers in the moderate climate of the Willamette Valley in Oregon. Small broomrape infestations can contaminate seed and forage harvests, reduce host crop yield, and even cause host plant death (Colquhoun and Mallory-Smith 2001). In Oregon, the primary host crop of small broomrape is red clover, which is grown for seed production and as an important rotational crop. Although many plant species have been reported be to small broomrape hosts, red clover is unique in that it is the only seed crop in the United States of America to be largely affected by this parasite (Colquhoun et al. 2001). Therefore, it is critical to examine the influence of small broomrape parasitism on red clover growth, with particular consideration to resource allocation within the host-parasite complex.

In Oregon, red clover that is grown for seed is typically planted in September or October. After winter and early spring (November to April) rainfall, a forage harvest is performed in May to remove superfluous vegetative growth and synchronize flowering within seed production fields. Red clover seed is harvested approximately 3 months later in August. This cropping system is ideal for the life cycle of small broomrape, as parasites attach to red clover plants in March when soil moisture is abundant (Eizenberg et al. 2005). Small broomrape shoots emerge approximately 2 months after attachment to host plants (Eizenberg et al. 2005), typically after the red clover forage harvest. Two negative consequences result from harvesting red clover foliage from parasitized plants. Red clover plants must re-establish a large portion of their photosynthetic surfaces while significant resources are diverted to small broomrape attachments. Lack of red clover re-growth can be detrimental to red clover seed yield, as the ability of red clover to flower after forage removal has been found to greatly affect seed yield (Steiner et al. 1995). The second consequence is that small broomrape growth often increases when red clover is injured or wounded (personal observation). This response may be the result of an increase in soil temperature or from ethylene production, which Zehhar et al.

(2002) reported to be capable of inducing germination in branched broomrape (*Orobanche ramosa* L.).

Current knowledge is limited on the complicated interactions between small broomrape and red clover grown for seed production. Therefore, the objective of this study was to determine the influence of small broomrape growth on the biomass accumulation and partitioning of red clover when host plants were managed with typical red clover seed production practices. This research will increase our biological understanding of this weed and lead to subsequent management tools.

MATERIALS AND METHODS

In 2003 and 2004, a greenhouse experiment was conducted in a randomized complete block design with three replications blocked in time. Each replication consisted of 80 red clover (cv. 'Kenland'[1]) plants (subsamples) that were established in individual 16 L pots with and without small broomrape seed. In each replication, small broomrape seed was placed in the root zone of 72 red clover plants to provide representative data for a highly variable parasite-host association and to produce sufficient data points for regression analysis. Eight non-parasitized red clover plants were grown in soil that did not contain small broomrape seed for each replication. Pots that included small broomrape seed were half-filled with 8 L of potting mix[2], layered with 6 L of small broomrape inoculated potting mix (approximately 60 mg of small broomrape seed), and covered with 2 L of potting mix. Small broomrape seed were collected from the inflorescences of small broomrape plants infesting an Oregon red clover seed production field in 2002. After planting, all pots were placed in a greenhouse where the temperature was approximately 22 C and light[3] was provided 12 h per day. Soil in pots was kept moist and watered as needed. Prior to initial flower, red clover plants were cut to approximately 5 cm from the soil surface, simulating a forage harvest commonly practiced by red clover growers. Forage subsamples were dried at 70 C for 72 h and weighed. Following red clover re-growth and full bloom, red clover inflorescence, roots, shoots and leaves, and attached small broomrape were separated, dried at 70 C for 72 h, and weighed. Forage harvest and final harvest timing were performed according to the non-parasitized red clover growth stage for each replication. Initial small broomrape stalk emergence was recorded for each pot.

Data were transformed to represent a percentage of the total red clover-small broomrape complex dry weight for red clover inflorescences, roots, shoots and leaves, and attached small broomrape. Ratios of red clover shoot (stem, leaf, and reproductive tissue) to root dry matter (shoot:root ratio), reproductive to above ground dry matter (reproductive index), and complex dry matter to day after planting (relative growth rate) also were calculated. Data were subjected to analysis using PROC MIXED for ANOVA and PROC REG for regression in SAS (SAS 1999). To test for legitimate combination of regression lines among blocks, 95% confidence intervals (Devore and Peck 1993) were used to compare slope and y-intercept.

RESULTS AND DISCUSSION

Effect of Small Broomrape on Red Clover Growth and Productivity

Small broomrape infection influenced several measures of red clover growth and productivity (Table 1); however, the large variability in this host-parasite complex was evident. The shoot:root ratio was not affected by parasitism, although the ratio of the parasitized plants was almost 35% greater. This result is similar to Hibberd et al. (1998), who found no difference in shoot to root quotient between control tobacco plants and plants parasitized by nodding broomrape (*Orobanche cernua* Loeffl.). Also similar to Hibberd et al.'s (1998) results, in this experiment the shoot:root ratio was significantly lower ($P < 0.001$) for parasitized red clover plants when parasite biomass was included with root dry matter (1.5 g g^{-1}). This result indicated that small broomrape caused a substantial change in red clover dry matter partitioning independent of any developmental changes. The reproductive index between parasitized and non-parasitized red clover also was not significant at the $\alpha = 0.05$ level, even though the index for parasitized plants was less than half of non-parasitized plants.

