AN ABSTRACT OF THE THESIS OF

Kyle C. Ross for the degree of Master of Science in Crop Science presented on March 4, 2003. Title: Integrated Small Broomrape (*Orobanche minor* Sm.) Management In Red Clover (*Trifolium pratense* L.).

Abstract approved

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Small broomrape, a holoparasitic weed, is a relatively new weed introduction in the Pacific Northwest that has contaminated a limited number of red clover fields in Oregon. Greenhouse and field studies were conducted to evaluate small broomrape response to common crop and weed species in the Pacific Northwest. Host species in the greenhouse or field study included alfalfa, arrowleaf clover, carrot, celery, common vetch, crimson clover, lettuce, prickly lettuce, red clover, spotted catsear, subterranean clover, white clover, and wild carrot. False-host species included barley, birdsfoot trefoil, creeping bentgrass, cucumber, field corn, fine fescue, flax, Italian ryegrass, nasturtium, oat, orchardgrass, perennial ryegrass, snap bean, sugar pea, sunflower, sweet corn, tall fescue, tomato, and wheat. Non-host species included sugar beet and curly dock. The greenhouse polyethylene bag system provided a rapid

and inexpensive screening for plant species host status to small broomrape. Germination and attachment to host roots are initiated by chemical exudates, that may change concentration in response to nutrient availability and microorganisms. Red clover was grown in varying concentrations of ammonium sulfate fertilizer with and without *Rhizobium* inoculation, and with small broomrape seeds. Neither *Rhizobium* inoculation nor ammonium concentration influenced the number of small broomrape attachments to red clover roots. A survey was conducted of red clover seed growers with small broomrape-contaminated fields in the Pacific Northwest. Red clover seed from six respondents were cleaned at the same cleaning facility, and the same respondents purchased their seed stock from this cleaning facility. Small broomrape was not identified in red clover fields prior to or during the first clover seed harvest of fall planted red clover in small broomrape-contaminated sites.

Integrated Small Broomrape (*Orobanche minor* Sm.) Management In Red Clover (*Trifolium pratense* L.).

by

Kyle C. Ross

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Kyle C. Ross, Author

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CONTRIBUTION OF AUTHORS

Dr. Carol A. Mallory-Smith and Dr. Jed B. Colquhoun guided in the design, data collection, analysis, data interpretation, and writing of each manuscript.

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1. INTRODUCTION

1.1 Introduction

Small broomrape (*Orobanche minor* Sm.) is a relatively new introduction in the Pacific Northwest and has contaminated limited acreage of the primary red clover (*Trifolium pratense* L.) seed stock production area of the world. Small broomrape is a federally listed noxious weed that is prohibited in interstate commerce. The Pacific Northwest produces a diverse array of crops shipped throughout the world for seed stock; therefore, preventing small broomrape from contaminating seed lots is vitally important for maintaining markets for Pacific Northwest agricultural products.

In 2001 there were 7,000 ha of red clover seed production in Oregon, with an $4.4 million annual seed value plus forage value (Young 2002). Oregon produces arrowleaf (*Trifolium vesiculosum* Savi.), crimson (*Trifolium incarnatum* L.), subterranean (*Trifolium subterraneum* L.), and white (*Trifolium repens* L.) clovers for seed in addition to red clover.

Cultural management of red clover for seed production varies among growers. Spring-seeded red clover intended for seed production is grown alone or as a companion crop with a small grain. Spring-seeded red clover is about 15 months old before the first seed harvest. Fall-seeded red clover planted alone is about 11 months old before the first seed harvest. Red clover seed crops are grown for up to two harvest cycles, with a forage harvest in late spring and a seed harvest in late summer of each harvest cycle. Spring forage harvest of red clover increases uniformity of blooming and provides greater economic return, while not decreasing seed yield. Red clover seed production adds nutrients to the soil and provides an opportunity to control weeds in a grass seed or wheat rotation.

In 1998, small broomrape was detected in a certified red clover seed field in Clackamas County, Oregon. The 1998 identification of small broomrape was the first observation of small broomrape in commercial agriculture in Oregon. The first time small broomrape was identified in Oregon was in 1923 near Portland (Oregon State University Herbarium 1923).

Small broomrape, (Orobanchaceae family) is an annual that reaches 15 to 50 cm in height (Rodriguez 1993). Flowers are self-pollinating in a terminal cluster (Rodriguez 1993). Flowering period is short, starting 1 week after emergence, with seed release beginning 1 month after emergence (Rodriguez 1993). Small broomrape is an obligate parasite, lacking chlorophyll, and obtaining all nutrients at the expense of the host (Rodriguez 1993). Nutrients are removed from the host to the small broomrape plant through a haustorium. An individual small broomrape plant produces over 1 million seeds (Pieterse 1979) that are 0.3 by 0.2 mm (Parker and Riches 1993). *Orobanche* spp. parasitize plants from Asteraceae (Romanova et al. 2001), Fabaceae (Goldwasser et al. 1997), and Solanaceae (Romanova et al. 2001) plant families. Major economic damage from small broomrape is restricted to the Fabaceae family and clover in particular (Miller et al. 1997).

Knowledge of small broomrape biology is essential for its management and prevention. Prevention is the primary and most effective management method for control of small broomrape in red clover.

1.2 Hosts, False-Hosts, and Non-Hosts

Plants are classified as hosts (parasitic seed germination and attachment to the host), false-hosts (parasitic seed germination and senescence prior to host attachment), or non-hosts (no parasitic seed germination or attachment) of small broomrape. Host and false-host plants produce chemical exudates that signal broomrape germination (Joel et al. 1995). Following germination, a second chemical signal is required for a haustorium to emerge and penetrate the host root (Lynn and Chang 1990). With false-hosts, the concentration of the exudates promoting haustorium initiation is not adequate to stimulate attachment after germination (Smith et al. 1990). Host plant roots are penetrated by the small broomrape haustoria through enzymatic action and mechanical pressure (Losner-Groshen et al. 1998).

Orobanche ramose L. is a parasite of some crucifers (Cruciferae), carrot (*Daucus carota* L.), celery (*Apium graveolens* L.), cotton (*Gossypium hirsutum* L.), hemps (*Cannabis* spp.), lettuce (*Lactuca sativa* L.), melons (*Cucumis* spp.), potato (*Solanum tuberosum* L.), safflower (*Carthamus tinctorius* L.), sunflower (*Helianthus annuus* L.), tobaccos (*Nicotiana* spp.), and tomato (*Lycopersicon esculentum* Mill.). Egyptian broomrape (*Orobanche aegyptiaca* Persoon) is a parasite of some crucifers, cotton, cucurbits (*Cucurbita* spp.), eggplant (*Solanum melongena* L.), potato, tobacco, and tomato. *Orobanche crenata* Forssk is a parasite of broadbean (*Vicia faba* L.), carrot, tomato, and pea (*Pisium sativa* L.). *Orobanche cumana* Wallr. is a parasite of eggplant, sunflower, tobacco, and tomato. *Orobache lutea* Baumgarten is a parasite of clovers (*Trifolium* spp.).

Small broomrape is a parasite of clovers and other species. Red clover (Yokota et al. 1998), carrot, English ivy (*Hedera helix* L.), geraniums (*Pelargonium* spp.), mock orange (*Philadelphus coronarius* L.), petunias (*Petunia* spp.) privets (*Ligustrum* spp.), spotted catsear (*Hypochaeris radicata* L.), tobacco, and white clover (Frost and Musselman 1980) are host plants to small broomrape. Root exudates from basils (*Ocimum* spp.), carrot, cucumber (*Cucumis sativa* L.), corn (*Zea mays* L.), onion (*Allium cepa* Linn.), and tomato-stimulated germination of small broomrape (Yoneyama et al. 2001); therefore are false-hosts. Research has not been conducted on the host status of many Pacific Northwest crop and weed species, or conducted in the climate and soil conditions of the Pacific Northwest. Typical crops produced in the Pacific Northwest include a wide array of crops from many plant families. Weed species also can assist in introduction and dispersal of small broomrape. Common weed species in the Pacific Northwest are also from a wide array of plant families.

The majority of methods aimed at controlling *Orobanche* spp. in host crops have failed (Goldwasser et al. 1997); therefore, *Orobanche* spp. must be controlled prior to growth of a host crop. False-host crops and resistant crops decreased *Orobanche crenata* Forsk. seedbank by 30% in one crop cycle (Linke et al. 1993). Longer life spans and root systems that are more extensive increased the effectiveness of a false-host crops ability to diminish *Orobanche* spp. from the soil seedbank.

1.3 Influence of Nutrients

Nitrogen (specifically ammonium nitrate) reduced branched broomrape (*Orobanche ramose*) seed germination in aseptic conditions (Abu Irmaileh 1994).

Ammonium in combination with potassium reduced Egyptian broomrape parasitism of tomato grown in potting media (Jain and Foy 1992). Under aseptic and hydroponic conditions, ammonium application decreased small broomrape parasitism of red clover (Sato et al. 2001; Westwood and Foy 1999), *Striga hermonthica* parasitism of grain (Cechin and Press 1993; Mumera and Below 1993), and Egyptian broomrape parasitism of tomato (Jain and Foy 1992).

The ammonium form of nitrogen decreased germination stimulant production by red clover roots, while the nitrate form of nitrogen increased germination stimulant production by red clover roots (Sato et al. 2001). Ammonium inhibits small broomrape radicle elongation (Westwood and Foy 1999). Soil borne bacteria continuously convert ammonium to nitrate through the process of nitrification. Evaluation of nitrogen's influence on small broomrape's ability to parasitize hosts has been conducted in hydroponic studies with limited small broomrape seed. A moderate small broomrape infestation creates a dense soil seedbank, often more dense than that experienced in the reported hydroponic studies. Bacteria cause differences in a soil system that would not exist in an aseptic system. Therefore, more research is needed to determine if nutrient amendments are an effective management method for small broomrape in the Pacific Northwest.

1.4 Influence of *Rhizobium*

Rhizobia are symbiotic bacteria that fix N_2 on roots of a variety of legume species, including red clover. *Rhizobium leguminosarum* Biovar. *trifolii* nodulation of red clover increased small broomrape parasitism of red clover in hydroponic aseptic

conditions (Morozov et al. 2000). However, small broomrape attachment does not require *Rhizobium* nodulation of red clover roots (Morozov et al. 2000). More research is needed to determine if *Rhizobium* inoculation of red clover is an effective management method for small broomrape in the Pacific Northwest.

1.5 Survey of Growers With Small Broomrape-Contaminated Sites

The Oregon Department of Agriculture (ODA) compiled a list of red clover seed growers with at least 1 small broomrape-contaminated field. These growers were asked to complete a survey with questions about their farming operations. The data from this survey will assist in understanding small broomrape biology, management, and introduction.

Farmers often are unutilized resources for understanding a newly introduced weed species. The affects of cultural and chemical management practices employed by growers on the success of weed species can provide insight into the biology of the species. The cardinal point to management of weed species is to understand the biology of the individual weed species, which is developed by observations under many conditions.

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2. EVALUATION OF SMALL BROOMRAPE (*Orobanche minor*) HOST AND FALSE-HOST SPECIES

2.1 Abstract

Small broomrape, a holoparasitic weed, was recently introduced to the Pacific Northwest and contaminates a limited number of red clover fields in Oregon. Greenhouse and field studies were conducted to evaluate small broomrape response to common crop and weed species in the Pacific Northwest. In greenhouse studies, plants were grown in a hydroponic polyethylene bag system to allow for continuous visibility of their roots and monitoring of small broomrape seed germination and attachment. Plants were classified as hosts (stimulate parasitic seed germination and attachment to the host), false-hosts (stimulate parasitic seed germination and senescence prior to host attachment), or non-hosts (no parasitic seed germination or attachment). Results of the greenhouse study were validated in a field contaminated with small broomrape. Host species in the greenhouse or field study included alfalfa, arrowleaf clover, carrot, celery, common vetch, crimson clover, lettuce, prickly lettuce, red clover, spotted catsear, subterranean clover, white clover, and wild carrot. False-host species included barley, birdsfoot trefoil, creeping bentgrass, cucumber, field corn, fine fescue, flax, Italian ryegrass, nasturtium, oats, orchardgrass, perennial ryegrass, snap bean, sugar pea, sunflower, sweet corn, tall fescue, tomato, and wheat. Non-host species included sugar beet and curly dock. The hydroponic polyethylene bag system provides a quick and inexpensive method for screening small broomrape host species.

