AN ABSTRACT OF THE DISSERTATION OF

Salam A. Al-thahabi for the degree of Doctor of Philosophy in Crop Science presented on November 9, 2006.

Title: Small Broomrape (*Orobanche minor*) Management Using Wheat (*Triticum aestivum*) as a False Host

Abstract approved:

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Carol A. Mallory-Smith

Small broomrape (*Orobanche minor*) is an obligate, chlorophyll-lacking parasite that parasitizes red clover (*Trifolium pratense*) roots. This study was conducted to develop and implement an integrated, biologically based control program for small broomrape by using wheat as a false host to reduce the soil seed bank. The relationship between temperature and small broomrape seed germination was investigated. Small broomrape seed germination increased as temperature increased from 5 to 20 C. The greatest germination was 75% with the 20 C treatment. For germination to occur, small broomrape must be stimulated by host or false plant exudates. The relationship between temperature and germination stimulant production by red clover and wheat was studied. There were differences in small broomrape germination when exudates from red clover grown for 8 wk were used. Small broomrape germination stimulated by exudates from wheat grown for 4 wk differed among germination temperatures

tested. The least small broomrape germination occurred with the 15 C treatment. The timing of small broomrape germination stimulant production by red clover and wheat relative to crop growth stage was studied. The maximum germination was 77% using exudates produced by red clover at the 3-trifoliolate stage. For wheat, the maximum germination was 25% using exudates produced by 1-leaf wheat. Germination stimulant produced after the tillering stage of wheat was minimal. Red clover produced germination stimulant at all growth stages, while wheat produced germination stimulant only at early stages of development. A study investigated the number of winter wheat rotation life cycles (WRC) that are required to reduce parasitism of subsequent red clover crops. The greatest number of small broomrape attachments and the greatest small broomrape biomass were observed with 0-wheat rotation cycles (0-WRC), and both were reduced if wheat was included in the rotation. Above ground red clover biomass was less with the 0-WRC treatment than any of the other treatments. Wheat in rotation with red clover has the potential to reduce small broomrape impact on red clover.

Small Broomrape (*Orobanche minor*) Management Using Wheat (*Triticum aestivum*) as a False Host

by

Salam A. Al-thahabi

A DISSERTATION

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APPROVED:

Major Professor, representing Crop Science

Head of the Department of Crop and Soil Science

Dean of the Graduate School

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

Salam A. Al-thahabi, Author

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CONTRIBUTION OF AUTHORS

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

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF APPENDIX FIGURES

DEDICATION

I would like to dedicate this project to my mother and my kids, Ghaith and Hadi.

Small Broomrape (*Orobanche minor*) Management Using Wheat (*Triticum aestivum*) as a False Host

CHAPTER 1

General Introduction

Broomrapes (*Orobanche* spp.) are obligate, chlorophyll-lacking root parasites that parasitize many dicotyledonous species and cause damage to vegetable and field crops worldwide (Parker and Riches 1993; Foy et al. 1989). Broomrapes are capable of inflicting yield losses, ranging from zero to complete crop failure, depending on the level of infestation (Foy et al. 1989; Manschadi et al. 1996), and present a major limitation to agriculture in Africa, Southern Europe, and the Middle East (Abu-Irmaileh 1998; Manschadi et al. 1996; Parker and Riches 1993).

The majority of broomrapes are found in the warm and temperate parts of the northern hemisphere, especially the Mediterranean region (Sauerborn 1991), but some species have spread to other parts of the world. Egyptian broomrape (*O. aegyptiaca)* occurs mainly in southeastern Europe, northeastern Africa, and the Middle East, while, branched broomrape (*O. ramose)*, which is closely related to Egyptian broomrape is mostly found in the Middle East. Crenate broomrape (*O. crenata)* is restricted to the Middle East. Small broomrape (*Orobanche minor* Sm.) is a native of Europe, but is

now found in the Middle East, Africa, New Zealand, Australia, and North America (Foy et al. 1989). In Oregon, small broomrape was reported in red clover fields in 1998. In 2000 and 2001, small broomrape was found in 15 and 22 red clover fields, respectively (Colquhoun et al. 2001). Small broomrape is a federally listed noxious weed in the United States that has quarantine significance for many of Oregon's trading partners (Colquhoun et al. 2001).

Small broomrapes are annuals that reproduce by seeds. Each small broomrape plant can produce over 1,000,000 seeds per plant (Pieterse 1979). The seeds are dust-like and can be spread by wind, water, machinery, contaminated crop seeds, and animals. Its seeds remain dormant in the soil for 10 years or more until induced to germinate by root exudates (Foy et al. 1989; Parker and Riches 1993). Upon germination, the small broomrape seed develops a tube-like radicle that attaches to the host root surface. After attachment to the host root, the radicle develops a haustorium, which penetrates the root and forms connections to the vascular system of the host plant (Parker and Riches 1993). The portion of the parasite remains outside of the root tissue then develops into a tubercle, that initiates a floral meristem that produces a floral spike (Foy et al. 1989; Parker and Riches 1993). The small broomrape flower stalks emergences from the tubercle about 4 to 5 months after initial parasitic attachment to red clover (Lins et al. 2005).

Small broomrape seeds require the presence of germination stimulant, a chemical signal for germination (Foy et al. 1989; Kasasian 1973). Small broomrape

germination stimulants include alectrol and orobanchol, which are analogues of strigolactones and have been isolated from the root exudates of the host red clover (Yokota et al. 1998). However, the seeds need to be exposed to a moist environment (called preconditioning) for several days at a suitable temperature (optimum 15 to 20 C) before seeds respond to germination stimulants (Kebreab and Murdoch 1999; Pieterse 1981; van Hezewijk 1994).

Several factors influence germination of broomrapes in the soil including temperature, moisture, pH, nutrients, soil type, and stimulants produced by host plants. Studies on the effect of temperature on germination of Egyptian broomrape, crenate broomrape, sunflower broomrape (*O. cumana)* indicated that each species had a specific optimum temperature range for germination and development which reflected its geographical distribution (Sauerborn 1991). Kebreab and Murdoch (1999b) reported that optimum temperatures for germination of Egyptian broomrape, small broomrape, and crenate broomrape were 18 to 21, 17 to 20 and 18 C, respectively. Kebreab and Murdoch (2000) found that Egyptian broomrape germination increased as temperature increased from 5 to 20 C and decreased above 26 C, and that maximum germination occurred at 20 to 26 C.