Table 4.1. Growth and productivity measurements for parasitized and non-parasitized red clover plants.

			Dry matter			Relative growth rate		
Treatment	Shoot root $^{-1}$ [a]	Reproductive index[b]	Forage cutting[c]	Red clover	Total complex	Forage cutting	Red clover	Total complex
	------------g g^{-1}----------		-------------g $plant^{-1}$-----------			---g dry matter $plant^{-1}$ day^{-1}---		
Parasitized	5.4	0.18	15.5	50.4	65.4	0.3	0.3	0.5
Non-parasitized	8.4	0.41	12.8	107.7	107.7	0.2	0.7	0.7
P-value	0.127	0.097	0.462	0.009	0.019	0.457	0.023	0.052

[a] Ratio of red clover above ground dry matter to root dry matter.
[b] Ratio of red clover inflorescence dry matter to above ground red clover dry matter at final harvest.
[c] Red clover shoot dry matter from forage cutting.

Neither dry matter nor relative growth rate differed between parasitized and non-parasitized red clover plants for the forage cutting, indicating no effect of parasitism on early above ground red clover growth. Similar results have been reported for other broomrape-crop associations (Barker et al. 1996; Hibberd et al. 1998; Manschadi et al. 1996). However, the effect of parasitism was evident in both red clover and total complex dry matter and relative growth rate. In each case, dry matter and relative growth of parasitized red clover plants were approximately half of non-parasitized plants, suggesting that small broomrape parasitism was responsible for a tremendous reduction in the ability of red clover to produce dry matter. These results are in stark contrast with the results of other broomrape-crop association studies. Barker et al. (1996) and Hibberd et al. (1998) reported dry matter reductions for host plant biomass in parasitized plants, but not when host plant dry matter included the parasite dry matter. Thus, in those two systems dry matter productivity was maintained at the level of non-parasitized plants and partitioning was shifted to include parasite growth. The authors attributed productivity maintenance in those parasitized systems to sustained production of leaf area, greater leaf area ratio, increased specific leaf area, and delayed leaf senescence; however, forage was not harvested in these crops. In our cropping system, the red clover forage harvest removes most of early leaf growth, forcing red clover plants to produce new leaves while effectively decreasing the leaf area and eliminating the delay in leaf senescence.

Effect of Small Broomrape on Red Clover Dry Matter Partitioning

A strong linear relationship was observed between small broomrape dry weight and total red clover-small broomrape complex dry matter accumulation, suggesting that small broomrape partitioned a set percentage of dry matter within the host-parasite complex regardless of host size (Figure 4.1). This result was particularly interesting because the amount of red clover dry matter partitioned to small broomrape does not appear to be parasite density-dependant (see Appendix, Figure A.1), and perhaps may be regulated to maintain survival of the host plant.

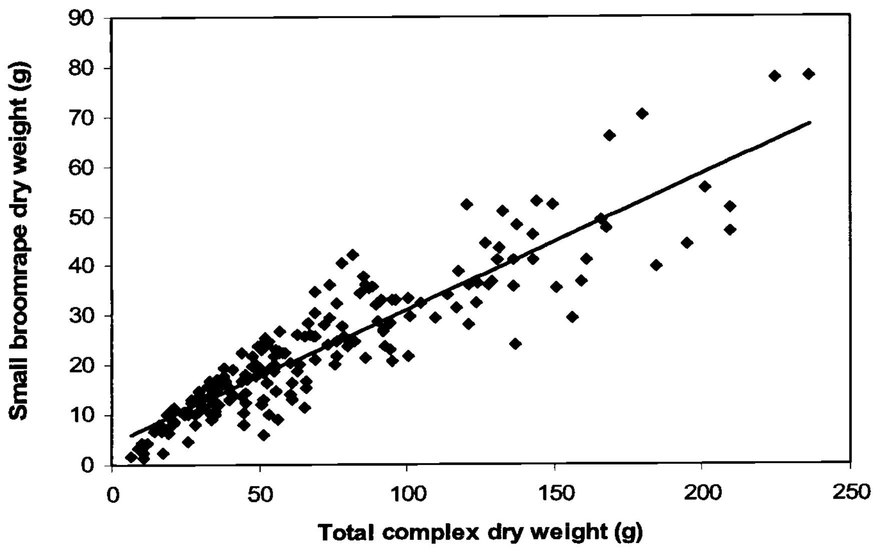

Figure 4.1. Relationship between small broomrape dry weight and total red clover-small broomrape complex dry weight. $Y = 3.8 + 0.28X$; $R^2 = 0.84$; $P < 0.0001$

Additionally, small broomrape parasitism caused differences in dry matter partitioning among red clover tissues (Figure 4.2). This is most evident in the relative amount of dry matter partitioned to the economically important red clover reproductive tissues, which were reduced by 70% as a result of small broomrape parasitism. Neither relative root nor shoot and leaf biomass were affected by parasitism; however, relative small broomrape dry matter averaged 35% of the host-parasite complex. Given that the calculated relative small broomrape dry matter and the slope of the regression analysis were similar (within 7%), it is clear that approximately 30% of the total complex dry matter was partitioned to small broomrape in this experiment.