Nomenclature: curly dock, *Rumex crispus* L. RUMCR; prickly lettuce, *Lactuca serriola* L. LACSE; small broomrape, *Orobanche minor* Sm. ORAMI; spotted catsear, *Hypochaeris radicata* L. HYPRA; wild carrot, *Daucus carota* L. DAUCA; alfalfa,

Medicago sativa L. MEDSA; arrowleaf clover, *Trifolium vesciculosum* Savi. TRIVE; barley, *Hordeum vulgare* L. HORVU; birdsfoot trefoil, *Lotus corniculatus* L. LOTCO; carrot, *Daucus carota* L. DAUCA; celery, *Apium graveolens* L. APIGR; common vetch, *Vicia sativa* L. VICSA; creeping bentgrass, *Agrostis stolonifera* L. AGRST; crimson clover, *Trifolium incarnatum* L. TRIIN; cucumber, *Cucumis sativa* L. CUCSA; field corn, *Zea mays* L. ZEAMA; fine fescue, *Festuca rubra* L. FESRU; flax, *Linum usitatissimum* L. LINUS; Italian ryegrass, *Lolium multiflorum* Lam. LOLMU; lettuce, *Lactuca sativa* L. LACSA; nasturtium, *Tropaeolum majus* L. TROMJ; oats, *Avena sativa* L. AVESA; orchardgrass, *Dactylis glomerata* L. DACGL; perennial ryegrass, *Lolium perenne* L. LOLPE; red clover, *Trifolium pratense* L. TRIPR; snap bean, *Phaseolus vulgaris* L. PHAVU; sugar beet, *Beta vulgaris* L. BETVU; sugar pea, *Pisium sativa* L. PISSA; subterranean clover, *Trifolium subterraneum* L. TRISU; sunflower, *Helianthus annuus* L. HELAN; sweet corn, *Zea mays* L. ZEAMA; tall fescue, *Festuca arundinaceae* Schreb. FESAR; tomato, *Lycopersicon esculentum* Mill. LYCES; wheat, *Triticum aestivum* L. TRIAE; white clover, *Trifolium repens* L. TRIRE.

Additional index words: parasitic weed, host analysis.

2.2 Introduction

Small broomrape is a relatively new introduction to the Pacific Northwest and has contaminated limited acreage of the world's primary red clover seed production area. The entire host range of small broomrape is unknown, but could include crops important to the diverse Pacific Northwest agricultural system. Management with herbicides or other methods is difficult; therefore, knowledge of the host range is essential to prevent the spread of small broomrape.

Plants species are classified as hosts (stimulate parasitic seed germination and attachment to the host), false-hosts (stimulate parasitic seed germination and senescence prior to host attachment), or non-hosts (no parasitic seed germination or attachment). Host and false-host plants produce chemical exudates that promote small broomrape germination (Joel et al. 1995). Following germination, a second chemical stimulant is required for a haustorium to emerge and penetrate the host root (Lynn and Chang 1990). Haustorium initiation stimulant is insufficient in false-hosts to stimulate attachment after germination (Smith et al. 1990). Host plant roots are penetrated by the small broomrape haustoria as a function of enzymatic action and mechanical pressure (Losner-Goshen et al. 1998). Carbon, nutrients, water, and other solutes move from the host through the haustorium to small broomrape and provide all resources for small broomrape development and reproduction. Removal of resources from the host crop can result in severe damage, yield loss, or death.

Orobanche spp. parasitize members of the Asteraceae (Romanova et al. 2001), Fabaceae (Goldwasser et al. 1997), and Solanaceae (Romanova et al. 2001) plant families. Major economic damage from small broomrape is restricted to the Fabaceae

family and clovers in particular (Miller et al. 1997). In previous studies of small broomrape biology, red clover (Yokota et al. 1998), carrot, English ivy (*Hedera helix* L.*)*, geranium (*Geranium* spp.), mock orange (*Philadelphus* spp.), petunia (*Petunia* spp.), privet (*Ligustrum* spp.), spotted catsear (*Hypochaeris radicata* L.), tobacco (*Nicotiana* spp.), and white clover (*Trifolium repens* L.) (Frost and Musselman 1980) were determined to be hosts for small broomrape. Root exudates from basil (*Ocimum* spp.), carrot, cucumber, corn, onion (*Allium* spp.), soybean (*Glycine max* (L.) Merr.), sunflower, sugar pea, and tomato stimulated germination of small broomrape (Yoneyama et al. 2001). However, research has not been conducted on the host status of many Pacific Northwest crop and weed species or been conducted in the climate of the Pacific Northwest.

Growth systems allowing continuous, unobtrusive observation of the test plant root system and small broomrape seed facilitate determination of host status. Methods developed to view host root growth and small broomrape seed fate, without disturbing the test plant include: glass cylinders with blotting paper (Wild 1948), filter paper with vermiculite or absorbent cotton (Visser et al. 1977), vertically oriented test tubes or petri dishes (Hameed et al. 1973), plexiglass with soil (Linke and Vogt 1987), glass plates with foam rubber and cloth (Jacobsohn et al. 1990), and hydroponic polyethylene bags with filter paper (Goldwasser et al. 1997; Parker and Dixon 1983; Porter et al. 1966). The hydroponic polyethylene bag system provides full visibility of the developing parasite, adequate access to the root system, and aseptic conditions at minimal cost.

The majority of methods aimed at controlling *Orobanche* spp. in host crops have failed (Goldwasser et al. 1997); therefore, *Orobanche* spp. must be controlled prior to production of a host crop. False-hosts can be utilized to diminish the small broomrape seedbank in the soil.

False-host crops decreased the *Orobanche crenata* Forsk. seedbank by 30% in one cropping cycle (Linke et al. 1993). The ability of a false-host species to deplete *Orobanche* spp. from the soil seedbank increased when the false-host species was a long-lived with an extensive root systems (Kleifeld et al. 1994).

The objectives of this research were to characterize the response of common Pacific Northwest crop and weed species to small broomrape, and to evaluate the efficacy of the hydroponic polyethylene bag system to determine the host status of plants in the field.

2.3 Materials and Methods

Hydroponic Polyethylene Bag Study

Experiment Design and Establishment

Greenhouse experiments were initiated on July 9 (Experiment 1), September 12 (Experiment 2), and September 26 (Experiment 3), 2001. Thirty-four plant species were tested for host status to small broomrape (Table 2.1). The study used a completely randomized design with either 3 (Experiment 1) or 4 (Experiments 2 and 3) replications.

Twelve seeds of each potential host species were germinated in petri dishes with moistened filter paper for 7 days (d) after which a healthy seedling was placed in the

hydroponic polyethylene bag system. The hydroponic polyethylene bag system consisted of a 17.8 by 20.3 cm polyethylene bag and a 15.3 by 19.1 cm Whatman®[1] glass microfiber filter paper (grade 934-AH) as described by Goldwasser et al. (1997). Small broomrape seeds were collected in 2000 from red clover fields contaminated with small broomrape. Glass microfiber filter paper was moistened with distilled water and evenly inoculated with 1 mg (approximately 350 seeds) of small broomrape seed. The glass microfiber filter paper was centered horizontally in the polyethylene bag and placed 1 cm below the top of the polyethylene bag. One seedling of each test species was centered on the glass microfiber filter paper with the junction of the stem and the roots placed 1 cm below the top of the glass microfiber filter paper. Distilled water was placed inside the polyethylene bag through a flexible straw to a level 1 cm above the bottom of the glass microfiber filter paper. Hydroponic polyethylene bags were placed in a greenhouse maintained at 18 C night and 24 C day with supplemental light[2]. Miracle Gro®[3] all-purpose plant food (15-30-15 with micronutrients) diluted to 0.5% concentration was applied with a cabinet sprayer at 187 L ha^{-1} every 7 d over 105 d.

Data Collection and Statistical Analysis

Small broomrape germination was determined by emergence of a radicle from the seed. Attachment was determined by an acute swelling of the host plant root and development of a tubercle. Germination and attachment were quantified every 7 d for 105 d. Total small broomrape seed number was counted in each polyethylene bag at the initiation of the experiment in order to determine final germination and attachment percentages.

Data were subjected to ANOVA. An experiment by treatment (species) interaction was significant; therefore, the data for each experiment were analyzed separately. Means were separated using Fisher's protected LSD test ($p = 0.05$).

Field Study

Experiment Design and Establishment

Field experiments were initiated during April and May of 2001 and 2002 in a field near Aurora, OR, contaminated with small broomrape seed. The soil was Quantama loam (fine loamy, mixed, mesic Aquultic Haploxerolls) with 0 to 8% slope. Field experiments consisted of 19 plant species (Table 2.2). The experimental design was a randomized complete block with 4 replications of plots measuring 15.2 by 1.8 m.

The experimental area was disced twice, harrowed, and rolled in September 2000. Prior to planting, on April 21, 2001 glyphosate was applied at 2245 g ai ha^{-1} tank mixed with a non-ionic surfactant at 0.25% v/v. On May 5, 2001, alfalfa, arrowleaf clover, birdsfoot trefoil, carrot, celery, common vetch, crimson clover, cucumber, flax, lettuce, red clover, snap bean, sugar beet, sugar pea, subterranean clover, sunflower, and white clover were seeded at common field rates in 5 rows spaced 24 cm apart. Tomatoes as 8-wk-old transplants, and potatoes as whole tubers with at least one eye, were planted in two rows 61 cm apart with 61 cm between plants in the rows. Perennial species were allowed to grow through the second year of the study and therefore were not removed or tilled.

In September 2001, plots to be planted in 2002 with annual crops were tilled with a rotary tiller and on April 1, 2002, glyphosate was applied at 2245 g ai ha^{-1} tank mixed with a non-ionic surfactant at 0.25% v/v prior to planting on April 15, 2002. Lettuce,

sunflower, snap bean, sugar pea, carrot, cucumber, celery, and flax were planted as seed, tomatoes were planted as 8-wk-old transplants, and potatoes were planted as whole tubers. Plots were drip irrigated in 2002 to assist crop germination.

Data Collection and Statistical Analysis

Four randomly selected plants from each plot were excavated from April through June in each year at 7 d intervals and inspected for attached small broomrape. The number of small broomrape plants per host was counted, dried for 1 d at 90 C, and weighed. Monthly precipitation, daily average air temperature, and daily average soil temperature at 5.1 cm depth were obtained from a meteorological site 1 km from the research area (Figure 2.1).

Data were subjected to ANOVA. There was an experiment by treatment (species) interaction was evident; therefore, the data for each experiment were analyzed separately. Means were separated using Fisher's protected LSD test ($p = 0.05$).

2.4 Results and Discussion

Hydroponic Polyethylene Bag Study

Hosts

Host species included dicot crop and weed species from the Apiaceae, Asteraceae, and Fabaceae families (Tables 2.4 and 2.5). Crop host species important to Pacific Northwest agriculture included alfalfa, arrowleaf clover, carrot, celery, common vetch, crimson clover, lettuce, red clover, subterranean clover, and white clover. Weed host species that are common weeds in Pacific Northwest agriculture included prickly lettuce, spotted catsear, and wild carrot.

Weed species that are hosts of small broomrape can grow in a false-host or non-host crop, resulting in small broomrape-contaminated crops and further spread of small broomrape seed. False and non-host crops are not inspected for small broomrape seed, thereby creating an easy and likely small broomrape seed transport mechanism. Weed species that are small broomrape hosts are common along roadsides, in ditches, and in other uncropped areas. Small broomrape contamination of non-cropland could provide a conduit for further spread of small broomrape and may prohibit the practicality of eradication.

Small broomrape germinated between 14 to 21 days after planting (DAP) and attached between 35 to 42 DAP to red clover in the hydroponic polyethylene bag system (Figure 2.2). Small broomrape germinated and attached most rapidly in lettuce (between 7 to 14 DAP and 21 to 28 DAP, respectively). Among the plants tested for host status, lettuce was the most rapid bioassay indicator of small broomrape seed contamination in soil. The time required to stimulate small broomrape seed germination may be a function of the speed and degree of small broomrape germination stimulant production by host plants.

False-Hosts

Small broomrape false-host species included dicots and monocots from the Asteraceae, Cucurbitaceae, Fabaceae, Gramineae, Linaceae, Solanaceae, and Tropaeolaceae families (Tables 2.3 and 2.6). False-host species important to Pacific Northwest agriculture included barley, birdsfoot trefoil, creeping bentgrass, cucumber, field corn, fine fescue, flax, Italian ryegrass, oats, orchardgrass, perennial ryegrass, snap bean, sugar pea, sunflower, sweet corn, tall fescue, and wheat.

False-hosts differed in their ability and rate of germination stimulation (Figure 2.3). Wheat stimulated rapid small broomrape germination that had a plateau at 25% in Experiment 1, 40% in Experiment 2, and 25% in Experiment 3. Birdsfoot trefoil stimulated a slow increase in small broomrape germination that reached 10% in experiment 1, 12% in experiment 2, and 8% in Experiment 3. Sweet corn stimulated small broomrape germination to 20% in Experiment 1 and 5% in Experiments 2 and 3. False-hosts that stimulate rapid small broomrape germination could be used as a cover crop and simultaneously deplete small broomrape from the soil seedbank, while adding organic matter and nutrients. The quantity and speed of small broomrape seedbank depletion must be analyzed along with economic crop production incentives to determine the most desirable false-host for small broomrape seedbank depletion systems.