Broomrapes parasitize members of many plant families including Asteraceae (Romanova et al. 2001), Fabaceae (Goldwasser et al. 1997), and Solanaceae (Romanova et al. 2001). Plants species are classified as hosts, false hosts, or non-hosts. False host plants produce chemical exudates that promote small broomrape

germination, but not attachment (Joel et al. 1995). False hosts crops have been identified for various broomrape species. Sorghum (*Sorghum bicolor* L.), corn (*Zea mays* L.), cucumber (*Cucumis sativus* L.) and flax (*Linum usitatissimum* L.) have been used as false host crops for branched broomrape (*O. ramose*) (Abu-Irmaileh 1984; Parker and Riches 1993), while sorghum, barley *(Hordeum vulgare* L.), and hairy fruit vetch (*Vicia dasycarpa* spp.) have been used as false hosts for crenate broomrape (*O. crenata*) (Kasasian 1973; Linke et al. 1991b). Yoneyama et al. (2001) reported basil (*Ocimum basilicum* L.), carrot (*Daucus carota* L.), cucumber, corn, onion (*Allium cepa* L.), and soybean [*Glycine max* (L.) Merr.], as false hosts for small broomrape (Yoneyama et al. 2001). Ross et al. (2004) identified wheat (*Triticum aestivum* L.) as a false host that induced 25 to 40% germination of small broomrape seeds, and Lins et al. (2006) reported that wheat induced 20 to 70% germination of small broomrape seeds.

Crop rotation with false hosts has been practiced as a control measure for parasitic weeds. False host crops decreased the crenate broomrape seed bank by 30% in one cropping cycle (Linke et al. 1993). Kleifeld et al. (1994) tested false hosts crops to decrease infestation of Egyptian broomrape (*O. aegyptiaca* Pers.), and reported that growing flax in two successive winter seasons or one summer cropping with mung beans (*Phaseolus aureus* Roxbg.) reduced early infestation of the parasite and significantly increased tomato (*Lycopersicum esculentum* Mill.) vigor and production. False host crops decreased the crenate broomrape seed bank by 30% in one cropping

cycle (Linke et al. 1993). In Oregon, USA, wheat was reported to be a false host of small broomrape (Ross et al., 2004), and therefore, has the potential to be included in an integrated small broomrape management system.

Chemical control of broomrapes has been achieved through soil fumigation and herbicide soil and foliar applications (Foy et al. 1989). Imazamox herbicide applied after small broomrape flower stalk emergence prevented further emergence but did not prevent small broomrape seed production, whereas imazamox applied before flower stalk emergence prevented small broomrape emergence and seed production (Colquhoun et al. 2002). Imazamox applied postemergence to red clover and preemergence to small broomrape provided excellent control in Oregon (Lins et al. 2005).

Small broomrape management in red clover seed production may prove to be difficult for several reasons including, the high amount of seed production, viability of seed in the soil over several years, lack of seed germination in the absence of a chemical trigger from a suitable host, the growth habit, and close association with the host crop. However, integrating chemical and cultural practices may lead to effective control.

The overall goal of this project was to help refine and provide data for an integrated, biologically based program for small broomrape control using wheat as a false host to reduce the soil seed bank. Objectives of the research were: 1) to determine the relationship between temperature and small broomrape seed

germination, 2) to determine the relationship between temperatures during growth of winter wheat and the red clover and small broomrape stimulant production, 3) to determine the timing of small broomrape germination stimulant production of wheat and red clover relative to crop growth stage, and 4) to determine the number of winter wheat life cycles required to reduce parasitism of the subsequent red clover crop.

CHAPTER 2

Effect of Temperature on Small Broomrape (*Orobanche minor*) Germination

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ABSTRACT

Small broomrape is a holoparasitic weed that has been identified in the Pacific Northwest and has contaminated a limited number of red clover fields in Oregon. The relationship between temperature and small broomrape seed germination was studied in controlled-environment growth chambers. Small broomrape seed germination increased as temperature increased from 5 to 20 C. Germination at 25 C was less than at 15 and 20 C. The greatest germination was 75% with the 20 C treatment. Maximum germination was 14 DAT for seeds exposed to 20 and 25 C, while maximum germination was delayed until 21 and 28 DAT at 15 and 10 C, respectively. There was no small broomrape seed germination at 5 C.

Nomenclature: Small broomrape, *Orobanche minor* Sm., red clover, *Trifolium pratense* L.

Key words: Germination stimulant, parasitic plant, seed germination, temperature.

Abbreviations: DAT, days after treatment.

INTRODUCTION

Broomrapes (*Orobanche* spp.) are obligate chlorophyll-lacking root holoparasites that parasitize many dicotyledonous species and cause severe damage to vegetable and field crops worldwide, especially in the Mediterranean area (Musselman 1980; Parker and Riches 1993). Broomrapes are capable of inflicting yield loss or complete crop failure, depending on the level of infestation (Foy et al. 1989; Manschadi et al. 1996). Red clover fields infested with small broomrape were documented in Oregon in 1998. This was the first report of a seed crop in the United States to be contaminated with small broomrape (Colquhoun et al. 2001). Small broomrape also is a parasite of several other crop and weed species (Ross et al. 2005).

Several researchers have studied indirectly the effect of temperature on broomrape parasitism by changing the host sowing date. Delaying the sowing dates of faba beans (*Vicia faba* L.) and lentils (*Lens culinaris* Medik.) from autumn to winter reduced crenate broomrape (*O. crenata* Forsk.) and Egyptian broomrape (*O. aegyptiaca* Pers.) infection levels (Foy et al. 1991; Grenz et al. 2005; Linke et al. 1991a; Manschadi et al. 2001; Mesa-Garcia and Garcia Torres 1986; ter Borg 1986; van Hezewijk 1994). Similar responses to delayed sowing were reported for crenate broomrape infection in carrot (*Daucus carota* L.) (Eizenberg et al. 2001), sunflower

(*Helianthus annuus* L.) (Castejon-Munoz et al. 1993), and chickpea (*Cicer arietinum* L.) (Kukula et al. 1985).

Broomrape seed requires a moist environment, called preconditioning, for a certain period of time at a suitable temperature (optimum 15 to 20 C) before the seeds become responsive to germination stimulants (Joel et al. 1995; Kebreab and Murdoch 1999a; Musselman 1980). Gibot-Leclerc et al. (2004) found that the optimum preconditioning temperature for branched broomrape (*O. ramosa* L.) was 20 C. Matusova et al. (2004) reported an optimal preconditioning temperature of 21 C for sunflower broomrape (*O. cumana* Wallr.) and 30 C for witchweed (*Striga hermonthica*). Song et al. (2005) found that the greatest germination of branched broomrape, Egyptian broomrape, and small broomrape seeds was observed in seeds preconditioned at 18 C for 7 d followed by germination stimulation at 18 C. Prolonged preconditioning particularly at lower conditioning temperatures may lead to an induction of secondary dormancy in crenate broomrape and Egyptian broomrape (Kebreab and Murdoch, 1999a; Song et al. 2005).