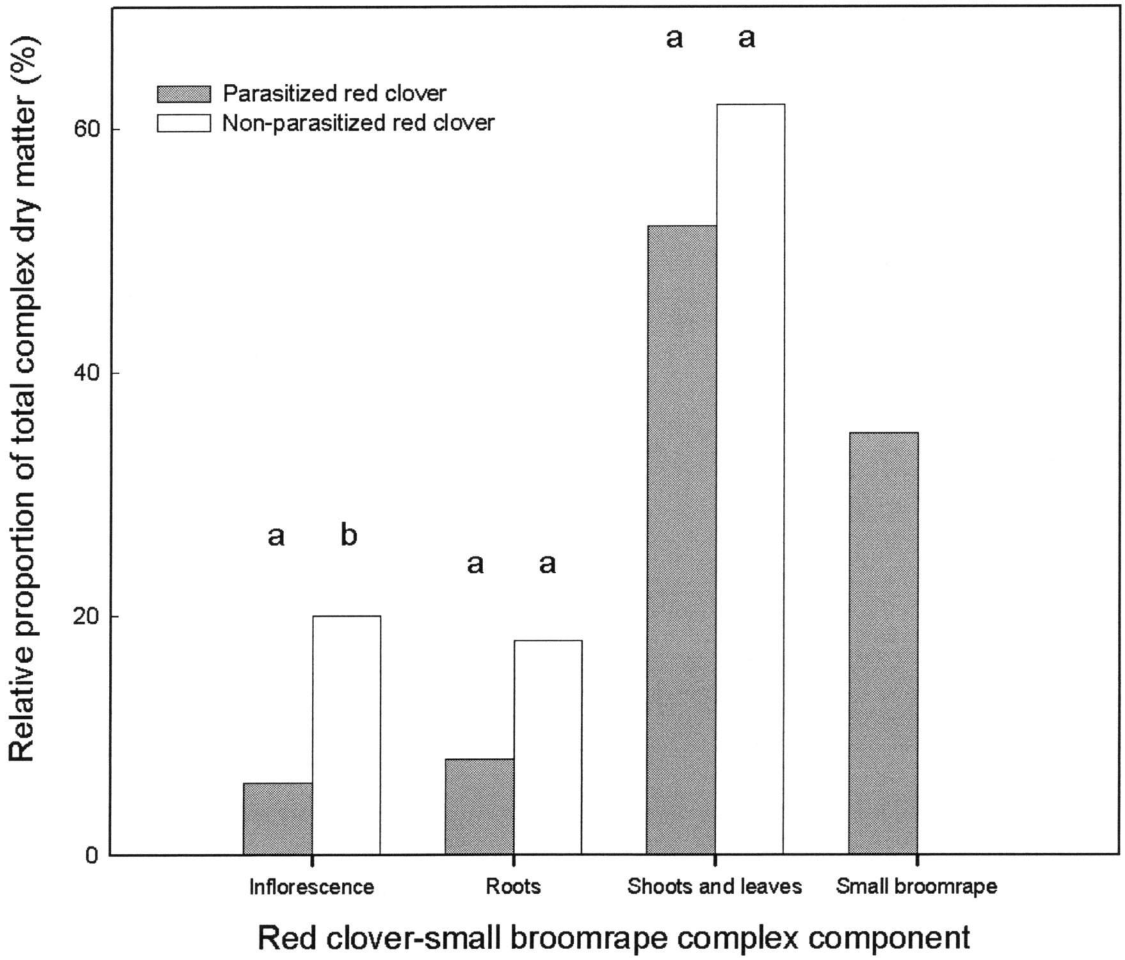

Figure 4.2. Dry matter partitioning among red clover inflorescence, roots, shoots and leaves, and small broomrape expressed as a percentage of the total complex dry weight. Means with the same letter are not significantly different at the $\alpha = 0.05$ level.

Lastly, a scatter plot was constructed to investigate the relationship between small broomrape soil emergence date and relative small broomrape dry matter accumulation (Figure 3). There was no relationship between those two factors, suggesting that crop damage can occur prior to small broomrape shoot emergence in red clover.

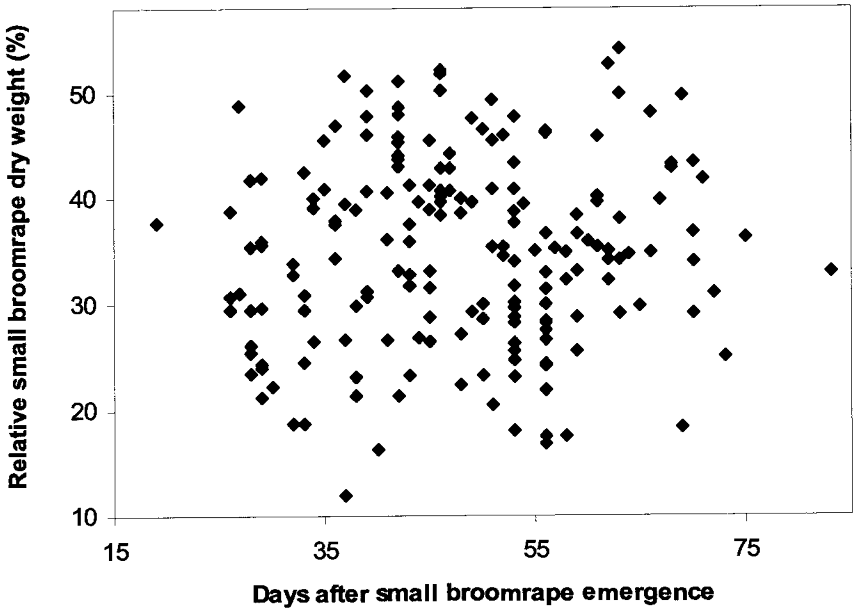

Figure 4.3. Scatter plot of small broomrape dry weight, relative to total complex dry weight, and days after small broomrape soil emergence.