Non-Hosts

Non-host species included curly dock from the Polygonaceae family and sugar beet from the Chenopodiaceae family (Tables 2.3 and 2.6). Small broomrape did not germinate in the control treatment without a planted test species, which indicates that small broomrape must be in the presence of plant roots to germinate.

Field Study

In 2001, no small broomrape attachments developed in the field experiment. In 2002, small broomrape attachments developed in the field experiment on alfalfa, arrowleaf clover, common vetch, crimson clover, red clover, subterranean clover, and white clover. While soil and air temperatures were similar between years, precipitation was below average and possibly limiting in January, February, and

March 2001 (Figure 2.1). The difference in precipitation between 2001 and 2002 could explain the absence of small broomrape attachment to host species in 2001 field plots.

Hosts

Small broomrape host species in the field study were limited to dicots from the Fabaceae family in 2002 (Tables 2.6 and 2.7). From May 15 to June 19 in 2002, the biomass of attached small broomrape plants increased on alfalfa, common vetch, red clover, subterranean clover, and white clover, while attachment to arrowleaf and crimson clover and parasite biomass decreased over the same period (Table 2.7). An increase in total small broomrape biomass per host plant was associated with an increase in the quantity of small broomrape attachments to alfalfa, common vetch, and red clover. An increase in total small broomrape biomass per host plant was associated with a decrease in the quantity of small broomrape attachments to subterranean clover and white clover. A decrease in total small broomrape biomass per host plant was associated with a decrease in the quantity of small broomrape attachments to arrowleaf clover and crimson clover.

One small broomrape plant was attached to both a subterranean clover plant and a prickly lettuce plant. Multiple simultaneous hosts are possible from different species and botanical families.

Non-Hosts or False-Hosts

In the field study, small broomrape false-host species were not distinguishable from non-host species due to characteristics of the soil system and size of the small broomrape seed (less than 1 mm in diameter). Non-host or false-host species included

dicots from the Apiaceae, Asteraceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, Linaceae, and Solanaceae families (Table 2.6).

Comparison of The Hydroponic Polyethylene Bag Study and The Field Study

Common vetch was a host in the field study but was a false-host in the hydroponic polyethylene bag study, while celery and lettuce were hosts in the hydroponic polyethylene bag study and not in the field study. Low soil moisture following planting of annual spring crops, such as lettuce, may have prevented parasitic attachment in the field study, while in the hydroponic polyethylene bag study there was abundant moisture and the same species acted as a host. The quantity of attachments per plant in the field study on alfalfa and common vetch increased between May 15 and June 19, while the quantity of attachments decreased over the same period on arrowleaf clover, crimson clover, red clover, subterranean clover, and white clover (Table 2.7). The decrease in attachments was suspected to be an outcome of plant maturation and decrease in soil moisture content from lack of spring and summer precipitation. As soil moisture content decreased, fine roots became more difficult to excavate, and small broomrape attachments may not have been identified.

Attachments of small broomrape to red clover were greater in the polyethylene bag study than in the field study. Differences in attachment number may have been due to differences in available moisture content in the field and hydroponic studies. Moisture was constant and adequate in the hydroponic polyethylene bag study, and therefore did not limit small broomrape development. Transfer of small broomrape germination stimulant from plant roots to the small broomrape seed is likely to be facilitated by greater moisture content. Additionally, small broomrape seed must be preconditioned,

where the seed absorb moisture and become receptive to the germination stimulant (Kebreab and Murdoch 2001). Low soil moisture would inhibit preconditioning and transfer of germination stimulant, thus resulting in reduced small broomrape germination.

Small broomrape germination varied among plant species and experiments (Table 2.4). The quantity of attached small broomrape varied by plant species at 77 DAP (p-value 0.021) (Table 2.5). Variability in results might be overcome by increasing species replicate number. Small broomrape germination requires a healthy host and a conducive environment. The hydroponic polyethylene bag study is a quick screening procedure to determine if a plant species is a host to small broomrape. However, all suspected non-host species should be screened in soil with small broomrape seed before commercial production in a small broomrape-contaminated field.

Implications of Host Species Range

Host and false-host species can be used to reduce the small broomrape seedbank in a contaminated site. Host species can be grown as a cover crop and destroyed prior to small broomrape emergence. False-hosts can be grown in contaminated sites in rotation with other crops to deplete the small broomrape seed in the seedbank. Bioassays with host plants such as lettuce, could be conducted to monitor the small broomrape soil seedbank until no small broomrape attachments to host roots occur in subsequent years. Host weeds and volunteer host crops must be controlled in contaminated sites to prevent further seed production and contamination of crop seed.

2.5 Sources of Materials

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

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

[3]Miracle Gro® water soluble all-purpose plant food, Miracle Gro®, P. O. Box 606, Maysville, OH 43040.

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Table 2.1. Plant species tested for small broomrape (*Orobanche minor*) host status in the hydroponic polyethylene bag study.

Common name	Latin name
Dicots	
Alfalfa	*Medicago sativa* L.
Arrowleaf clover	*Trifolium vesiculosum* Savi.
Birdsfoot trefoil	*Lotus corniculatus* L.
Carrot	*Daucus carota* L.
Celery	*Apium graveolens* L.
Common vetch	*Vicia sativa* L.
Crimson clover	*Trifolium incarnatum* L.
Cucumber	*Cucumis sativa* L.
Flax	*Linum usitatissimum* L.
Lettuce	*Lactuca sativa* L.
Nasturtium	*Tropaeolum majus* L.
Red clover	*Trifolium pratense* L.
Snap bean	*Phaseolus vulgaris* L.
Sugar beet	*Beta vulgaris* L.
Sugar pea	*Pisium sativa* L.
Subterranean clover	*Trifolium subterraneum* L.
Sunflower	*Helianthus annuus* L.
Tomato	*Lycopersicon esculentum* Mill.
White clover	*Trifolium repens* L.

Table 2.1. (Continued) Plant species tested for small broomrape (*Orobanche minor*) host status in the hydroponic polyethylene bag study.

Common name	Latin name
Monocots	
Barley	*Hordeum vulgare* L.
Creeping bentgrass	*Agrostis stolonifera* L.
Field corn	*Zea mays* L.
Fine fescue	*Festuca rubra* L.
Italian ryegrass	*Lolium multiflorum* Lam.
Oats	*Avena sativa* L.
Orchardgrass	*Dactylis glomerata* L.
Perennial ryegrass	*Lolium perenne* L.
Sweet corn	*Zea mays* L.
Tall fescue	*Festuca arundinaceae* Schreb.
Wheat	*Triticum aestivum* L.
Weed Species	
Curly dock	*Rumex crispus* L.
Prickly lettuce	*Lactuca serriola* L.
Spotted catsear	*Hypochaeris radicata* L.
Wild carrot	*Daucus carota* L.
Control – no planted test species	

Table 2.2. Plant species tested for small broomrape (*Orobanche minor*) host status in field studies in 2001 and 2002.

Common name	Latin name
Dicots	
Alfalfa	*Medicago sativa* L.
Arrowleaf clover	*Trifolium vesiculosum* Savi.
Birdsfoot trefoil	*Lotus corniculatus* L.
Carrot	*Daucus carota* L.
Celery	*Apium graveolens* L.
Common vetch	*Vicia sativa* L.
Crimson clover	*Trifolium incarnatum* L.
Cucumber	*Cucumis sativa* L.
Flax	*Linum usitatissimum* L.
Lettuce	*Lactuca sativa* L.
Potato	*Solanum tuberosum* L.
Red clover	*Trifolium pratense* L.
Snap bean	*Phaseolus vulgaris* L.
Sugar beet	*Beta vulgaris* L.
Sugar pea	*Pisium sativa* L.
Subterranean clover	*Trifolium subterraneum* L.
Sunflower	*Helianthus annuus* L.
Tomato	*Lycopersicon esculentum* Mill
White clover	*Trifolium repens* L.

Table 2.2. (Continued) Plant species tested for small broomrape (*Orobanche minor*) host status in field studies in 2001 and 2002.

Common name	Latin name
Control – no planted test species	

Table 2.3. Plant species response to small broomrape (*Orobanche minor*) in the hydroponic polyethylene bag study.

Non-host	Host[a]	False-host[b]
Curly dock	Alfalfa	Barley
Sugar beet	Arrowleaf clover	Birdsfoot trefoil
	Carrot	Common vetch
	Celery	Creeping bentgrass
	Crimson clover	Cucumber
	Lettuce	Field corn
	Prickly lettuce	Fine fescue
	Red clover	Flax
	Spotted catsear	Italian Ryegrass
	Subterranean clover	Nasturtium
	White clover	Oats
	Wild carrot	Orchardgrass
		Perennial ryegrass
		Snap bean
		Sugar pea
		Sunflower
		Sweet corn
		Tall fescue
		Tomato
		Wheat

[a] Based on observation of at least one attached small broomrape per test plant.

[b] Based on observation of at least one germinated small broomrape per test plant without attachment.

Table 2.4. Small broomrape (*Orobanche minor*) germination per test plant in the hydroponic polyethylene bag study as quantified 77 days after planting (DAP).

Species	Experiment 1	Experiment 2	Experiment 3
		% [a]	
Dicots			
Alfalfa	4.60	3.85	5.52
Arrowleaf clover	21.62	19.44	10.96
Birdsfoot trefoil	9.92	11.96	6.45
Carrot	17.25	15.87	26.55
Celery	39.98	11.15	9.40
Common vetch	5.59	29.28	23.76
Crimson clover	21.68	28.59	28.89
Cucumber	1.20	0.00	0.28
Flax	33.14	39.57	45.93
Lettuce	46.54	26.32	21.54
Nasturtium	10.91	28.03	30.71
Red clover	24.37	27.15	30.82
Snap bean	12.21	20.26	15.45
Sugar beet	0.00	0.00	0.00
Sugar pea	8.40	26.28	19.83
Subterranean clover	38.63	28.63	21.94
Sunflower	1.59	3.38	0.93
Tomato	3.85	1.48	1.34

Table 2.4. (Continued) Small broomrape (*Orobanche minor*) germination per test plant in the hydroponic polyethylene bag study as quantified 77 days after planting (DAP).

Species	Experiment 1	Experiment 2	Experiment 3
		% [a]	
Dicots (Continued)			
White clover	22.12	14.70	30.33
Monocots			
Barley	4.54	1.97	0.51
Creeping bentgrass	9.47	6.97	8.21
Field corn	--	9.52	7.50
Fine fescue	11.99	11.53	9.15
Italian ryegrass	0.42	6.62	1.46
Oats	8.58	9.22	4.00
Orchardgrass	12.69	10.04	4.82
Perennial ryegrass	1.16	1.20	1.69
Sweet corn	20.52	5.74	4.99
Tall fescue	1.06	1.44	1.19
Wheat	25.95	41.34	21.79
Weed Species			
Curly dock	0.00	0.00	0.00
Prickly lettuce	37.90	44.91	23.70
Spotted catsear	26.93	11.73	7.47

Table 2.4. (Continued) Small broomrape (*Orobanche minor*) germination per test plant in the hydroponic polyethylene bag study as quantified 77 days after planting (DAP).

Species	Experiment 1	Experiment 2	Experiment 3
		% [a]	
Weed Species (Continued)			
Wild carrot	21.94	30.19	3.21
Control – no planted test species	0.00	0.00	0.00
LSD (p = 0.05)	17.37	10.79	8.89

[a] Percent germination = number of germinated seeds / total seeds x 100.

Table 2.5. Small broomrape (*Orobanche minor*) attachments per host in the hydroponic polyethylene bag study as quantified 77 days after planting (DAP).

Species	Experiment 1	Experiment 2	Experiment 3
	attachments plant $^{-1}$		
Dicots			
Alfalfa	0.67	0.00	0.25
Arrowleaf clover	1.33	1.25	0.25
Carrot	1.33	1.00	0.25
Celery	0.00	0.25	1.00
Crimson clover	0.00	0.25	0.50
Lettuce	1.00	0.00	0.25
Red clover	3.67	0.50	0.50
Subterranean clover	0.00	0.25	0.00
White clover	2.33	0.00	1.75
Weed Species			
Prickly lettuce	4.00	1.00	0.00
Spotted catsear	0.33	0.00	0.25
Wild carrot	0.67	0.50	0.25
LSD (p = 0.05)	2.95	0.87	0.84

Table 2.6. Plant species host status to small broomrape (*Orobanche minor*) in the field study in 2002.