The effect of temperature on seed germination of broomrape also has been studied. Kasasian (1973) reported a maximum germination at 18 C for Egyptian broomrape seeds conditioned at temperatures between 8 to 28 C. Pieterse (1979) considered temperatures of about 20 to 25 C to be most favorable for germination of broomrape seeds. Kebreab and Murdoch (1999a) modeled in vitro the effect of temperature on the germination rate of four broomrape species. Maximum germination

of small broomrape was obtained after 2 wk at a temperature range of 17 to 20 C. Kebreab and Murdoch (2000) found that Egyptian broomrape germination increased with increased temperature from 5 to 20 C and decreased above 26 C, and that maximum germination occurred from 20 to 26 C.

This study was conducted to determine the relationship between temperature and small broomrape seed germination.

MATERIALS AND METHODS

Small broomrape seeds were collected from inflorescences of plants parasitizing red clover in a field in Oregon. The inflorescences were dried at room temperature and seeds were cleaned using 100-µm sieves and stored in the dark at room temperature.

Approximately 25 small broomrape seeds were placed in 1 cm diameter glass fiber filter paper (GFFP) disks. Four GFFP disks were placed in a Petri dish containing 2 layers of 7 cm diameter filter paper moistened with 2.5 ml deionized water. To precondition the small broomrape seeds, the Petri dishes were wrapped with aluminum foil and placed in a growth chamber at 20 C for 14 d preconditioning (as suggested by Kroschel 2001). After preconditioning, 4 GFFP discs with 25 seeds each were transferred to a Petri dish containing 2 layers of 7 cm diameter filter paper moistened with 2.5 ml of 0.001 mg/ L GR_{24} a synthetic broomrape germination

stimulant. Petri dishes were sealed and wrapped with aluminum foil and placed in growth chambers set at 5, 10, 15, 20, and 25 C for 4 wk.

Germinated small broomrape seeds were counted 7, 14, 21, and 28 d after treatment (DAT) using a stereoscopic microscope. Percent germination was determined from 4 discs in each Petri dish, and the cumulative germination percentage was calculated 7, 14, 21, 28 DAT.

The experiment was arranged in a completely randomized design with 6 replicates of each treatment and was repeated. Non-linear models of seed germination percentage at each observation as a function of temperature were fitted separately. The normality of the data was investigated using the UNIVARIATE procedure of SAS and data were log transformed to improve normality but the back transformed data are presented. Data was analyzed using PROC MIXED for ANOVA and PROC REG for regression in SAS (SAS 2002) and treatment means were separated at the 95% confidence limit ($p \leq 0.05$).

RESULTS AND DISCUSSION

There was no an experiment by treatment interaction, and therefore, experimental results were combined. All treatments received an identical and optimal conditioning treatment (14 d at 20 C in water), so the differences in the subsequent germination can be attributed to differences in temperature.

Correlations were found between small broomrape cumulative germination and temperature ($y = -20.2 + 2.22 x - 1.02 x^2$, $R^2 = 0.95$). There were significant quadratic interactions for total small broomrape germination and temperature ($p<0.0001$). Cumulative small broomrape germination percentage 28 DAT was greatest at 20 C where germination was 75%, while the lowest germination was 0% at 5 C (Figure 1). Germination increased as temperature increased from 5 to 20, but dropped at 25 C.

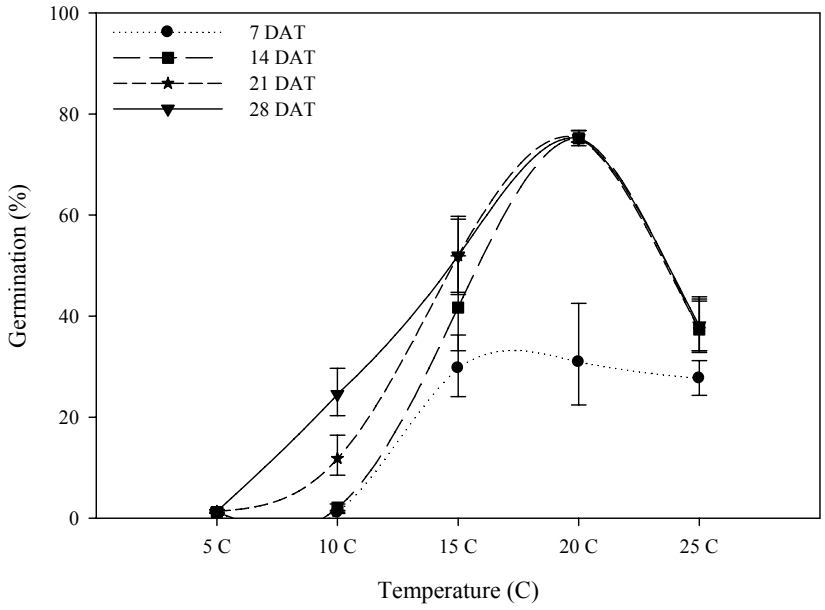

Figure 2.1: Effect of temperature on small broomrape germination rate when exposed to GR_{24}. Bars indicate 95% confidence limits.

There were differences among treatments ($p <0.0001$) when the small broomrape seeds were tested 7, 14, 21, and 28 DAT (Figure 2). Maximum small

broomrape germination was obtained 14 DAT at 20 and 25 C, while maximum small broomrape germination was delayed until 21 and 28 DAT at 15 and 10 C, respectively. No small broomrape seed germinated at 5 C.

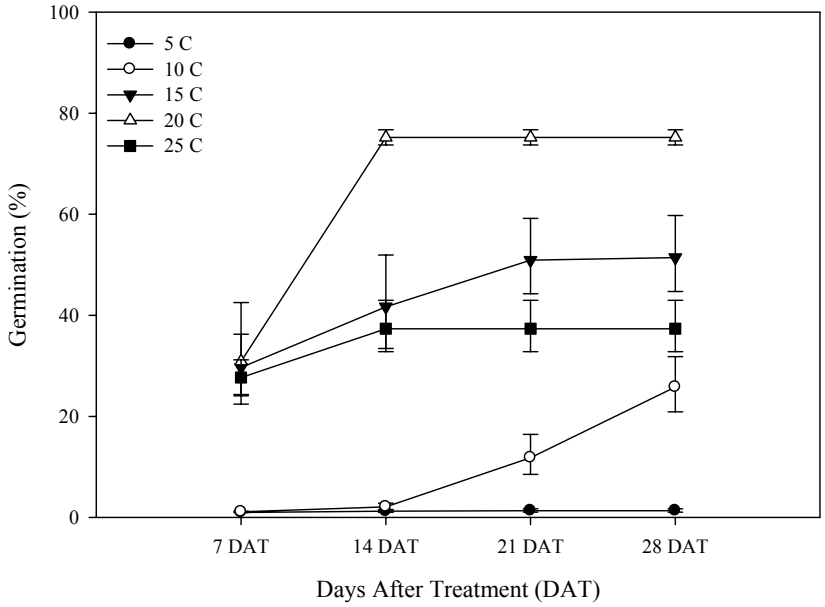

Figure 2.2: Effect of days after treatment (DAT) and temperature on the germination rates of small broomrape when exposed to GR_{24}. Bars indicate 95% confidence limits.