Implications for Small Broomrape Management

Significant red clover dry matter loss due to small broomrape parasitism occurs after forage harvest. While this forage harvest may be devastating to parasitized red clover, through a combination of parasite stimulation and leaf loss, it may provide an excellent opportunity to maximize the effectiveness of an herbicide application.

Eizenberg et al. (2005) developed and validated a growing degree day-based model for small broomrape development in Oregon. This model predicts small broomrape emergence soon after a red clover grower would typically perform a forage harvest. Therefore, a red clover grower could apply an herbicide, such as imazamox (Lins et al. 2005, Colquhoun et al. 2005), after a forage harvest when the majority of the parasites are attached to the red clover plants, but not emerged from the soil. Red clover plants would also be actively re-growing and be able to translocate the herbicide to small broomrape attachments. Imazamox application to small broomrape before soil emergence will provide effective control (up to 100%) and prevent viable small broomrape seed production (Colquhoun and Mallory-Smith 2001).

SOURCES OF MATERIALS

[1]Tangent Seed Lab Int'l, 33731 Highway 99E, P.O. Box 331, Tangent OR, 97389.

[2] Sunshine Mix #1 potting mix, Sun Gro Horticulture, Inc., 110th Avenue NE, Suite 490, Bellevue, WA 98004.

[3] Sun System III lighting systems with 430-watt metal halide bulb, Sunlight Supply, 408 Northeast 88th Street, Vancouver, WA 98665.

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CHAPTER 5

Red Clover (*Trifolium pratense*) Establishment with Winter Wheat for Small Broomrape (*Orobanche minor*) Management

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ABSTRACT

Small broomrape seed germination is stimulated in the presence of wheat, but the parasite senesces prior to host attachment. Therefore, wheat has the potential to be utilized as a cultural control method to reduce the small broomrape soil seedbank in affected sites, while producing a marketable commodity. The objective of this research was to evaluate red clover establishment when intercropped with winter wheat in a small broomrape management system. Red clover was grown in monocrop, fall intercropped with wheat, and spring interseeded into wheat grown in narrow and wide rows. Spring (February, March, and April) seeding red clover into winter wheat avoids parasite-host contact in small broomrape infested fields. However, red clover that was spring interseeded into wheat produced minimal dry matter and ground cover by the following summer, regardless of wheat row spacing. Red clover fall intercropped with wheat produced enough ground cover for stand retention in one of two sites. Wheat yield was not affected by row spacing or red clover competition. Fall intercropping red clover with wheat produced wheat grain yield equivalent to monocrop wheat and an established red clover stand in the summer following wheat harvest. This red clover-wheat intercropping system can be combined with herbicides in an integrated small broomrape management plan.

Nomenclature: ORAMI; red clover, *Trifolium pratense* L.; winter wheat, *Triticum aestivum* L.; Small broomrape, *Orobanche minor* Sm.

Key words: Intercropping, integrated weed management.

INTRODUCTION

Small broomrape is a parasitic weed that presents a significant threat to the future of red clover seed production in Oregon (Colquhoun et al. 2001). Red clover is a valuable commodity in Oregon (approximately $8 million annually) and is an important rotational crop in grass seed production systems. Since 1998 after identification on a single Oregon red clover seed production farm, the number of infestations has increased to 15 in 2000 and 22 in 2001. Small broomrape reduces host crop yield through the disruption of nutrient and water transport in host plants (Parker and Riches 1993), and contaminates seed crops by producing up to 1 million dust-like seeds per plant (Pieterse 1979). Small broomrape is a federally prohibited noxious weed that is restricted from export as a seed contaminant. In addition to red clover, small broomrape parasitizes other members of the legume family, as well as several other crop and weedy plant species in Oregon (Ross et al. 2004).

Management of this parasite in red clover seed production is difficult given the reproductive capabilities of small broomrape. Herbicides control small broomrape attached to red clover (Colquhoun and Mallory-Smith 2001; Lins et al. 2005a), but cultural practices such as false-host intercropping may lead to effective integrated small broomrape management systems. False-host plant species stimulate parasitic plant seed germination with death prior to host plant attachment. False-hosts differ from host plants in that false-host species release exudates that only promote parasitic seed germination and not attachment. Broomrape (*Orobanche spp.*) false-hosts have been identified and can be used to deplete parasites from the soil seedbank (Hershenhorn et al. 1996; Lins et al. 2005b). Parker and Riches (1993) reported sorghum (*Sorghum* *vulgare* Pers.*)*, corn (*Zea mays* L.), mung bean (*Phaseolus aureus* Roxb.), and cucumber (*Cucumis sativus* L.) as false hosts for branched broomrape (*Orobanche ramosa* L.), while similar results were reported with sweet pepper (*Capsicum anuum* L.) as a false host for Egyptian broomrape (*Orobanche aegypiaca* Pers.) (Bischof and Koch 1974; Hershenhorn et al. 1996). Research by Lins et al. (2005b) identified wheat to be an effective false-host for small broomrape. The small broomrape soil seedbank could be reduced in infested red clover fields by incorporating wheat into red clover seed production.