Non-host or false-host	Host[a]
Birdsfoot trefoil	Alfalfa
Carrot	Arrowleaf clover
Cucumber	Common vetch
Celery	Crimson clover
Flax	Red clover
Lettuce	Subterranean clover
Potato	White clover
Snap bean	
Sugar beet	
Sugar pea	
Sunflower	
Tomato	
Control	

[a] Based on observation of at least one attached small broomrape per test plant.

Table 2.7. Small broomrape (*Orobanche minor*) attachment quantity and biomass per host plant in the field study in 2002.

	Attachment			
	Quantity		Biomass	
Species	May 15	June 19	May 15	June 19
	— number plant $^{-1}$ —		—— g plant^{-1} ——	
Dicots				
Alfalfa	0.00	0.25	0.00	1.63
Arrowleaf clover	0.25	0.00	0.22	0.00
Common vetch	0.25	0.75	0.05	2.83
Crimson clover	0.50	0.00	0.50	0.00
Red clover	0.75	2.25	11.50	11.90
Subterranean clover	0.75	0.50	5.01	5.85
White clover	2.00	1.75	2.69	5.23
LSD (p = 0.05)	1.62	1.69	5.01	5.94

Figure 2.1. Precipitation, average air temperature, and average soil temperature at 5.1 cm soil depth in 2001 and 2002 at the North Willamette Experiment Station, Aurora, OR, about 1 km from the experiment site.

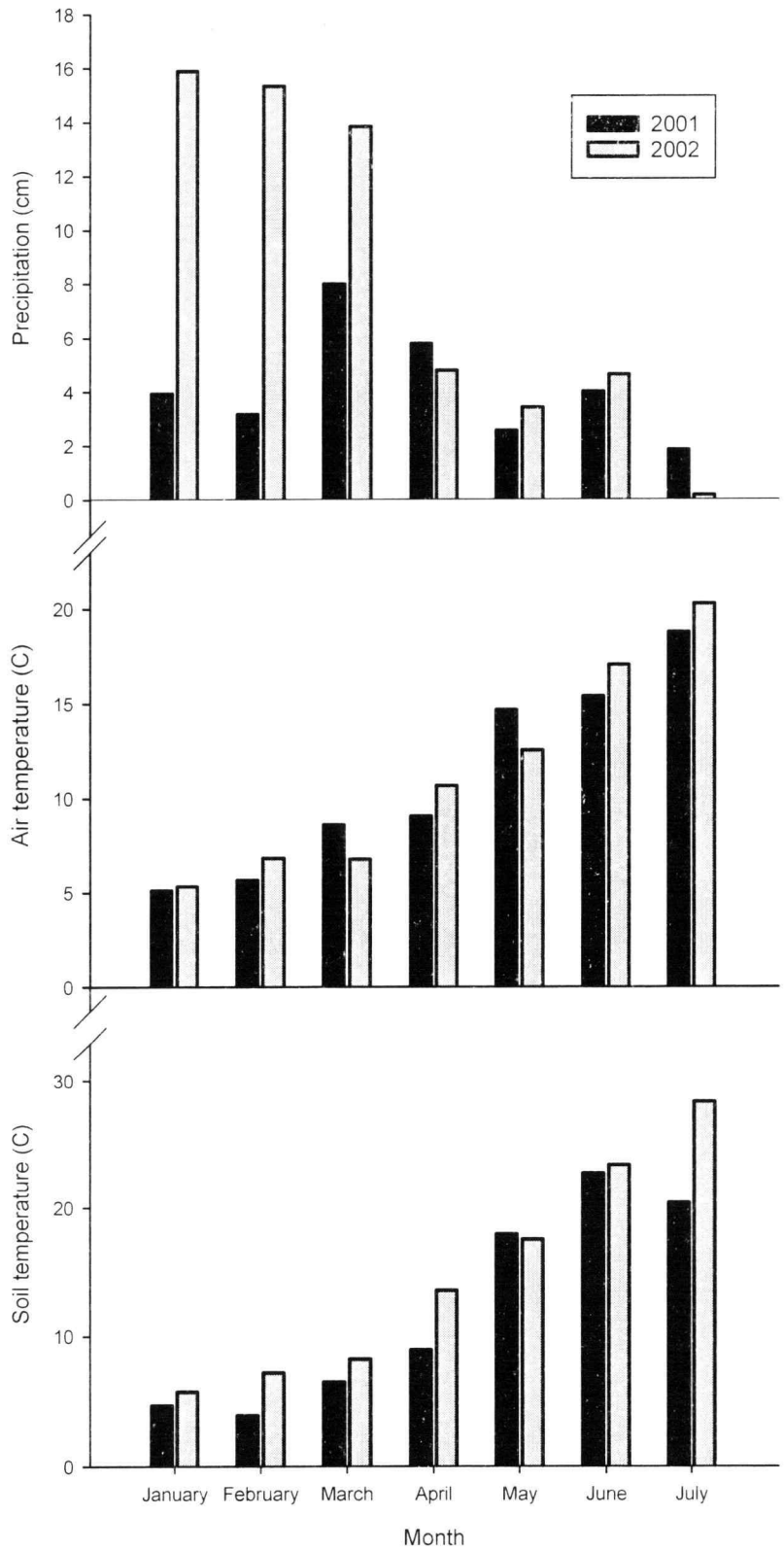

Precipitation (cm)
18
16
14
12
10
8
6
4
2
0
2001
2002
Air temperature (C)
20
15
10
5
0
Soil temperature (C)
30
20
10
0
January
February
March
April
May
June
July
Month

Figure 2.2. Small broomrape (*Orobanche minor*) germination and attachment to red clover (*Trifolium pratense*) in the hydroponic polyethylene bag study. Error bars represent one standard deviation from the mean ($p = 0.05$).

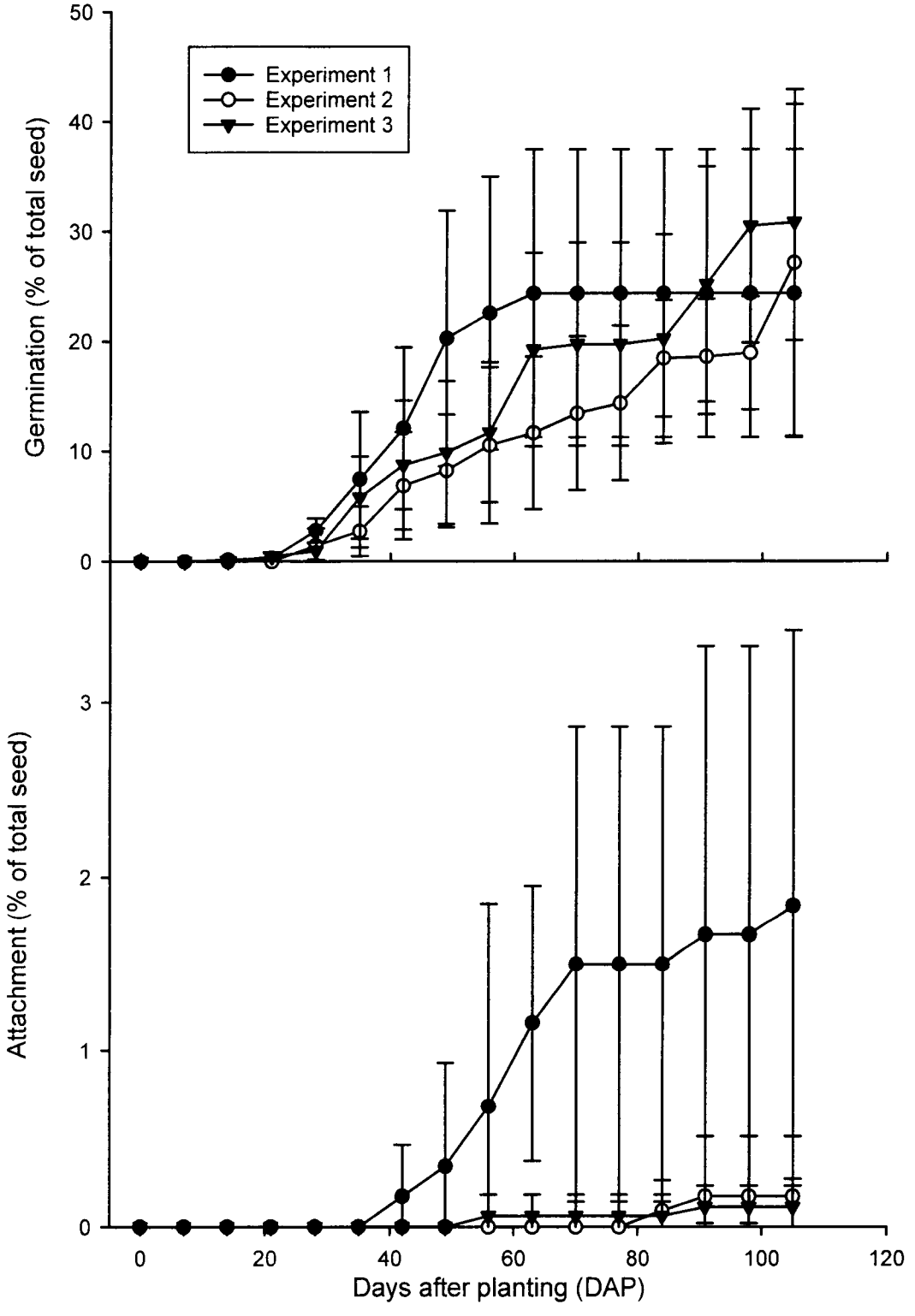
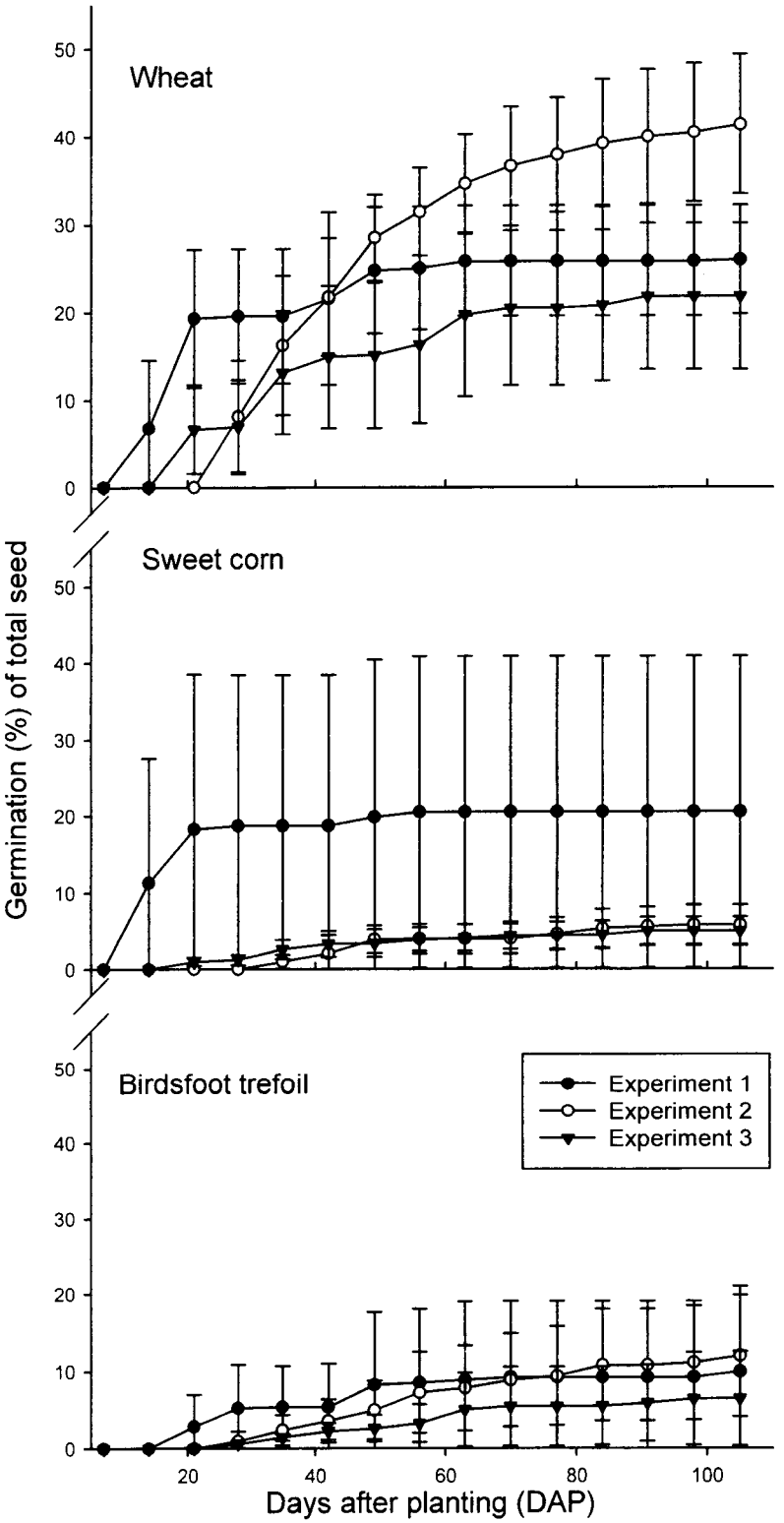

Experiment 1
Experiment 2
Experiment 3
Germination (% of total seed)
Attachment (% of total seed)
Days after planting (DAP)
0
10
20
30
40
50
1
2
3
0
20
40
60
80
100
120

Figure 2.3. Comparison of small broomrape (*Orobanche minor*) germination when grown with false-host crops in the hydroponic polyethylene bag study. Error bars represent one standard deviation from the mean ($p = 0.05$).