Small broomrape is an annual plant that in Oregon germinates in early spring and produces seed in summer. Temperature and small broomrape parasitism are strongly related (Eizenberg et al. 2004, 2005). Small broomrape parasitism often increases as temperature increases. In our study, we found temperatures of 15 to 25 C to be most favorable for small broomrape seed germination. This finding is in

agreement with the results obtained by Kebreab and Murdoch (2000) who reported that germination of Egyptian broomrape increased as temperature increased from 5 to 20 C and decreased above 26 C. Pieterse (1979) considered temperatures of 20 to 25 C to be most favorable for germination of broomrape seeds. However, Kebreab and Murdoch (1999b) modeled the effect of temperature on germination rate of four broomrape species and found that optimum temperatures for germination of small broomrape was obtained after 2 wk at a temperature range of 17 to 20 C.

The temperature dependent susceptibility to broomrape was observed in legume crops. Lentils, faba beans, and chickpea were more sensitive to infection by crenate broomrape in early fall plantings than in winter plantings (Kukula et al. 1985). Mesa-Garcia and Garcia-Torres (1986) reported that delay of faba bean planting from mid-October to mid-December and mid-January reduced crenate broomrape infection with an increase in crop yield. Eizenberg et al. (2005) studied the temperature-dependent relationship between small broomrape and red clover, and reported that small broomrape parasitism and temperature were strongly related. In addition, small broomrape tubercle initiation was delayed by low temperature.

In our study, no small broomrape seed germination was obtained at 5 C and very low germination at 10 C. Temperature has a primary influence on seed dormancy and germination, affecting both the capacity for germination by regulating dormancy and the rate or speed of germination in non-dormant seeds. Kebreab and Murdoch (1999a) developed a quantitative model for the effects of temperature on primary and

secondary dormancy in broomrape seeds. They reported a positive, linear relationship between the rate of loss of primary dormancy and temperature from 10 to 30 C in Egyptian broomrape and from 10 to 25 C in crenate broomrape. In addition, the effects of temperature on the induction of secondary dormancy vary among broomrape species. Generally, secondary dormancy occurred most slowly at 20 to 25 C and increased as temperature decreased to 10 C or increased to 30 C; however, secondary dormancy is less likely to be induced in small broomrape than in other broomrape species (Kebreab and Murdoch 1999a).

In conclusion, temperature had a substantial effect on small broomrape sensitivity to germination in our study. Small broomrape seed germination increased as temperature increased 5 to 20 C. the results of the research will be used to help refine the predictive small broomrape development models developed by Eizenberg et al. (2005) for the Pacific Northwest USA.

SOURCES OF MATERIALS

Whatman® qualitative filter paper. Whatman International ltd, Maidstone, England.

VWR qualitative filter paper. VWR Scientific Product, West Chester, PA 19380

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CHAPTER 3

The relationship between temperature and plant age on small broomrape germination stimulant production by red clover (*Trifolium pratense*) and wheat (*Triticum aestivum*)

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ABSTRACT

Small broomrape is a holoparasitic plant that lives on roots of red clover and several other host crop and weed species. Wheat (*Triticum aestivum*) has been shown to be a false host for small broomrape. Hosts and false hosts produce stimulants that induce small broomrape germination. The relationship between temperature and the germination stimulant production by red clover and wheat was studied in controlled-environment growth chambers at 10, 15, 20, and 25 C for either 4 or 8 wk. There were no differences in small broomrape germination when exudates of red clover grown for 4 wk were used. There were differences in germination when exudates from red clover grown for 8 wk were used. The greatest germination percentage was 35% with the 25 C treatment, while the lowest germination percentage was 2% with the 10 C treatment. Small broomrape germination stimulated by exudates from wheat grown for 4 wk differed by temperature. The greatest germination percentage was 24% with the 10 C treatment, and the least germination of 7% occurred with the 15 C treatment. There were no differences in germination when exudates from wheat exudates grown for 8 wk were used.

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

Key words: False host, germination stimulant, integrated weed management, parasitic plant, red clover, seed germination, temperature**,** wheat.

INTRODUCTION

Small broomrape is a holoparasitic plant that lives on roots of red clover and several other crop and weed species. Broomrapes lack photosynthetic capacity, so they rely completely on hosts for carbon, water, and nutrients needed for growth (Musselman 1980; Parker and Riches 1993). Broomrapes are capable of inflicting high yield losses on affected crops and present a major limitation to agriculture in Africa, Southern Europe, and the Middle East (Abu-Irmaileh 1998; Manschadi et al. 1996; Parker and Riches 1993). Small broomrape is a federally listed noxious weed in the USA, and red clover is the first seed crop in the USA to be contaminated with small broomrape (Colquhoun et al. 2001, 2002).

Each small broomrape plant can produce over 1,000,000 seeds (Pieterse 1979). The seeds remain dormant in the soil for long periods of time until induced to germinate by root exudates. Host plant species produce chemical compounds that trigger the parasitic seed germination and attachment, while false host plants produce chemical exudates that only promote broomrape germination (Joel et al. 1995). Wheat has been identified as a false host for small broomrape (Ross et al. 2004; Lins et al. 2005). Two germination stimulants for small broomrape, alectrol and orobanchol, have been isolated from the root exudates of red clover (Yokota et al. 1998). In addition, small broomrape seeds respond to synthetic germination stimulants,

strigolactone analogues (Abdel Halim et al. 1975; Cook et al. 1966, 1972; Johnson et al. 1976; Saghir 1986; Stewart and Press 1990; Zahran 1982).

Broomrape seeds require preconditioning; exposure to a moist environment called for certain period of time at a suitable temperature (optimum 15 to 20 C), before the seeds become responsive to germination stimulants (Joel et al. 1995; Kebreab and Murdoch 1999a; Musselman 1980). Song et al. (2005) found that the greatest germination of branched broomrape (*O. ramosa*), Egyptian broomrape (*O. aegyptiaca*) and small broomrape seeds was observed in seeds preconditioned at 18 C for 7 d followed by germination at 18 C. Several researchers have studied indirectly the effect of temperature on broomrape parasitism by changing the host plants sowing dates. Delaying the sowing dates from autumn to winter reduced the infection levels (Foy et al. 1991; Kukula et al.1985; Linke et al. 1991a; Mesa-Garcia and Garcia-Torres 1986; ter Borg 1986; van Hezewijk 1994). The effect of temperature on seed germination of broomrape also has been studied. Kasasian (1973) reported that 18 C is the optimal temperature for crenate broomrape (*O. crenata*) seed preconditioning and germination. Pieterse (1979) considered temperatures of 20 to 25 C to be most favorable for broomrape germination. Kebreab and Murdoch (1999b) reported that optimum temperatures for germination of Egyptian broomrape, small broomrape, and crenata broomrape were 18 to 21, 17 to 20 and 18 C, respectively.