In western Oregon, small broomrape attaches to red clover plants from late February to late March (Eizenberg et al. 2005). This is the optimal time for a false-host, such as winter wheat, to reduce the parasitic soil seedbank. However, the potential for small broomrape parasitism would increase for red clover plants that are present in the wheat crop during this time. Delaying host crop planting past the optimum time of broomrape attachment is an effective management option to reduce the likelihood of parasitism (Cubero and Moreno 1979). Thus, small broomrape parasitism could be reduced by seeding red clover into a winter wheat crop in the spring (February to April) to avoid parasite-host contact.

Forage legume establishment with a grass hay crop to increase the amount of forage harvested in the first cutting and to suppress weeds has long been a common practice (Peters 1961). Additionally, Fairey and Lefkovitch (1991), Gomm et al. (1976), and Steiner and Snelling (1994) reported legume seed crop establishment with annual hay and cereal crops. Intercropping in these systems is cost effective to legume seed growers because it allows for the harvest of hay or grain in the first year of legume establishment when seed production is minimal. However, no research has been conducted on the agronomic aspects of a red clover-winter wheat intercropping system, particularly with the secondary purpose of managing a small broomrape infestation. The agronomic success of a red clover-wheat intercropping system may offer red clover growers a practical option for integrated control of small broomrape, while producing wheat grain to offset costs in the red clover establishment year. Therefore, the objective of this research was to investigate the agronomic feasibility of a red clover-winter wheat intercropping system with regard to wheat yield and red clover establishment.

MATERIALS AND METHODS

In 2003, two field experiments were established at the Hyslop Research Farm near Corvallis, OR. Experiments used red clover (cv. Kenland), oat (cv. Cayuse) and winter wheat (cv. Foote) to compare wheat yield and red clover establishment among intercropping systems. Experimental design was a randomized complete block with ten treatments, four replications, and a plot size of 2.4 by 12.2 m. Treatments included red clover monocropped in 30 cm rows, wheat monocropped in 15 cm rows (conventional wheat system), a common red clover establishment system of red clover broadcast-seeded into 30 cm oat rows at time of planting, red clover broadcast into 15 cm wheat rows at time of planting, and red clover spring-broadcast (February, March, April) into fall-planted 15 and 30 cm wheat rows. The 15 cm wheat row width was chosen for the fall red clover interseeding treatment to maximize wheat yield and the release of small broomrape germination exudates. This treatment was also included with the intention of a spring imazamox application to an imazamox-tolerate wheat crop for small broomrape control. Seeding rates were 9, 90, 70, and 140 kg ha^{-1} for red clover, oat, and narrow and wide row wheat, respectively. Red clover seed was inoculated with the appropriate *Rhizobium* spp. prior to planting.

For both experiments, oat, wheat, and fall interseeded red clover were planted on October 14, 2003 in a Woodburn silt loam (fine-silty, mixed, mesic Aquultic Argixeroll) with a pH of 5.6 and 2.7% organic matter. Plot areas were mold-board plowed the previous spring, summer fallowed, fall disked, and then spring disked and harrowed to prepare a suitable seedbed. Additionally, plot areas were fertilized prior to planting with 54 kg ha^{-1} of N, P, and K and supplemented with 90 kg ha^{-1} of N on March 22, 2004. Spring red clover interseeding dates were February 18, March 15, and April 19, 2004.

Treatments were grown with conventional best management practices. Monocrop and spring-interseeded wheat treatments received a preemergence application of flufenacet and metribuzin at 0.35 and 0.09 kg ai ha^{-1}, respectively, for weed control. Pyridate (1.05 kg ai ha^{-1}) and clethodim (0.14 kg ai ha^{-1}) were applied to red clover monoculture during the first year for weed control. Weed species included annual bluegrass (*Poa annua* L.), persian speedwell (*Veronica persica* Poir.), shepherd's-purse (*Capsella bursa-pastoris* (L.) Medic.), and spiny sowthistle (*Sonchus asper* (L.) Hill). Azoxystrobin (0.15 kg ai ha^{-1}) was applied twice for wheat stripe rust (*Puccinia striiformis*) prevention.

Red clover monocrop and red clover-oat treatments were harvested for forage at 25% red clover stand flowering on May 12, 2004, leaving a stubble height of approximately 5 cm. Clethodim (0.14 kg ai ha^{-1}) was applied to red clover-oat treatments to prevent oat re-growth. Wheat grain was harvested on July, 23 2004. Wheat stubble was flail chopped and removed. Clethodim (0.14 kg ai ha^{-1}) was applied to the experimental area to control volunteer wheat sprout and grass weeds on September 9, 2004.