Wheat
Sweet corn
Birdsfoot trefoil
Experiment 1
Experiment 2
Experiment 3
Germination (%) of total seed
Days after planting (DAP)
0
10
20
30
40
50
20
40
60
80
100

3. INFLUENCE OF NITROGEN AND *RHIZOBIUM* ON SMALL BROOMRAPE (*Orobanche minor*) ATTACHMENT TO RED CLOVER (*Trifolium pratense*)

3.1 Abstract

Small broomrape, a holoparasite, is a relatively new introduction in the Pacific Northwest and has contaminated a limited number of red clover fields in Oregon. Germination and attachment to host roots are initiated by chemical exudates whose concentration may change in response to nutrient availability. Cultural control of small broomrape was not facilitated through manipulation of soil ammonium concentration and *Rhizobium* spp., a clover nodulating and atmospheric nitrogen fixating bacteria. In related research ammonium treatments in hydroponic growth systems decreased small broomrape radicle length and percent germination. *Rhizobia* penetrates the root cortex of red clover through a wound to form a symbiotic relationship for fixing atmospheric nitrogen. Red clover was grown with and without *Rhizobium* inoculate, in pots that contained small broomrape seeds and varying levels of ammonium. Neither *Rhizobium* inoculation nor ammonium application rate influenced the number of small broomrape plants parasitizing red clover.

Nomenclature: red clover, *Trifolium pratense* L., TRIPR; *Rhizobium leguminosarum* RHILE; small broomrape, *Orobanche minor* Sm. ORAMI.

Additional index words: parasitic weed, nodulation, ammonium, nitrogen fixation.

3.2 Introduction

Small broomrape (*Orobanche minor* Sm.) is a relatively new introduction in the Pacific Northwest and has contaminated limited acreage of the primary red clover (*Trifolium pratense* L.) seed production area of the world. The Pacific Northwest produces a diverse array of crops that are planted worldwide as seed stock; therefore, preventing this weed from contaminating seed lots is vitally important in maintaining markets for Pacific Northwest agriculture products. Management with herbicides is difficult; therefore, cultural control procedures are essential.

Small broomrape is a holoparasite that lacks chlorophyll and the ability to photosynthesize (Frost and Musselman 1980); therefore, it must obtain nutrients from the host. Host plants produce chemical exudates that signal small broomrape to germinate (Joel et al. 1995). After germination, a second chemical signal, called the haustorium initiation stimulant, is required for a haustorium to emerge and penetrate the host root (Lynn and Chang 1990). Haustorium initiation stimulant is required in greater concentrations than germination stimulant; less than adequate haustorium initiation stimulant results in senescence of any small broomrape seedlings (Smith et al. 1990). Red clover roots are penetrated by small broomrape haustoria through enzymatic action and mechanical pressure (Losner-Goshen et al. 1998). Carbon, nutrients, water, and other solutes move through the haustorium to small broomrape and provide resources for small broomrape development and reproduction. Removal of nutrients by small broomrape causes severe reduction in the vigor, biomass, and reproductive ability of red clover.

Rhizobia are symbiotic bacteria that fix N_2 in the nodules present on the roots of a variety of legume species, including red clover. *Rhizobium leguminosarum* Biovar. *trifolii* nodulation of red clover was suggested to increase small broomrape parasitism of red clover in hydroponic aseptic conditions (Morozov et al. 2000). However, small broomrape does not require *Rhizobium* nodulation of red clover roots for attachment (Morozov et al. 2000). Ammonium application in hydroponic aseptic conditions decreased small broomrape parasitism of red clover (Sato et al. 2001; Westwood and Foy 1999), *Striga hermonthica* parasitism of grain (Cechin and Press 1993; Mumera and Below 1993), and Egyptian broomrape (*Orobanche aegytiaca* Pers.) parasitism of tomato (*Lycopersicon esculentum* Mill.) (Jain and Foy 1992).

The ammonium form of nitrogen decreased germination stimulant production by red clover roots, while the nitrate form of nitrogen increased germination stimulant production by red clover roots (Sato et al. 2001). Ammonium inhibits small broomrape radicle elongation (Westwood and Foy 1999). Soil-borne bacteria continuously convert ammonium to nitrate through the process of nitrification (Havlin et al. 1999). Evaluation of nitrogen's influence on small broomrape host parasitism has been conducted in hydroponic studies with limited small broomrape seed. An individual broomrape plant produces over 1 million seeds (Pieterse 1979); therefore, a moderate small broomrape infestation creates a dense soil seedbank, often more dense than that experienced in the hydroponic studies. In a dense soil seedbank, an extremely high reduction in small broomrape attachment is required to reduce the small broomrape infestation.

Management of small broomrape by manipulating *Rhizobium* inoculation and ammonium-based fertilizer applications could be a cost-effective strategy in red clover seed production. Therefore, the objective of this study was to determine the relationship between *Rhizobium*, ammonium-based fertilizer, and red clover parasitism by small broomrape.

3.3 Materials and Methods

Site Description and Experimental Design

Greenhouse experiments were initiated on May 15, June 27, September 11, and September 25 in 2001 and May 7 in 2002. Ten treatments were applied to 'Kenland' red clover with 4 replications in each study. Within each replication, red clover seed in 5 pots received *Rhizobium* inoculation, while red clover seed in the other 5 pots received no *Rhizobium* inoculation. Individual pots with *Rhizobium* inoculated and non-inoculated red clover seed received one of five ammonium treatments, 0.0, 5.6, 11.2, 22.4, or 44.8 kg ha^{-1}. Treatments were completely randomized within each experiment.

Experiment Establishment

Commercial grade ammonium sulfate (21-0-0-24) was ground in a blender. Ammonium sulfate was passed through a screen with a opening size of 0.246 x 0.246 mm. Small broomrape seed were collected in 2000 from red clover fields contaminated with small broomrape. Commercial 'Kenland' red clover seed was used as a seed source. One g of Urbana Laboratories alfalfa/clover *Rhizobium* and 3 ml

with 1% fat cow milk were used to inoculate 15 g of red clover seed. These products were thoroughly mixed and allowed to air dry at 21 C for 3 h.

Black plastic pots (14 cm^2) were filled with dry loose perlite to the level with the top perimeter of the pot. Pots were watered with a mist of tap water to moisten and consistently compress the perlite to 2 cm below the brim of the pot. Six inoculated or non-inoculated red clover seeds were randomly placed on the perlite. Ten mg of small broomrape seed and the ammonium sulfate treatments were evenly dusted on the perlite. Fifteen ml of dry perlite were evenly applied to the surface of the compressed perlite to cover the red clover seeds, small broomrape seed, and ammonium sulfate. Tap water was applied as a mist above the pots. Pots were sub-irrigated with tap water to prepare and maintain the media for red clover and small broomrape germination and growth. Red clover was grown in a greenhouse at 18 C night and 24 C day with artificial light[1] supplied from 6:00 to 22:00. Red clover plants were thinned to 3 per pot at 28 days after planting (DAP) and grown to 105 DAP, when data were collected.

Data Collection and Statistical Analysis

Red clover plants were excavated and the roots were washed and inspected for small broomrape attachment 105 DAP.

Data were subjected to ANOVA. An experiment by treatment interaction was significant; therefore, the data for each experiment were analyzed separately. Means were separated using a Fisher's protected LSD test ($p = 0.05$). Treatments with *Rhizobium* inoculum were contrasted with those without *Rhizobium* inoculum and treatments with nitrogen were contrasted with those without nitrogen.

3.4 Results and Discussion

Nitrogen

Nitrogen affected the quantity of small broomrape attachments to red clover only in one of five experiments (Table 3.1). Within each experiment, the quantity of small broomrape attachments per red clover plant was similar among treatments and varied across replication in both *Rhizobium* inoculated and non-inoculated red clover seed (Figure 3.1). Differences in small broomrape attachment among experiments were too great for data to be combined (Table 3.2). Red clover plants experienced nitrogen toxicity at 44.8 kg of ammonium ha^{-1}; therefore, ammonium was not bound to the perlite.

Experiments were not aseptic, creating the possibility that autotrophic bacteria, *Nitrosomonas, Nitrosolobus, Nitrospria,* and *Nitrosovibrio,* converted NH_4^+ to NO_2^- through nitrification (Havlin et al. 1999). The autotrophic bacteria *Nitrobacter* converts NO_2^- to NO_3^- through oxidation (Havlin et al. 1999). The resulting nitrate is subject to rapid leaching from field soils and probably the perlite growth system. The high water solubility and mobility of the nitrate form of nitrogen allows for rapid nitrate leaching losses (Havlin et al. 1999).

Nitrosomonas, Nitrosolobus, Nitrospria, Nitrosovibrio, and *Nitrobacter* are abundant in field soils (Havlin et al. 1999). Soil conditions that decrease the activity of these autotrophic bacteria are an unfavorable environment for red clover. If a high level of NH_4^+ was required to eliminate small broomrape from attaching to red clover, it would be difficult to maintain without resulting in NH_4^+ toxicity to the red clover.

Nitrapyrin, a nitrification inhibitor could, be used to postpone the nitrification process and maintain a high quantity of NH_4^+ in the soil.

Ammonium has been associated with decreased production of small broomrape germination stimulant, while the nitrate form of nitrogen has been associated with an increase in production of small broomrape germination stimulant (Sato et al. 2001). If some ammonium was converted to nitrate in the perlite, it is thereby likely to cancel out any effect that ammonium had on decreasing small broomrape attachment to red clover.

In prior aseptic experiments, broomrape parasitism decreased when ammonium was applied to red clover (Westwood and Foy 1999; Van Hezewijk and Verkleij 1996). Ammonium sulfate at 4 mM in the germination medium decreased germination of *Orobanche crenata* Forsk. from 50% to 16% and ammonium sulfate plus *Nitrapyrin*, a nitrification inhibitor, reduced germination from 55% to 2% in aseptic conditions (Van Hezewijk and Verkleij 1996). At dense small broomrape seed concentration in the soil, ammonium could decrease germination rates without significantly decreasing attachment rates. Three thousand five hundred small broomrape seeds per pot at 16% germination results in 560 germinated seeds dispersed around the roots of three red clover plants, which would present a high likelihood for attachment. An individual broomrape plant produces over 1 million seeds (Pieterse 1979). Seed production from 1 small broomrape plant dispersed on 5.5 m^2 is equivalent to 3500 small broomrape seeds per pot. An infestation of 1 or greater small broomrape plants per 5.5 m^2 in a single year, would make the soil seedbank more dense then that experienced by red clover plants in this experiment. Red clover plants

in this experiment and prior experiments tended to have less than 3 small broomrape attachments per red clover plant. Differences in small broomrape attachment numbers to individual red clover plants may be observed at sparse and high densities of small broomrape seed in the soil seedbank.

Rhizobium

Rhizobia nodules were observed in minimal quantities on non-inoculated red clover plants. *Rhizobia* nodules in all inoculated treatments were pink in color, indicating active N_2 fixation. *Rhizobia* nodules on the red clover roots did not increase susceptibility to penetration of the small broomrape haustorium. Therefore, small broomrape is not dependent on *Rhizobia* nodules for successful attachment. Root exploration and biomass were reduced when small broomrape parasitized red clover (Figure 3.2). Less root area and root exploration would decrease the probability of parasitism by subsequent small broomrape seedlings. Decreased root area, root exploration, and above ground biomass were likely due to removal of nutrients from red clover for small broomrape growth and development. *Rhizobium* inoculation of red clover affected the quantity of small broomrape attachments in one of five experiments (Table 3.1).

In aseptic conditions, small broomrape parasitism increased in *Rhizobium* inoculated red clover compared to non-inoculated red clover (Morozov et al. 2000). Absence of microbiological soil organisms in aseptic lab conditions and lack of nitrogen may have caused the relationship among small broomrape, red clover, and *Rhizobium* to be different. Red clover root development and defense mechanisms are likely to be different when no nitrogen is present from *Rhizobium* and fertilizers,

compared to red clover plants receiving nitrogen from *Rhizobium* or fertilizer. There are low levels of *Rhizobia* present in field soils that would cause red clover plants not inoculated with *Rhizobium* to develop *Rhizobium* nodulations in field soil and would therefore receive nitrogen.