The objective of this study was to determine the effect of temperature on production of germination stimulant by red clover and wheat and subsequent small broomrape germination.

MATERIALS AND METHODS

Small broomrape seed was collected from inflorescences of plants parasitizing red clover in a field in Oregon. The inflorescences were dried at room temperature, and seeds were cleaned using 100-µm sieves and stored in the dark at room temperature until studies were initiated.

'Kenland' red clover and 'Foote' winter wheat plants were grown in 10 cm^2 pots filled with pure sand. Plants were watered regularly with deionized water to maintain adequate moisture. Immediately after planting, pots were transferred to controlled environment growth chambers supplying 12 hours of supplemental light. Growth chamber temperature treatments were 10, 15, 20, and 25 C. Plants were thinned to 1 in each pot 2 wk after planting. Ten ml of half-strength Hoagland's nutrient solution was added to the pots once a week.

Four and 8 wk after planting (WAP) the plants were removed from the sand, washed, and immersed in deionized water in a flask. Water in each flask was maintained at 100 ml and 125 ml for wheat and red clover, respectively, during the 5 d

period. The seedling was supported in the flask with non-absorbent cotton so that the roots were immersed in water, and was allowed to grow for 5 d.

Approximately 25 broomrape seeds were placed in 1 cm diameter glass fiber filter paper (GFFP) disks. Four GFFP disks were placed in a Petri dish containing 2 layers of 7 cm diameter filter paper moistened with 2.5 ml deionized water. The Petri dishes were wrapped with aluminum foil and placed in a growth chamber at 20 C for 14 d for preconditioning (as suggested by Kroschel 2001). After preconditioning, 4 GFFP discs with 25 seeds each were transferred to a Petri dish containing 2 layers of 7 cm diameter filter paper moistened with 2.5 ml of root exudates from the extraction described previously. Petri dishes were sealed and wrapped with aluminum foil and placed in dark growth chambers set at 20 C for 2 wk.

Weekly observations were made by using a stereoscopic microscope to determine the rate of small broomrape seed germination. Percent germination was determined from 4 discs in each Petri dish and the average percentage of small broomrape germination calculated. The experiment was arranged in a completely randomized design with six replications for each treatment, and the experiment was repeated. Statistical analysis was performed using SAS and ANOVA was conducted. Treatment means were separated using 95% confidence limits.

RESULTS AND DISCUSSION

There was no experiment by treatment interaction. Therefore, experimental results were combined and represented as 12 replications for each treatment. All treatments received an identical and optimal conditioning treatment (14 d at 20 C in water), so the differences in the subsequent germination must be due to variability in germination stimulant production during the germination test.

Small Broomrape Seed Germination with Red Clover Exudates

There were no differences among treatments ($P < 0.1581$) when the exudates from red clover grown for 4 wk at any temperature were used. The germination ranged from 21% with the 20 C treatment to 32% with the 10 C treatment (Figure 3 1).

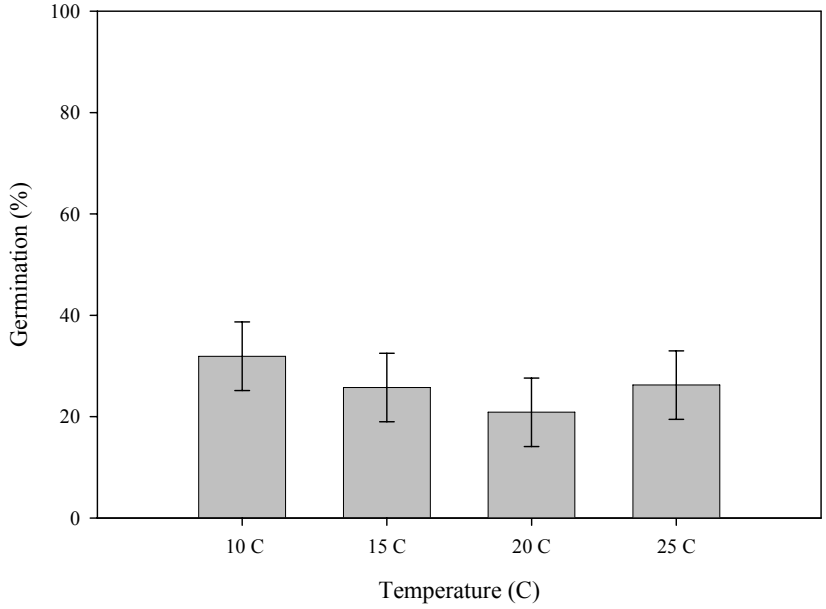

Figure 3.1: Relationship between temperature and red clover germination stimulant production 4 WAP on small broomrape seed germination. Bars indicate 95% confidence limits.

There were differences among treatments ($P < 0.0001$) when the exudates from red clover grown for 8 wk were used. There was a reduction in germination when the exudates from red clover plants grown at 10 C for 8 wk were used when compared to plants grown at 15, 20, and 25 C. The greatest germination was observed in the 25 C treatment where germination was 35%, while the lowest germination was 2% in the 10 C treatment (Figure 3.2).

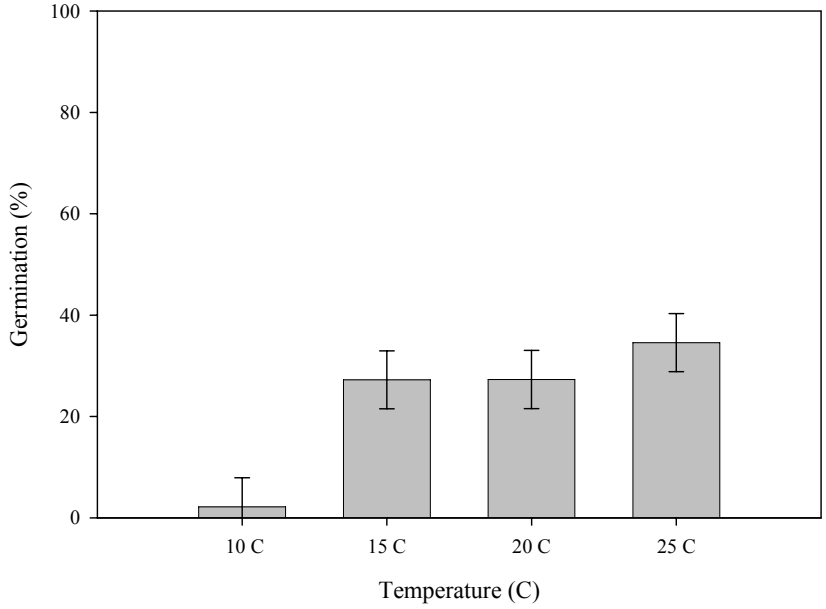

Figure 3.2: Relationship between temperature and red clover germination stimulant production 8 WAP on small broomrape seed germination. Bars indicate 95% confidence limits.