Cereal crops, red clover, and weed dry matter were sampled (0.25 m) on April 10 and June 24, 2004 for Site 1 and on April 24 and June 24, 2004 for Site 2. Samples were dried at 70 C for 72 h and weighed. Photosynthetic photon flux density (PPFD) ($\mu mol\ m^{-2}\ s^{-1}$) was measured at the top of the cereal crop canopy and at the top of the interseeded red clover understory with a line quantum sensor[1] to determine the percentage of photosynthetically active radiation (PAR) reaching interseeded red clover for each treatment. PPFD was measured in three random sites in each plot and the sensor was positioned perpendicular to the cereal rows and parallel to the ground. The PPFD was quantified December 3, 2003, and January 22, March 2, April 10, and June 1, 2004, on clear sunny days within 2 h of noon. Second year red clover establishment was determined by placing a transect down the middle of each plot and calculating percent red clover ground cover on March 25, 2005. A predetermined level of 70% red clover ground cover was used to designate a successfully established red clover seed production stand. This percentage was based on a typical stand retention threshold used by local red clover growers (G. Gingrich, personal communication).

Data were subjected to analysis of variance using PROC GLM in SAS (SAS 1999). Data between sites were not combined for analysis because of differences in crop growth. Fisher's protected LSD was used to separate treatment means, while contrasts were used to make comparisons between grouped cropping systems.

RESULTS AND DISCUSSION

Intercrop Dry Matter and Light Measurements

Cereal (oat and wheat) dry matter differed among cropping systems for the April sampling date at Site 2, but not at Site 1 (Table 5.1). The conflicting results from the two sites can be attributed to differences in oat and red clover growth between sites. Oat at Site 1 was highly vigorous and intercepted a greater percentage of PAR than wheat treatments through the first two light sampling dates (Table 5.2). Oat at Site 2 was less vigorous and allowed as much or more relative PAR to reach interseeded red clover than wheat interseeded treatments. Generally, fall-seeded red clover was more vigorous at Site 2 as compared to Site 1, thus increasing resource competition at Site 2. However, red clover competition did not affect wheat dry matter accumulation at either site for the June sampling date. Wheat dry matter did not differ among wheat treatments when oat was removed from the analysis at the later sampling date. Additionally, wheat dry matter from the fall interseeded wheat and the wheat monocrop did not differ at any site or sampling date. Row spacing also did not affect wheat dry matter between narrow (15 cm) and wide (30 cm) spring interseeded wheat at the June sampling date for Site 2. Wide row wheat was able to accumulate as much dry matter as narrow row wheat even with fewer plants per area.

Red clover dry matter differed among cropping systems at both sites and sampling dates. The red clover monocrop produced considerably greater red clover dry matter in both sampling dates than all other treatments at Site 1; however by the June sampling date at Site 2, red clover dry matter did not differ between the red clover monocrop and the oat intercrop. As mentioned previously, this was the result of relatively poor oat growth and competitive red clover at Site 2. At the April light sampling, PAR at the red clover canopy in the oat intercrop and fall wheat intercrop was 22 and 56% greater respectively, at Site 2 compared to Site 1. While red clover was competitive in fall interseeded treatments at Site 2 in the June sampling, spring seeded red clover dry matter was negligible at both sites and wheat row spacings. Red clover dry matter produced in wide-row wheat was insignificant even though these plants received a greater proportion of PAR than red clover in narrow row wheat.

Weed dry matter in monocropped wheat and all red clover-wheat intercrop systems was greatly reduced compared to the red clover monocrop and red clover-oat intercrop system on the April sample date. This result was principally due to the high efficacy of the preemergence herbicide application that was applied to the monocroped and spring interseeded wheat. However, herbicide was not applied to fall interseeded wheat and weed dry matter was still suppressed better than the red clover-oat intercrop at both sites in the April sampling date. There were no differences between red-clover oat intercrop weed dry matter and the systems that included wheat by the June sampling date, but the red clover-oat intercrop had been harvested for forage and treated with a graminicide after cutting to prevent oat re-growth.

Table 5.1. Cereal, red clover, and weed dry matter for red clover monocrop, wheat monocrop and red clover-cereal intercrops.

	Dry matter											
	Site 1 sample date						Site 2 sample date					
	April 10			June 24			April 28			June 24		
Cropping system	Cereal[a]	Red clover	Weeds	Wheat	Red clover	Weeds	Cereal	Red clover	Weeds	Wheat	Red clover	Weeds
	kg ha^{-1}											
Red clover monocrop	-	66.7	30.5	-	228.7	161.5	-	95.5	11.3	-	322.9	131.9
Wheat monocrop	605.0	-	0.0	2443.5	-	5.4	648.8	-	7.0	2626.5	-	2.7
Red clover-oat intercrop	611.1	26.4	35.5	-	121.1	5.4	417.4	55.7	51.4	-	341.8	0.0
Fall red clover-wheat intercrop	631.3	13.7	10.2	2650.7	43.1	2.7	740.9	44.1	28.3	2855.2	105.0	0.0
February red clover-wheat (15 cm)	683.3	1.1	1.1	2790.6	8.1	2.7	737.6	0.5	12.1	2572.7	8.1	2.7
March red clover-wheat (15 cm)	708.3	0.8	0.0	2941.3	8.1	2.7	799.8	0.8	8.6	2419.3	8.0	0.0
April red clover-wheat (15 cm)	680.0	0.0	0.0	3054.4	5.4	16.2	721.2	0.0	8.1	2715.3	8.1	2.7
February red clover-wheat (30 cm)	546.3	0.5	0.0	1854.1	10.8	5.4	593.1	0.8	32.6	2088.3	10.8	8.1
March red clover-wheat (30 cm)	478.7	1.1	2.4	2147.5	10.8	8.1	571.9	0.5	28.5	2440.8	10.8	0.0
April red clover-wheat (30 cm)	548.7	0.0	0.8	2451.6	10.8	2.7	578.3	0.0	21.5	2188.3	10.7	5.4
LSD 0.05	NS	14.2	14.0	NS	59.1	24.8	149.7	24.4	16.4	NS	57.2	58.9
Contrasts[b]												
Spring intercrop row width	***	NS	*	**	NS	NS	***	NS	***	NS	NS	NS
Fall vs. spring wheat intercrop	NS	**	NS	NS	NS	NS	NS	***	NS	NS	***	NS