The perlite growth system is likely to be more similar to the growing system present in field soils. Experiments using soil in place of perlite would incorporate unknown factors into the analysis and interactions from other variables. Greater rates of ammonium could not be utilized in red clover plants because of nitrogen toxicity at levels greater than 44.8 kg ammonium ha^{-1}. Ammonium treatment and *Rhizobium* do not reduce small broomrape parasitism of red clover and are not effective management tools.

3.5 Sources of Materials

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

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Westwood, J. H. and C. L. Foy. 1999. Influence of nitrogen on germination and early development of broomrape (*Orobanche* spp.). Weed Sci. 47:2-7.

Table 3.1. Contrast of number of attached small broomrape (*Orobanche minor*) in response to ammonium sulfate and *Rhizobium* inoculant treatments.

		P value				
Contrast	df	Exp[a] 1	Exp 2	Exp 3	Exp 4	Exp 5
Inoculant	1	0.2640	0.3905	0.6629	0.1645	0.0417
Nitrogen	1	0.0005	0.3176	1.0000	0.2441	0.8539

[a] Exp = Experiment

Table 3.2. ANOVA of number of attached small broomrape (*Orobanche minor*) in response to ammonium sulfate and *Rhizobium* treatments.

		P value				
Source of variation	df	Exp[a] 1	Exp 2	Exp 3	Exp 4	Exp 5
Replication	3	0.8838	0.8177	0.5532	0.4880	0.5873
Treatment	9	0.0621	0.2698	0.8326	0.3737	0.5149
LSD (p=0.05)		0.8044	0.7885	0.5205	0.4816	2.4672

[a] Exp = Experiment

Figure 3.1. Treatment mean quantity of small broomrape (*Orobanche minor*) plants per red clover (*Trifolium pratense*) plant.

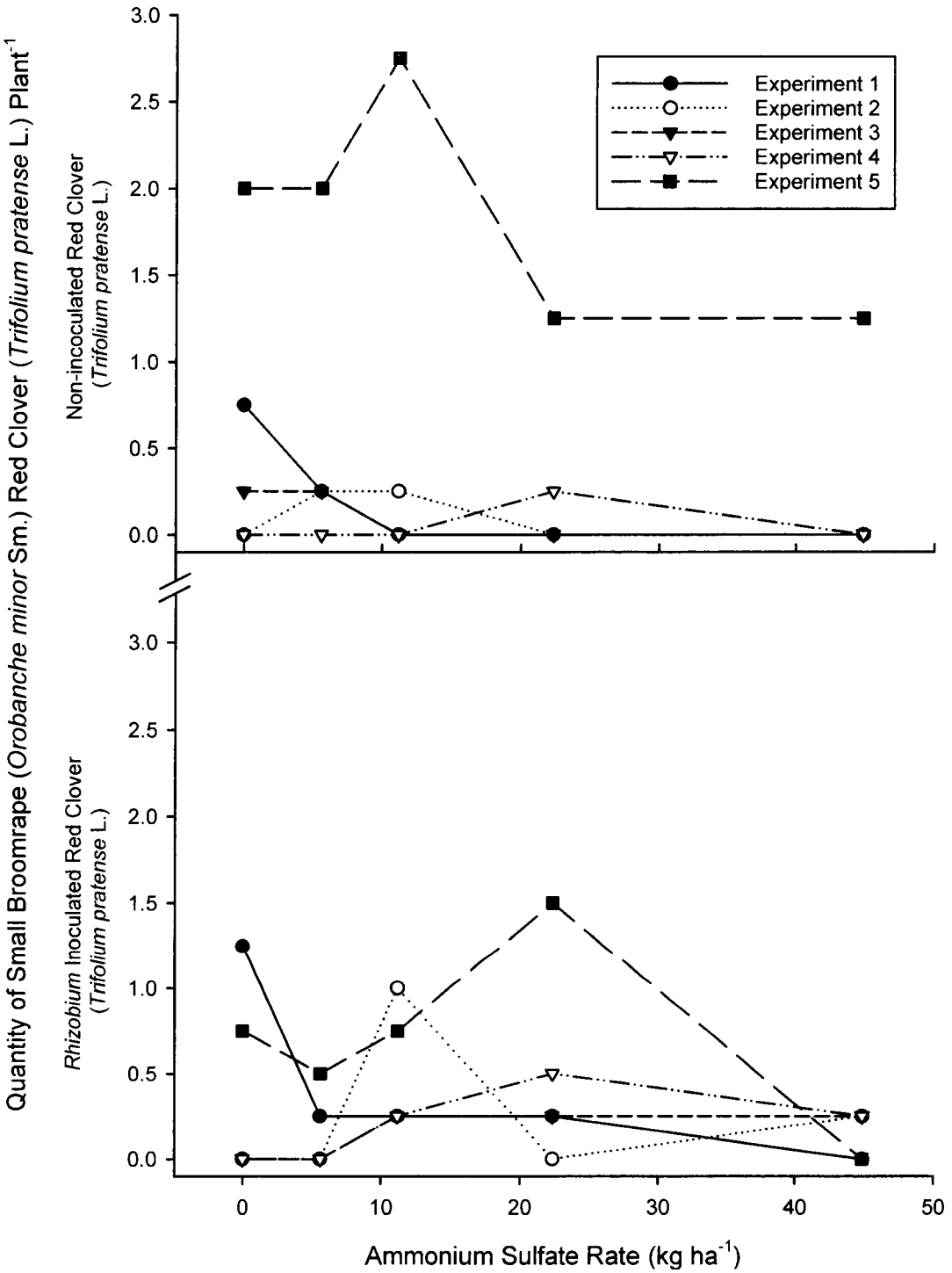
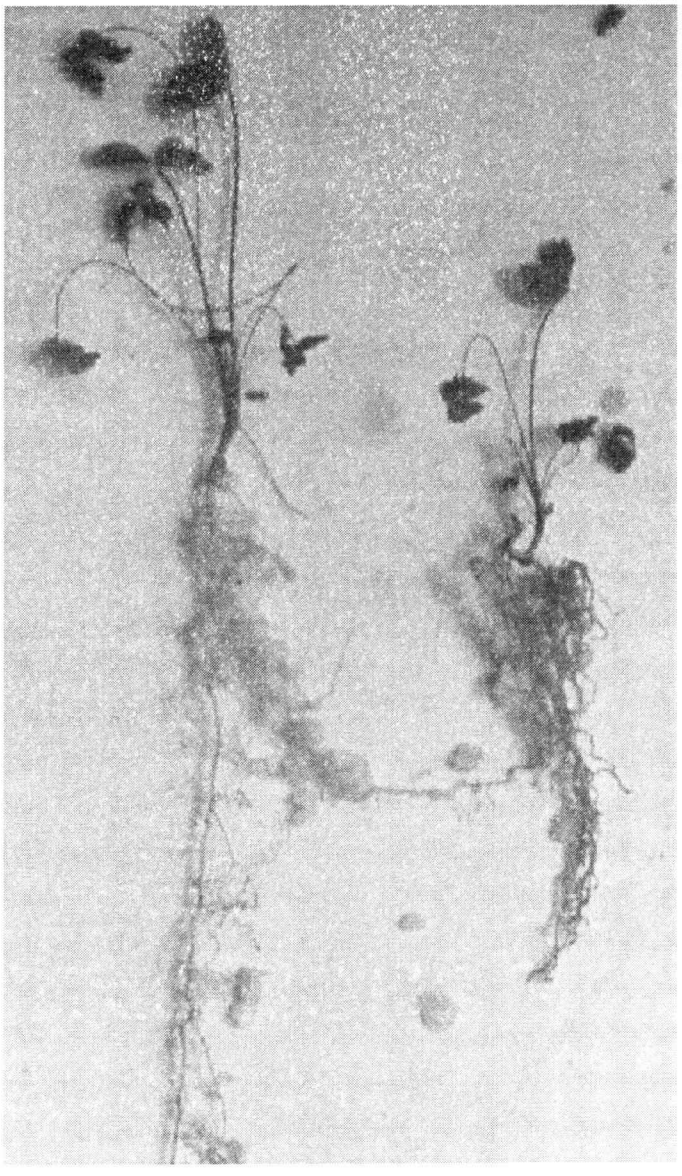

Quantity of Small Broomrape (*Orobanche minor* Sm.) Red Clover (*Trifolium pratense* L.) Plant^{-1}
Non-incoculated Red Clover (*Trifolium pratense* L.)
Rhizobium Inoculated Red Clover (*Trifolium pratense* L.)
Experiment 1
Experiment 2
Experiment 3
Experiment 4
Experiment 5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
Ammonium Sulfate Rate (kg ha^{-1})

Figure 3.2. Inoculated red clover (*Trifolium pratense*) plants. The red clover plant on the right has been parasitized by small broomrape (*Orobanche minor*) while the red clover plant on the left has not been parasitized.



4. A GROWER SURVEY: INTRODUCTION, DISPERSAL, AND MANANGEMENT OF SMALL BROOMRAPE (*Orobanche minor*) IN RED CLOVER (*Trifolium pretense*) SEED PRODUCTION

4.1 Abstract

Small broomrape, a holoparasitic weed, is a relatively new introduction in the Pacific Northwest and contaminates a limited number of red clover fields in Oregon. A survey was conducted of red clover seed growers with small broomrape-contaminated fields. The survey consisted of 19 questions aimed at capturing red clover seed growers' experiences with small broomrape biology, introduction, dispersal mechanisms, and current management practices. Twenty of 25 surveys were completed and returned. The red clover seed lots from 6 individual respondents were cleaned at the same cleaning facility and these 6 respondents also purchased red clover seed stock from this facility. Small broomrape was not discovered prior to or during harvest of the first seed crop from red clover planted in the fall; however, it was observed in second-year fall and spring-planted red clover and first year spring-planted red clover. Red clover was the only clover grown by 12 respondents, while eight respondents grew other species of clover in addition to red clover. This survey can provide information on small broomrape dispersal and to prioritize future research and extension efforts.

Nomenclature: small broomrape, *Orobanche minor* Sm. ORAMI.

Additional index words: parasitic weed, red clover, grower survey.

4.2 Introduction

Small broomrape is a relatively new introduction in the Pacific Northwest and has contaminated limited acreage of the primary red clover (*Trifolium pratense* L.) seed production area of the world. In 2001 there were 7,000 ha of red clover seed production in Oregon, with an $4.4 million annual seed value plus forage value (Young 2002). In 1998, small broomrape was detected in a certified red clover seed field in Clackamas County, Oregon (Suverly and Mallory-Smith 2000). Although small broomrape was first documented in Oregon in 1923, the 1998 observation was the first in Oregon commercial agriculture (Oregon State University Herbarium 1923). Small broomrape is a federally listed noxious weed that is prohibited in inter-state commerce.

Beginning in 1999, Oregon Department of Agriculture implemented temporary administrative rules on known small broomrape-contaminated fields in Oregon. Small-broomrape-contaminated fields were quarantined and were not allowed to be planted to known small broomrape host crops. Prior to harvest, isolated small broomrape plants in red clover fields were required to be removed and destroyed. Harvest of the portions of red clover fields heavily infested with small broomrape was not allowed. Seed harvest from small broomrape-contaminated fields were kept in secured sealed containers. All red clover seed lots were required to be tested for small broomrape seed prior to sale and shipment out of Oregon.

In 2000, the Oregon Department of Agriculture (ODA) received funds from the Interstate Pest Control Compact (IPCC) and United States Department of Agriculture

(USDA) to complete a comprehensive survey of the North Willamette Valley clover fields to determine the extent of the small broomrape dispersal.

Red clover is grown in a production system utilizing either fall or spring planting. In the fall planting system, red clover is planted in monoculture in late August to early October, and harvested in late May to early June for forage. In late August to early September, the red clover is then harvested for seed and grown for an additional year, contributing another forage and seed harvest. In the spring planting system, red clover is broadcast over an established grain crop in late winter or early spring, sown with spring grain, or seeded alone. In the spring planting system, the grain is harvested at maturity and the clover is not harvested the first year. Red clover is typically grown for 2 forage and seed harvests over 2 years, similar to the fall planting system.

The objective of this study was to survey Oregon red clover seed growers who have small broomrape on their farms about the introduction of small broomrape and the management practices utilized on their farms.

4.3 Materials and Methods

Data Collection Technique

Survey recipients were red clover seed growers who had 1 or more red clover seed fields contaminated with small broomrape in 1999, 2000, or 2001. Growers were asked 19 questions about their farming operation in order to determine characteristics of their farms, assess the accuracy of small broomrape identification by farmers, history and culture of the small broomrape-contaminated site, and potential introduction and dispersal mechanisms. Names of individuals, farms, custom

operators, agronomy companies, seed companies, and seed cleaning facilities were kept confidential.