Small broomrape is an annual plant that germinates in early spring and produces seed in summer in Oregon. Temperature and small broomrape parasitism are strongly related (Eizenberg et al. 2005). Small broomrape parasitism often increases as temperature increases. In our results, a reduction in germination was observed when the exudates from plants grown at 10 C for 8 wk were used. This reduction in germination might be due to long exposure to low temperature which resulted in low production of the germination stimulant, while short exposure to low temperature had no effect on the germination stimulant. Extracts from red clover grown at 10 C for 8

wk were not effective at stimulating germination. However, temperatures of 15 to 25 C seem to be suitable for the production of the red clover germination stimulant.

The findings of our study are in agreement with those results of Eizenberg et al. (2004). They reported that small broomrape parasitism and temperature were strongly related and that low temperature delayed tubercle initiation. In other research, temperature-dependent susceptibility to broomrape was observed in legume crops. Lentil (*Lens culinaris* Medik.), faba beans (*Vicia faba* L.), and chickpea (*Cicer arietinum* L.) were more sensitive to infection by crenate broomrape in early fall plantings than in winter plantings (Kukula et al. 1985). Delay of faba bean planting from mid-October to mid-December and mid-January reduced crenate broomrape infection with an increase in crop yield (Mesa-Garcia and Garcia-Torres 1986). Manschadi (2001) suggested that the timing of germination, attachment and further development stages of crenate broomrape were related to soil temperature and not affected by faba bean growth stage. Similar dependence on temperature also was observed in sunflower (*Helianthus annuus* L.) where early planting dates in cool winter temperatures reduced sunflower broomrape (*Orobanche cumana* Wallr.) infection (Castejon-Munoz et al. 1993). Our study with root extracts partly explained small broomrape infection of red clover in the field with low temperatures in the early fall and winter (Eizenberg et al. 2005). These results can be interpreted as an ecological mechanism for small broomrape adaptation to its main crop host.

Small Broomrape Seed Germination with Wheat Exudates

Differences existed among treatments ($P < 0.0053$) when the exudates from wheat grown for 4 wk at 10, 15, 20, and 25 C were used. Small broomrape germination was greatest in response to wheat grown at the 10 C treatment where germination was 24%, while the lowest germination (8%) was observed in the 15 C treatment. There was no difference between 20 C and 25 C treatments, where germination was 14%, and 21%, respectively (Figure 3.3).

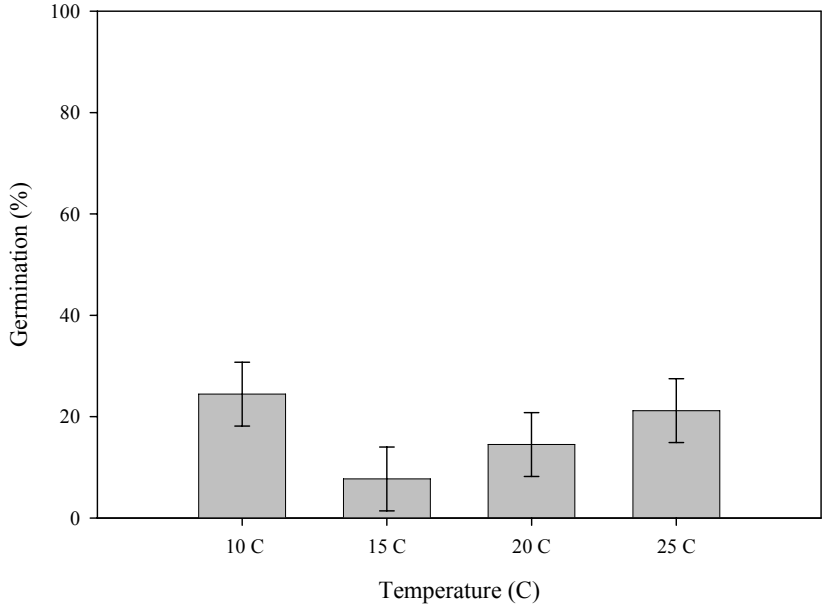

Figure 3.3: Relationship between temperature and wheat germination stimulant production 4 WAP on small broomrape seed germination. Bars indicate 95% confidence limits.

There was no difference among treatments (P <0.2793) when the exudates from wheat grown for 8 wk at any temperature were used. The germination ranged from 3% to 9% (Figure 3.4).

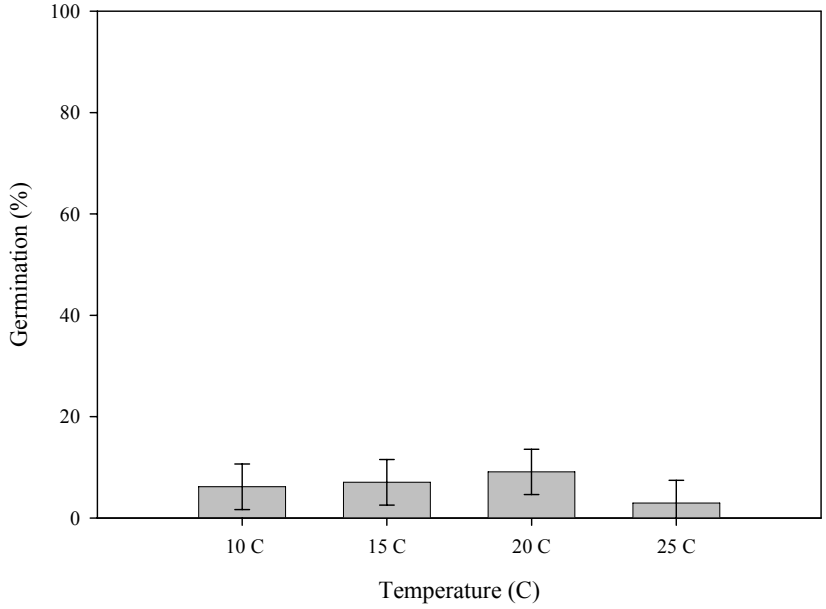

Figure 3.4: Relationship between temperature and wheat germination stimulant production 8 WAP on small broomrape seed germination. Bars indicate 95% confidence limits.