[a] Cereal columns include oat and wheat dry matter.

[b] Contrasts significant at the P = 0.1, 0.01, and 0.001 level are indicated with *, **, ***, respectively. Non-significant comparisons are indicated with NS.

Table 5.2. Relative level of photosynthetically active radiation (PAR) at the top of the red clover subcanopy within oat and wheat intercrops.

	PAR at top of red clover subcanopy									
	Site 1 sample date					Site 2 sample date				
Cropping system	December	January	February	April	May	December	January	February	April	May
	% of available PAR									
Red clover-oat intercrop	90	70	35	10	17	95	81	84	66	85
Fall red clover-wheat intercrop	95	77	27	5	7	97	92	52	27	28
February red clover-wheat (15 cm)	94	91	37	5	2	97	94	76	10	5
March red clover-wheat (15 cm)	95	91	38	5	3	98	82	62	9	5
April red clover-wheat (15 cm)	95	93	37	6	4	97	93	65	8	4
February red clover-wheat (30 cm)	97	98	55	13	7	97	96	87	25	13
March red clover-wheat (30 cm)	99	95	50	13	7	99	98	81	21	12
April red clover-wheat (30 cm)	96	97	55	11	5	98	96	84	22	16
LSD 0.05	4	7	8	4	4	2	10	13	9	8
Contrasts[a]										
Spring intercrop row width	**	***	***	***	***	NS	**	***	***	***

[a] Contrasts significant at the P = 0.01 and 0.001 level are indicated with ** and *** respectively. Non-significant comparisons are indicated with NS.

Wheat Yield and Red Clover Establishment

Wheat grain yield was typical of 2004 winter wheat yields in the Willamette Valley, OR, and did not differ among cropping systems (Table 5.3). Wheat yield was similar between wheat row widths, monocropped versus intercropped wheat, and fall versus spring red clover interseeding timing. These results suggest that wheat yield from interseeded treatments was not affected by red clover growth compared to monocropped wheat, and that wide row wheat was able to compensate for a lower wheat plant density than narrow row wheat to produce an equivalent yield. Similar results were obtained by Steiner and Snelling (1994), who reported that winter wheat yields were not affected by intercropped kura clover (*Trifolium ambiguum* M. Bieb.) when compared to a wheat monocrop. Legumes typically have little effect on companion cereal crops because cereal crops germinate faster and compete more effectively for moisture and light (Fairey and Lefkovitch 1991, Smith et al. 1954). However, wide row yield compensation in our study was contrary to results reported by Steiner and Snelling (1994) where narrow-row (15 cm) wheat generally produced greater yield than wide-row (30 cm) wheat. This difference may be partially accounted for by our use of a wheat cultivar with greater capacity for yield component compensation, such as an increase in tiller number when plant density was reduced (Puckridge and Donald 1967).

Table 5.3. Effect of cropping system on wheat yield and red clover ground cover.

Cropping system	Wheat yield Site 1	Wheat yield Site 2	Red clover ground cover Site 1	Red clover ground cover Site 2
	------------ kg ha^{-1} ----------		------------- % --------------	
Red clover monocrop	-	-	98	100
Wheat monocrop	6642	6856	-	-
Red clover-oat intercrop	-	-	99	100
Fall red clover-wheat intercrop	6140	6495	13	77
February red clover-wheat (15 cm)	6024	6544	2	14
March red clover-wheat (15 cm)	6152	6165	0	0
April red clover-wheat (15 cm)	6342	6984	0	0
February red clover-wheat (30 cm)	5455	6330	43	39
March red clover-wheat (30 cm)	6097	6305	9	33
April red clover-wheat (30 cm)	6226	6250	0	0
LSD 0.05	NS	NS	19	20
Contrasts[a]				
Spring intercrop row width	NS	NS	***	***
Wheat mono vs. Intercrop wheat	NS	NS	-	-
Fall vs. spring intercrop	NS	NS	-	-

[a] Non-significant comparisons are indicated with NS.

The percentage of the ground covered by red clover was used as a measure to indicate stand establishment in the second spring following seeding (Table 5.3). At both sites red clover stand establishment was 98% or greater for the red clover monocrop and the red clover-oat intercrop. Conversely, red clover ground cover in red clover-wheat intercrops only exceeded 70% for one treatment between both sites. At Site 1, red clover ground cover was not sufficient for stand retention in any red clover-wheat intercrop treatment. At Site 2, only the fall-seeded red clover-wheat intercrop produced red clover ground cover sufficient for stand retention. Red clover ground cover was greater in wide rows as compared to narrow rows, even though red clover in spring interseeded treatments did not produce more than 43% ground cover in any case.