A cover letter describing the purpose of the survey, a small broomrape information sheet with small broomrape biology, ecology, and description, and the survey were mailed to each grower. Growers were given an opportunity to add comments in addition to the survey questions.

Survey Area and Farm Characteristics

The survey covered Clackamas, Columbia, Linn, Marion, Multnomah, Washington, and Yamhill counties, and thus included the primary red clover production area in Oregon. This section of Oregon is in the Northern Willamette Valley and is characterized by a variety of soils that are typically deep, well drained, fertile, and surrounded by suburban development. Crop rotation varied among farms and among fields within an individual farm. Cropping systems were both dryland and irrigated.

Farm demographics were requested in two questions: annual average red clover (or any other contaminated crop) land area; and the total (owned and leased) farm land area.

Identification

Two questions assessed the accuracy of small broomrape identification relative to included photographs. The questions were: had you seen small broomrape prior to the year your red clover seed field was surveyed by ODA; and have you seen small broomrape in sites other than those identified by ODA.

History and Culture of Contaminated Sites

Seven questions asked growers about their management techniques and cultural practices to determine potential correlations between cropping systems, clover species, and red clover cultivars and small broomrape infestation. The questions were: have you seen small broomrape on crops other than clover; was the small broomrape-contaminated red clover field planted in the spring or fall; how many seed crops had been harvested prior to when ODA determined the red clover field was contaminated with small broomrape; what other crops were raised on your farm; what was the cropping history of the small broomrape-contaminated field in reverse chronological order beginning with the year small broomrape was discovered in the field; which species and cultivars of clover were contaminated with small broomrape; and which other species and cultivars of clover were raised in the small broomrape-contaminated field(s) or other fields.

Small Broomrape Introduction and Distribution

Cultural and Management Practices

Four survey questions asked growers about cultural and management practices that may have increased their possibility of contaminating a red clover field with small broomrape. Questions focused on associations between companies, individuals, custom operations, and seed lots. The questions were: have any custom operations been conducted in the small broomrape-contaminated field, and if so, what was the operation, when was the operation conducted, and who conducted the operation; where was the red clover seed stock purchased for planting the small broomrape-contaminated field and what was the seed lot number; what was the name of the

cleaning facility that cleaned the small broomrape-contaminated red clover seed lot; and after cleaning, did the red clover seed lot harvested from the small broomrape-contaminated field pass the Oregon State University (OSU) Seed Laboratory test for small broomrape contamination of red clover seed.

Environment

Three survey questions examined the possible correlation of wildlife habitats and water flow with the spread of small broomrape seed from one location to another. The questions were: does surface water flow across the small broomrape-contaminated field; has the field been irrigated from a water source other than a well; and have geese been seen on the small broomrape-contaminated site(s) or water sources that could reach fields.

Statistical Analysis

Data were pooled, averaged, and presented graphically and in tables.

4.4 Results and Discussion

Data Collection Technique

Twenty (80%) complete surveys were received, 1 (5%) survey was undeliverable, and the remaining 4 (15%) surveys were not returned. All questions in returned surveys were complete. The survey procedure utilizing a cover letter describing the purpose of the survey, a small broomrape information sheet with small broomrape biology, ecology, and description, small broomrape pictures and illustrations, and written survey questions made this survey successful in acquiring returned completed surveys.

Respondents were allowed to provide additional information on the survey, a few respondents described their management tactics for their small broomrape infestation, which included selective removal by hand, application of glyphosate, and mowing, followed by harvest or destruction of seed. A second focus of respondents additional comments was the role of wildlife, deer and geese, as possible mechanisms of small broomrape seed introduction and dispersal.

Survey Area and Farm Characteristics

Red clover was grown on limited land area on most farms as a rotation crop for control of weeds and volunteer crops, and fertility enhancement between monocotyledonous crops. Eighteen (90%) of the farms grew less then 20 ha, and 2 (10%) farms grew more than 20 ha of red clover seed. The majority of farms were single family ownership with 11 (55%) farms operating on less then 100 ha, 8 (40%) farms operating between 100 ha and 400 ha, and 1 (5%) farm operating over 400 ha. Farms less than 100 ha in size were typically operated by 1 individual, farms between 100 ha and 400 ha were typically operated by 2 to 4 individuals, and farms over 400 ha were typically operated by more than 4 individuals.

Identification

Eighteen (90%) of the respondents had not seen small broomrape prior to the year their field was found contaminated with small broomrape. The ODA marked small broomrape plants with flags in contaminated fields and showed them to growers for educational purposes and to assist growers in identifying small broomrape-contaminated fields. Five (25%) respondents recognized small broomrape in

neighboring red clover seed fields after the ODA survey. Growers were more aware of other small broomrape infestations after viewing infestations in their own fields.

History and Culture of Contaminated Sites

One (5%) respondent claimed to have seen small broomrape parasitizing strawberries (*Fragaria* spp.); however, strawberries have not been previously reported as a small broomrape host. Nineteen (95%) growers had not seen small broomrape parasitizing any other plant species.

Nine (45%) respondents had small broomrape parasitize spring-planted red clover prior to harvest of the first seed crop. Five (25%) respondents had small broomrape parasitize spring-planted red clover prior to harvest of the second seed crop. Six (30%) respondents had small broomrape parasitize fall-planted red clover prior to harvest of the second seed crop, but not prior to harvest of the first seed crop. No respondents had small broomrape parasitize fall planted red clover prior to or during the harvest of the first crop (Figure 4.1).

Cropping history and crop rotations varied among respondents, but generally consisted of a monocotyledonous crop followed by a dicotyledonous crop. This crop rotation allows growers to rotate mechanical and chemical weed control mechanisms and meet seed certification requirements. Nineteen (95%) growers raised wheat in rotation with red clover; however, other crops raised in rotations were diverse among respondents (Table 4.1).

Twelve (60%) respondents grew red clover, 7 (35%) respondents grew red clover and crimson clover (*Trifolium incarnatum*), and 1 (5%) respondent grew red clover and arrowleaf clover (*Trifolium vesciculosum*) in addition to their other crops (Figure 4.2). The red clover grown by all respondents were medium red clover cultivars. Eight (40%) respondents grew 'Kenland', 1 (5%) respondent grew 'Hedges', and 11 (55%) respondents were unsure of the cultivar. Kenland and Hedges red clover have no varietial resistance to small broomrape (Eizenberg et al. 2003).

Small Broomrape Introduction and Distribution

Cultural and Management Practices

Seven (35%) respondents had custom operations conducted on the small broomrape-contaminated field. Custom spraying and fertilizing occurred by separate third parties on 2 (10%) respondents' fields. One (5%) respondent's land was leased to a third party who had no fields contaminated by small broomrape. Custom forage harvest occurred on small broomrape-contaminated sites of 2 (10%) respondents by a common third party. Forage harvest most likely occurs prior to small broomrape emergence; therefore, small broomrape contamination is likely to only occur with transfer of soil with forage harvest. Custom seed harvest occurred on small broomrape-contaminated sites of 2 (10%) respondents by third parties having small broomrape-contaminated sites. Custom field operations do not appear to be the main mechanism for small broomrape dispersal.

No respondents reported the seed lot number of the red clover seed stock used to plant the small broomrape-contaminated fields. Companies that sold or cleaned red clover seed are referred to as "Company A thru I". Seed company "A" cleaned and

provided red clover seed stock to 6 (30%) of the respondents (Table 4.2). Other companies had a lower potential for dispersing small broomrape seed because they cleaned fewer small broomrape-contaminated red clover seed lots and sold less red clover seed stock that was grown in small broomrape-contaminated fields (Table 2). Company "A" may have unintentionally assisted in dispersal of small broomrape to new sites and seed lots; however, all seed lots after 1999 were tested by OSU's Seed Laboratory and certified free of small broomrape seed.

Environment

Seeds are frequently dispersed from one location to another by floating on the surface of water (Radosevich et al. 1997). Surface water frequently runs across 6 (30%) of the respondents' small broomrape-contaminated fields. Seven (35%) respondents have irrigated their small broomrape-contaminated sites with water from a source other than a well. Streams and water holding facilities that are open to the environment typically contain weed seed floated in by water, blown in by wind, or brought in by birds and wildlife (Radosevich et al. 1997). Geese frequently inhabit 11 (55%) respondents' fields. Geese may have the ability to move small broomrape seeds on their feet or after ingestion from contaminated fields or water. Fourteen (70%) respondents' small broomrape-contaminated fields have surface water run across the field, irrigated the field from a water source other then a well, or had geese inhabit the field. These three mechanisms are likely to have assisted in dispersal of small broomrape seed.

Implications

In the fall, red clover planted in the spring with no harvest is similar in size and development to red clover that has been harvested 1 or more times. In the fall, red clover that was planted the same fall with no harvest, is smaller and less developed, compared to red clover that been harvested 1 or more times or spring planted red clover that has not been harvested. Red clover planted in the fall at the beginning of the cold rainy season may not be developed enough to promote parasitism by small broomrape.

It is hypothesized that small broomrape begins germination during wet winter and early spring months when soil moisture is high. The late fall to early spring is the only time period were soil moisture is great enough in the dryland production systems of the Oregon's North Willamette Valley to allow in small broomrape parasitism of a host. The inability of small broomrape to parasitize seedling red clover may be due to a relationship between either germination or haustorium initiation stimulants and soil temperature and soil moisture levels. Production of germination and haustorium initiation stimulants by seedling red clover may be inadequate for small broomrape to respond due to the small size of the red clover seedling, low soil temperature, and high soil water content. Germination and haustorium initiation stimulants are diluted and quickly leached away from small broomrape seeds that are in adequate proximity to successfully attach to a red clover seedling. A red clover plant with a larger root system may produce more stimulants to overcome the effect of lower stimulant production that is possibly created by low temperature and dilution and leaching

effects from water. A larger root system has more root to soil contact and is likely to come in contact with more small broomrape seeds than a small root system

The specific mechanism behind dispersal of small broomrape is difficult to determine because the parasite is only visible after emergence in the presence of a host. Small broomrape is likely to have been dispersed by a combination of contaminated machinery, seed lots, water, and wildlife.

While respondents were able to accurately identify small broomrape, the subsequent management practices and management of small broomrape-contaminated fields varied. Some management practices utilized by respondents promote dispersal of weed seeds. This suggests that further education is appropriate, particularly in the areas of small broomrape prevention and control. Furthermore, the process of seed cleaning and certification of red clover seed lots should be examined.

4.5 Acknowledgments

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Table 4.1. Crops included in rotation with red clover in fields contaminated with small broomrape (*Orobanche minor*).

Crop	Latin name	Grower response
		%[a]
Alfalfa	*Medicago sativa* L.	5
Arrowleaf clover	*Trifolium vesciculosum* Savi.	5
Barley	*Hordeum vulgare* L.	5
Common vetch	*Vicia sativa* L.	5
Crimson clover	*Trifolium incarnatum* L.	35
Field corn	*Zea mays* L.	5
Oat	*Avena sativa* L.	45
Perennial ryegrass	*Lolium perenne* L.	25
Radish	*Raphanus sativus* L.	10
Red clover	*Trifolium pratense* L.	100
Snap bean	*Phaseolus vulgaris* L.	5
Strawberry	*Fragaria* spp.	5
Sugar beet	*Beta vulgaris* L.	5
Sugar pea	*Pisium sativa* L.	10
Sweet corn	*Zea mays* L.	10
Tall fescue	*Festuca arundinaceae* Schreb.	25
Wheat	*Triticum aestivum* L.	95

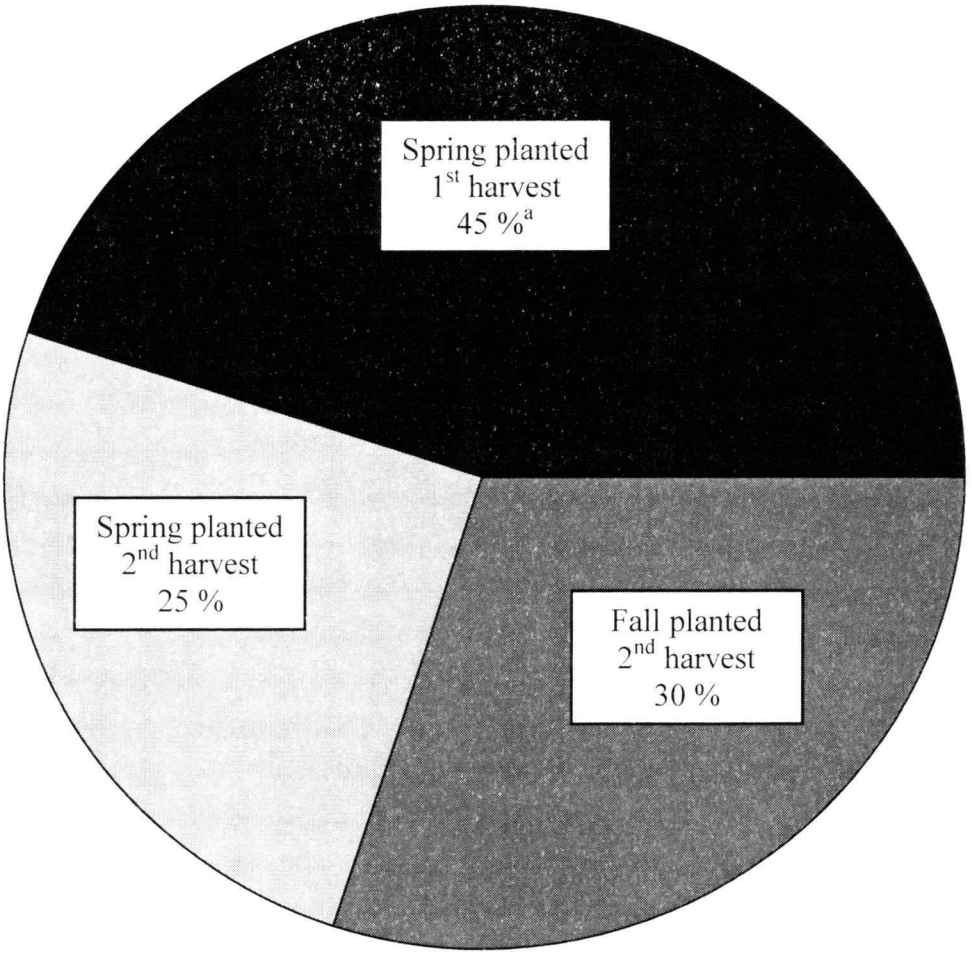
[a] survey responses that included each crop in rotation / number of survey responses x 100.