Wheat produces chemical exudates that promote small broomrape germination, but not further development. Ross et al. (2004) reported 25% to 40% small broomrape germination with wheat. In our results, significant differences in germination percentage among temperatures were observed when the exudates from wheat grown for 4 wk were used. Germination ranged from 8% to 24% with germination greatest with the 10 C treatment. There are at least two possible explanations for this result. First, wheat produced more germination stimulant at 10 C compared to other temperatures. Second, the 10 C temperature slowed the growth of wheat which

resulted in wheat at 2- to 3- leaf stages, and this growth stage produced more germination stimulant than is produced by wheat at later growth stages. In another study, wheat produced maximum germination stimulant at the 1- leaf stage and germination stimulant produced after tillering was minimal (see Chapter 4).

Very low germination percentages were observed when the exudates from wheat grown for 8 wk were used. This result is in agreement with Sunderland (1960), who concluded that the synthesis of *Striga hermonthica* and small broomrape germination stimulants decreased with maturity of maize roots. Hameed et al. (1973) reported that root exudates of marigold (*Tagetes erecta* L.) and peanut (*Arachis hypogaea* L.*)* exhibit qualitative and quantitative changes in their composition with the increasing age of the plant.

Our results show that wheat and red clover produce stimulants differently in response to different temperatures; this is in agreement with Awad et al. (2006) who reported that 5-deoxy-strigol was one of major strigolactones, which stimulate small broomrape germination stimulant (in the root exudates of gramineous plants). However, root exudates of red clover that produced orobanchol and alectrol did not contain detectable amounts of 5-deoxy-strigol (Yokota et al. 1998).

In conclusion, long exposure to low temperature reduced germination stimulant production in red clover. This suggests that germination stimulants are either not produced or are degraded at low temperatures leading to low parasitism in the winter. Wheat exudates were much less effective at inducing small broomrape

germination, suggesting that either the quantity or activity of stimulant produced was lower or different germination stimulants were produced.

SOURCES OF MATERIALS

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Whatman® qualitative filter paper. Whatman International ltd., Maidstone, England.

VWR qualitative filter paper. VWR Scientific Product, West Chester, PA 19380

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CHAPTER 4

The timing of small broomrape germination stimulant production by red clover (*Trifolium pratense*) and wheat (*Triticum aestivum*)

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ABSTRACT

Broomrapes (*Orobanche* spp.) are root holoparasitic plants that cause severe damage to economically important crops, especially in Mediterranean countries. In Oregon, some red clover fields are infested with small broomrape (*O. minor)*. Small broomrape seeds germinate only in response to germination stimulants present in exudates from roots of hosts or false hosts. False hosts produce chemical exudates that promote small broomrape germination, but not further development. In previous research, wheat was shown to be a false host. Two studies were conducted to investigate the timing of small broomrape germination stimulant production by red clover and wheat relative to crop growth stage. Red clover produced germination stimulant at all growth stages tested. The maximum germination was 77% using exudates produced by red clover at the 3-trifoliolate stage. Minimum germination of 29% occurred when red clover exudates from the 1-trifoliolate stage was used. For wheat, the maximum germination was 25% using exudates produced by 1-leaf wheat. Germination stimulant produced after the tillering stage was minimal. Although wheat exudates resulted in less small broomrape germination than did the exudates from red clover, growing wheat in an infested field could be an important component of a plan for small broomrape integrated control.

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

Key words: False host, germination stimulant, integrated weed management, parasitic plant, red clover, seed germination, wheat.

INTRODUCTION

Broomrapes (*Orobanche* spp.) are obligate, chlorophyll-lacking root parasites that parasitize many dicotyledonous species and cause severe damage to vegetables and field crops worldwide (Parker and Riches 1993). Broomrapes obtain water, minerals and organic compounds from the host plant which results in lower host biomass accumulation (Baker et al. 1995). Small broomrape, a federally listed noxious weed in the USA was found in Oregon in 1998 (Colquhoun et al. 2002). Small broomrape parasitizes red clover, as well as other weedy plant species in Oregon (Ross et al. 2004).

Small broomrape seeds require the presence of germination stimulant, a chemical signal for germination (Foy et al. 1989; Kasasian 1973). However, before seeds respond to germination stimulants they need to be exposed to a moist environment (called preconditioning or conditioning) for several days at a suitable temperature (optimum 15 to 20 C). A conditioning period for 7 to 21 days is generally required for a maximize response to germination stimulants (Kebreab and Murdoch 1999b; Pieterse 1981), but prolonged preconditioning results in a decrease in responsiveness of seeds to the stimulants (Brown et al. 1951; Kebreab and Murdoch 1999; van Hezewijk 1994).

Plant species are classified as host plants, false host plants, and non-host plants. Host plant species produce chemical exudates that promote broomrape germination and attachment, while false host plants produce chemical exudates that only promote broomrape germination (Joel et al. 1995). Parker and Riches (1993) reported sorghum (*Sorghum bicolor* L.), corn (*Zea mays* L.), mung bean (*Phaseolus aureus* Roxb.), and cucumber (*Cucumis sativus* L.) as false hosts for branched broomrape (*O. ramose* L.). Research by Ross et al. (2004) and Lins et al. (2005) identified wheat to be an effective false host for small broomrape. Small broomrape seed false hosts can be used to deplete parasites from the soil seed bank (Ross et al. 2004).

Three germination stimulants (known as strigolactones) have been identified from host and non-host plant root exudates and are widely used to induce germination of Striga and broomrape seeds in laboratory studies. Germination stimulants for Striga produced by maize and sorghum were identified as strigol (Siame et al. 1993) and sorgolactone (Hauck et al. 1992), respectively. Small broomrape germination stimulants include alectrol and orobanchol, which are analogues of strigolactones and have been isolated from the root exudates of the host red clover (Yokota et al. 1998).

The timing of small broomrape germination stimulant production by red clover and wheat has not been determined. Therefore, this research was conducted to determine the germination stimulant production timing and subsequent small

broomrape germination in relation to crop growth stage for red clover, a host, and, wheat, a false host, in controlled-environment growth chambers.

MATERIALS AND METHODS

Small broomrape seed was collected from small broomrape inflorescences parasitizing red clover in a field in Oregon. The inflorescences were dried at room temperature and seeds were cleaned using 100-µm sieves and stored in the dark at room temperature.

'Kenland' red clover and 'Foote' winter wheat plants were grown in 10 cm^2 pots filled with pure sand in a growth chamber at 20 C for 12 hr light and 12 hr dark. Plants were thinned to 1 in each pot 2 wk after planting. Ten ml half-strength Hoagland's nutrient solution was added to each pot once a week.