These experiments confirm that spring interseeding red clover into conventionally managed winter wheat is not agronomically viable for red clover establishment. However, our results do provide valuable insight to further the design of red clover seed production systems for small broomrape management. Sufficient red clover establishment was variable between sites for the fall interseeded red clover-wheat cropping system. With this in mind, such a system could be adjusted to increase the probability of successful red clover establishment. Fall seeding red clover into wider wheat rows, 30 cm for example, to provide red clover with greater relative PAR would be one option. Although this would likely reduce the amount and coverage of small broomrape germination stimulant released from wheat and increase small broomrape parasitism of interseeded red clover, it appears that wheat grain yield would not be reduced as a result of red clover competition. Additionally, imazamox-tolerant wheat could be integrated into this system, in which imazamox would be applied in March to late April in Oregon to control small broomrape attached to the red clover intercrop. While this system would require an imazamox application, the small broomrape soil seedbank in infested fields could be greatly reduced by the combination of false-host induced "suicidal germination" and herbicide activity on attached parasites. Another option may be to simply increase the seeding rate of red clover for the fall interseeded red clover-wheat system at the narrow wheat row spacing. This system would also need to incorporate the previously mentioned imazamox herbicide program. Further research is needed to evaluate both the agronomic and economic viability of these systems and to investigate implementation in small broomrape infested fields.

SOURCES OF MATERIALS

[1] LI-191SB. LICOR Inc., Environmental Division, 4421 Superior Street, Lincoln, NE 68504.

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CHAPTER 6

General Conclusion

These studies were conducted to gain a better understanding of small broomrape biology in Oregon red clover seed production, while exploring small broomrape management options. Our results revealed a number of biologically significant small broomrape growth characteristics that can be used to design integrated management systems in red clover seed production. The herbicide combination of imazamox plus bentazon applied to emerged small broomrape eliminated further emergence and reduced the density of emerged small broomrape in red clover, while maintaining crop safety. Red clover growers could use an imazamox plus bentazon application to limit small broomrape damage to their crop if it is detected after emergence. However, these herbicides did not prevent small broomrape seed production. Given the ability of small broomrape to produce large amounts of seed, the weed seed bank will likely increase even when herbicides are applied to emerged small broomrape. Additionally, considerable amounts of dry matter may have already been partitioned to small broomrape attachments by the time of soil emergence. An integrated management system is necessary that reduces the small broomrape soil seed bank and controls attached parasites prior to economic damage

Wheat caused a large proportion of small broomrape seed to germinate, thereby reducing the likelihood of parasitism on red clover plants. Therefore, growing wheat on infested sites would reduce the small broomrape soil seed bank. Our results also suggested that wheat and red clover could be successfully intercropped. With the addition of imazamox-resistant wheat, this system would truly be integrated; wheat would reduce the small broomrape soil seed bank through 'suicidal germination,' while a well-timed imazamox application would control parasites attached to interseeded red clover plants.

Future research on small broomrape management should investigate the following areas: 1) intercropping imazamox-resistant wheat and red clover on a small broomrape infested field site; and 2) locating the genes in wheat responsible for regulating the production of small broomrape germination stimulant.

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APPENDIX

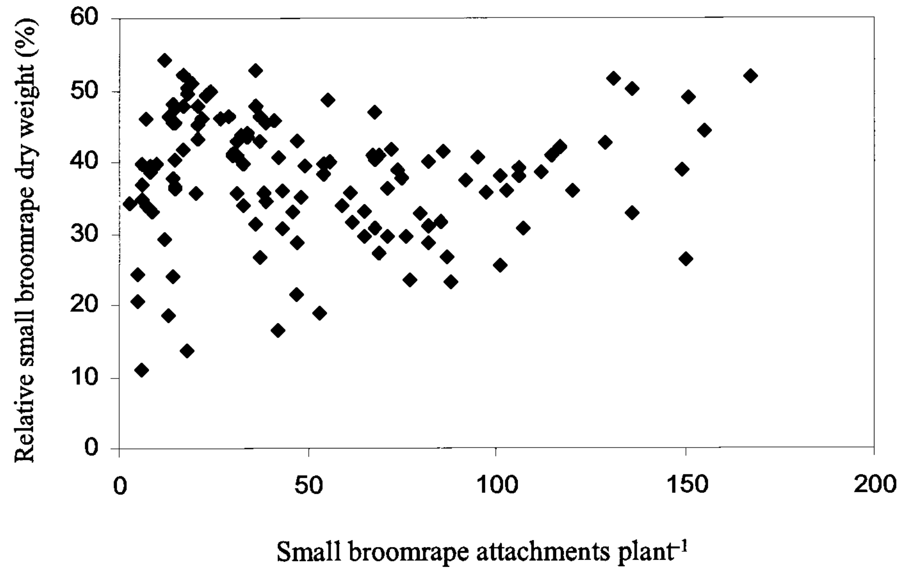

Figure A.1. Scatter plot of small broomrape dry weight, relative to total complex dry weight, and number of parasitic attachments per red clover plant.