Table 4.2. Seed company that supplied red clover (*Trifolium pratense*) seed stock for planting or cleaned the red clover seed from a small broomrape (*Orobanche minor*) infested field.

Seed company[a]	Seed lots purchased	Seed lots cleaned
	number	
A	6	6
B	2	0
C	1	0
D	1	1
E	1	3
F	2	1
G	0	2
H	0	1
I	0	1
Unknown	7	0
Destroyed seed	0	1

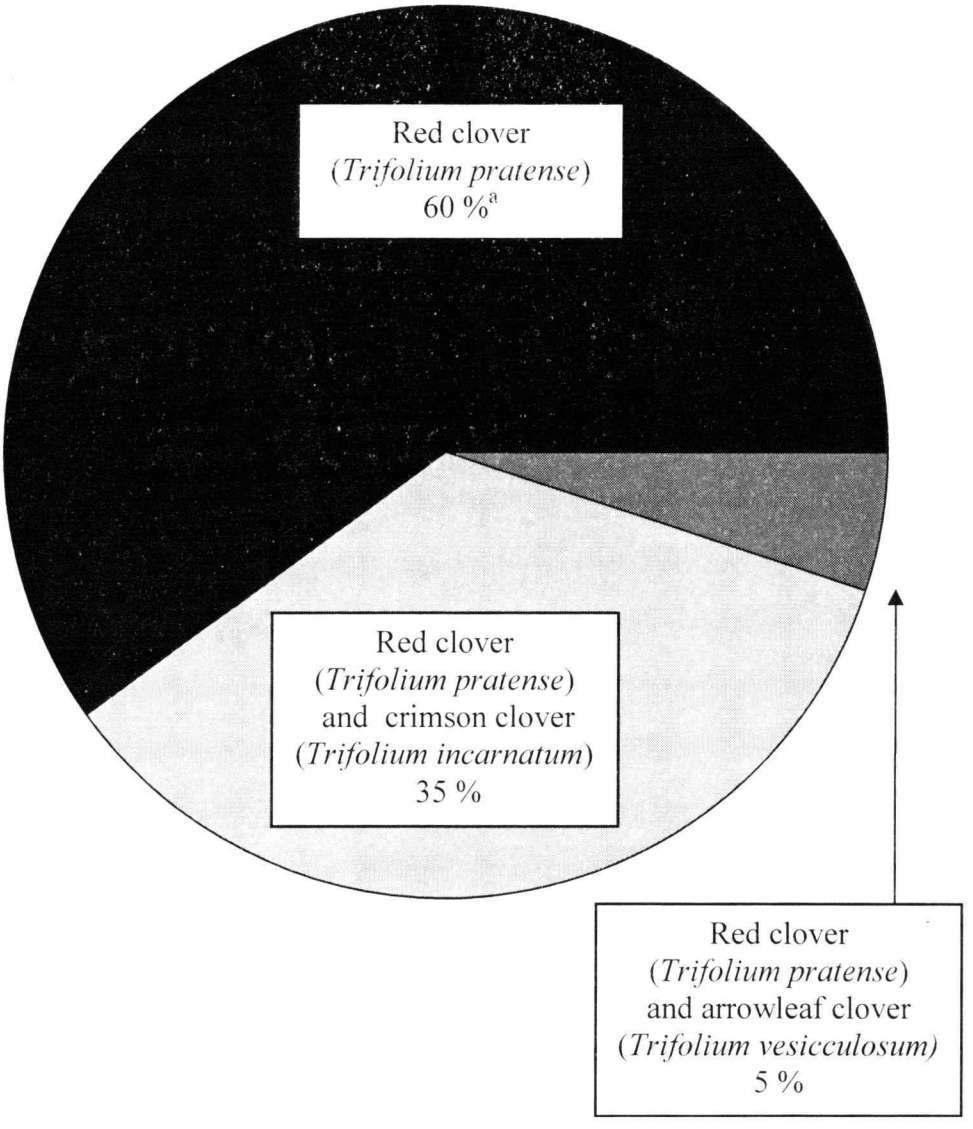
[a]Seed company referred to as "A" thru "I", "Unknown" if the seed company is unknown, or "Destroyed seed" if the seed lot was destroyed.

Figure 4.1. Planting and harvesting regime of red clover (*Trifolium pratense*) grown for seed in small broomrape (*Orobanche minor*) contaminated fields.



[a] Survey responses that included each planting and harvest regime / number of survey responses x 100.

Figure 4.2. Clover seed species grown by respondents of the Oregon small broomrape (*Orobanche minor*) survey.



[a] Survey responses that included each combination of crop species / number of survey responses x 100.

5. SUMMARY AND CONCLUSIONS

5.1 Introduction

A sound understanding of small broomrape (*Orobanche minor* Sm.) biology will provide growers with more cropping system options and tools to manage small broomrape-contaminated sites. Cropping systems for small broomrape-contaminated sites should include crops that decrease the quantity of small broomrape seed from the soil seedbank and provide economic return to the growers. Understanding the introduction and dispersal of small broomrape can prevent further dispersal of small broomrape and other weed species.

5.2 Hosts, False-Hosts, and Non-Hosts

Small broomrape host species important to Pacific Northwest agriculture included alfalfa (*Medicago sativa* L.), arrowleaf clover (*Trifolium vesiculosum* Savi.), carrot (*Daucus carota* L.), celery (*Apium graveolens* L.), common vetch (*Vicia sativa* L.), crimson clover (*Trifolium incarnatum* L.), lettuce (*Lactuca sativa* L.), red clover (*Trifolium pratense* L.), subterranean clover (*Trifolium subterraneum* L.), and white clover (*Trifolium repens* L.). Host crops should not be planted in small broomrape-contaminated sites, unless 100% control of small broomrape can be obtained. Common weed host species in Pacific Northwest agriculture included prickly lettuce (*Lactuca serriola* L.), spotted catsear (*Hypochaeris radicata* L.), and wild carrot (*Daucus carota* L.). Host weeds should be controlled in small broomrape-contaminated sites to prevent further addition of small broomrape seed to the soil seedbank and contamination of other crops with small broomrape seed. Crop false-host species important to Pacific Northwest agriculture included barley (*Hordeum*

vulgare L.), birdsfoot trefoil (*Lotus corniculatus* L.), creeping bentgrass (*Agrostis stolonifers* L.), cucumber (*Cucumis sativa* L.), field corn (*Zea mays* L.), fine fescue (*Festuca rubra* L.), flax (*Linum usitatissimum* L.), Italian ryegrass (*Lolium multiflorum* Lam.), oat (*Avena sativa* L.), orchardgrass (*Dactylis glomerata* L.), perennial ryegrass (*Lolium perenne* L.), snap bean (*Phaseolus vulgaris* L.), sugar pea (*Pisium sativa* L.), sunflower (*Helianthus annuus* L.), sweet corn (*Zea mays* L.), tall fescue (*Festuca arundinaceae* Schreb.), and wheat (*Triticum aestivum* L.). False-host crops can be used to decrease the quantity of small broomrape seed in a small broomrape-contaminated site. Sugar beet (*Beta vulgaris* L.) is a non-host crop important to Pacific Northwest agriculture. Non-host weeds and crops are not likely to change the small broomrape soil seedbank.

Small broomrape attachment quantity to host species was greater in the hydroponic polyethylene bag study than the field study. Available moisture content may have contributed to the differences in small broomrape attachment quantity between field and hydroponic studies. Small broomrape germination and attachment quantity varied among plant species, indicating that plant species produce varying quantities of germination and attachment exudates, or may be more easily parasitized. The hydroponic polyethylene bag screening procedure is a quick and inexpensive method for determining a species host status to small broomrape. However, all species should be evaluated in the field for their host status to small broomrape.

5.3 Influence of Nitrogen

Nitrogen and *Rhizobium* inoculation affected small broomrape attachment to red clover in 1 of 5 experiments. Ammonium treatment to red clover did not increase red clover susceptibility to small broomrape haustorium penetration. Red clover treatment with ammonium based fertilizer is not an effective control measure for small broomrape.

5.4 Influence of *Rhizobium*

Treatments, which included *Rhizobium* inoculation and non-inoculation, affected small broomrape attachment to red clover in only 1 of 5 experiments. *Rhizobia* nodules on the red clover roots did not increase red clover susceptibility to small broomrape haustoria penetration. Red clover inoculation with *Rhizobium* is not a factor in small broomrape attachment to red clover.

5.5 Introduction and Dispersal

Red clover is grown as a rotational crop to enhance fertility, break disease cycles, control weeds, and provide economic return between monocotyledonous crops. Red clover production has likely assisted in the introduction and dispersal of small broomrape in Pacific Northwest.

Eighty percent of the growers with small broomrape-contaminated sites completed the Oregon red clover survey. Growers managed small broomrape contamination by spraying glyphosate, selective hand removal, or mechanical destruction of small broomrape plants.

Parasitism occurred prior to harvest of the first and second seed crops on spring-seeded red clover. However, in fall-seeded red clover, parasitism occurred prior to harvest of the second seed crop but after harvest of the first seed crop. The inability of small broomrape to parasitize first year fall-seeded red clover is possibly due to incompatibility between crop development, weed physiology, soil temperature, and available soil moisture. Fall planting with only 1 seed harvest followed by crop destruction may be a method to utilize red clovers' rotational crop advantages of enhancing fertility, breaking disease cycles, controlling weeds, and providing an economic return between monocotyledonous crops. Growing fall-seeded red clover for one crop cycle in small broomrape contaminated soil requires further research before implementation. Crop rotations among fields and farms were very diversified. Many red clover cultivars were parasitized by small broomrape. Custom operations were not likely to be the introduction or dispersal mechanism for small broomrape with the majority of respondents having no custom operation conducted on their farms. One seed cleaning facility provided 30% of the respondents with red clover seed stock and cleaned 30% of the respondents' seed. The survey results provided evidence that seed cleaning and specifically one seed cleaning facility may have unintentionally assisted in small broomrape seed dispersal; however, all seed lots were tested by Oregon State University's Seed Laboratory and certified to be free from small broomrape seed. Seventy percent of the small broomrape-contaminated sites had either surface water frequently flow across the field, irrigated the field from a water source other than a closed well, or had geese inhabit the field. These mechanisms likely assisted in the dispersal of small broomrape seed.

5.6 Future Research

Future research involving the use of false-hosts should explore the rate at which each false host species depletes small broomrape seed from the soil seedbank. The longevity of small broomrape seed should be determined. The use of polymerase chain reaction (PCR) may be a useful tool to determine the longevity of small broomrape seed, the rate at which false-host species deplete small broomrape seed from the soil seedbank, and in determining if a site or seed lot is contaminated with small broomrape seed.

Development of small broomrape-resistant red clover cultivars would be a management technique for growers with small broomrape-contaminated sites. Herbicide screening and understanding the fate of herbicides in small broomrape and hosts would be useful tools for small broomrape management.

Utilization of false-host in small broomrape-contaminated sites is one method to help manage small broomrape. Further research is needed to understand the biology of small broomrape and to create a successful management system.

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