Red clover exudates harvest timings included 1-trifoliolate, 3-trifoliolate, 5-trifoliolate, 24 wk after planting, and 29 wk after planting. Exudates harvest timings from wheat were 1-leaf, beginning of tillering, first node visible, flag leaf visible, boot, and 27 wk after planting (corresponding to Feekes growth stages 1, 2, 6, 8, 10, and 10.5). At each harvest timing, the plants were removed from the sand, washed, and immersed in deionized water in a flask. The amount of water in each flask was maintained at 100 ml and 125 ml for wheat and red clover, respectively, during the 5 d

period. The seedling was supported in the flask with non-absorbent cotton so that the roots were immersed in water, and was allowed to grow for 5 d.

Approximately 25 small broomrape seeds were placed in 1 cm diameter glass fiber filter paper (GFFP) disks. Four GFFP disks were placed in a Petri dish containing 2 layers of 7 cm diameter filter paper moistened with 2.5 cm deionized water. The Petri dishes were wrapped with aluminum foil and placed in a growth chamber at 20 C for 14 d for preconditioning (as suggested by Kroschel 2001). After preconditioning, 4 GFFP discs with 25 seeds each were transferred to a Petri dish containing 2 layers of 7 cm diameter filter paper moistened with 2.5 ml of root exudates from the extraction described previously. Petri dishes were sealed and wrapped with aluminum foil and placed in dark growth chambers at 20 C for 2 wk.

Weekly observations were made by using a stereoscopic microscope to determine the rate of small broomrape seed germination. Percent germination was determined from four discs in each Petri dish and the average percentage of seed germination was calculated. The experiment was arranged in a completely randomized design with six replications for each treatment, and the experiment was repeated. Statistical analysis was performed using SAS and ANOVA was conducted. Treatment means were separated using 95% confidence limits ($P \leq 0.05$).

RESULTS AND DISCUSSION

There was no experiment by treatment interaction; therefore, experimental results were combined and represented as 12 replications for each treatment.

Red Clover Study

Significant differences existed among treatments ($P < 0.0001$). All treatments received an identical and optimal conditioning treatment (14 d at 20 C in water), so the differences in the subsequent germination must be due to differences in germination stimulant production during the germination test. The greatest germination (77%) was observed with exudates from the 3-trifoliolate stage, while the least germination (29%) was observed with exudates from the 1-trifoliolate stage (Figure 4.1).

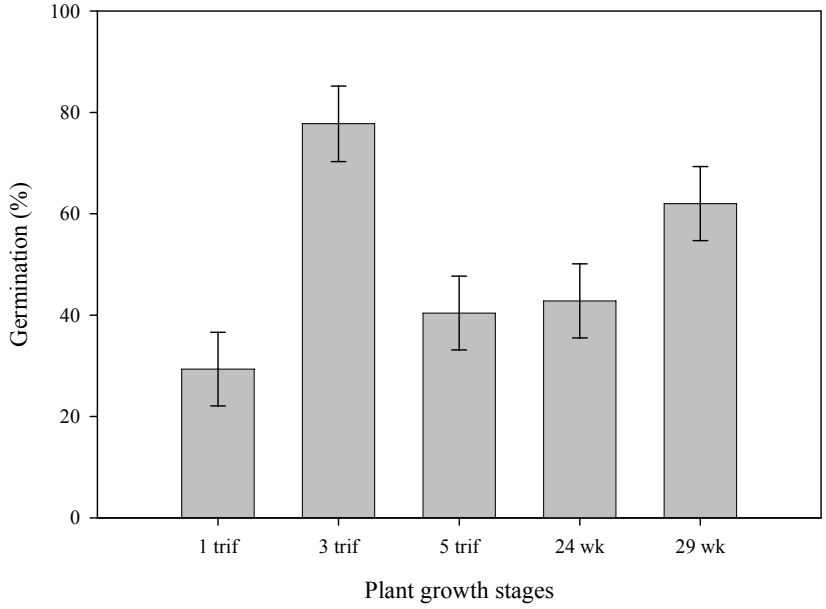

Figure 4.1: Relationship between red clover growth stages and small broomrape seed germination. Bars indicate 95% confidence limits.

The germination percentage declined at the 5-trifoliolate and the 24 wk after planting stages, where germination percentage was 40% and 43%, respectively, with no difference noticed between the two treatments. Germination increased to 62% when the exudate of the red clover at the 29 wk after planting stage was used.

In our study, the least germination was observed with the 1-trifoliolate exudates which might be due to the low level of germination stimulant produced by small plants. However, germination increased when the exudates from the large 3-trifoliolate and 29 wk after planting were used. Ma et al. 1996 reported that 1 wk old *Menispermum dauricum* DC. root cultures induced 87% Striga germination while 2 to

3 wk old root culture elicited negligible germination. However, root extracts from 6 to 8 wk induced 100% Striga seed germination.

Our results also indicated a reduction in germination percentage when the exudates from the 5-trifoliolate and 24 wk after planting stage plants were used compared to the 3-trifoliolate stage plants. There are two possible explanations for this reduction in the germination percentage. First, an increase in the concentration of the germination stimulant as a result of increase in the plant age and root size, or second, the presence of an inhibitory compound in the root extract. The second hypothesis seems plausible given that it has been suggested that root exudates contain both germination stimulants and inhibitors (Mallet 1973; Whitney 1978). In addition, many of the root exudate molecules have allelopathic effects (Gadkar et al. 2003). Brown et al. (1951) reported that high concentrations of host root exudates inhibited small broomrape germination. Johnson et al. (1976) reported that an analogue of strigol stimulated branched broomrape germination at low concentrations but inhibited germination at higher concentrations. Whitney and Carsten (1981) showed that host root exudates affected the germination of broomrape seeds and also contained inhibitory components that influenced the size and direction of growth of the resulting radicle. The natural substances from root exudates that inhibit broomrape germination have not been isolated; However, natural and chemical analogues of stimulating substances have been shown to decrease the percentage of germination once an

optimal concentration has been exceeded (Johnson et al. 1976; Saghir 1986; Stewart and Press 1990; Zahran 1982).

In related research, Ariga et al. (1996) suggested that aqueous extracts of cowpea roots and shoots contain both inhibitory and stimulatory substances for Striga seed germination. Emechebe et al. (2003) reported that regardless of crop species, 1 g of excised root or shoot resulted in greater germination of Striga than 2 g did and suggested a relative increase in inhibitory materials with increase in amount of plant material.

Red clover has been reported to produce more than one stimulant; therefore, it may produce individual stimulants at different levels under different growth conditions or at different growth stages. Therefore, the reduction in the germination percentage of small broomrape might be due to an excess of stimulatory substances or higher concentrations of the inhibitory substances produced at certain red clover growth stages.

Wheat Study

Significant differences existed among wheat growth stage treatments ($p < 0.0001$). Small broomrape germination was greatest in response to wheat in the 1-leaf stage (25%) and beginning of tillering stage (22%). Germination decreased with later growth stage treatments and was 0% at 27 wk after planting (Figure 4.2).

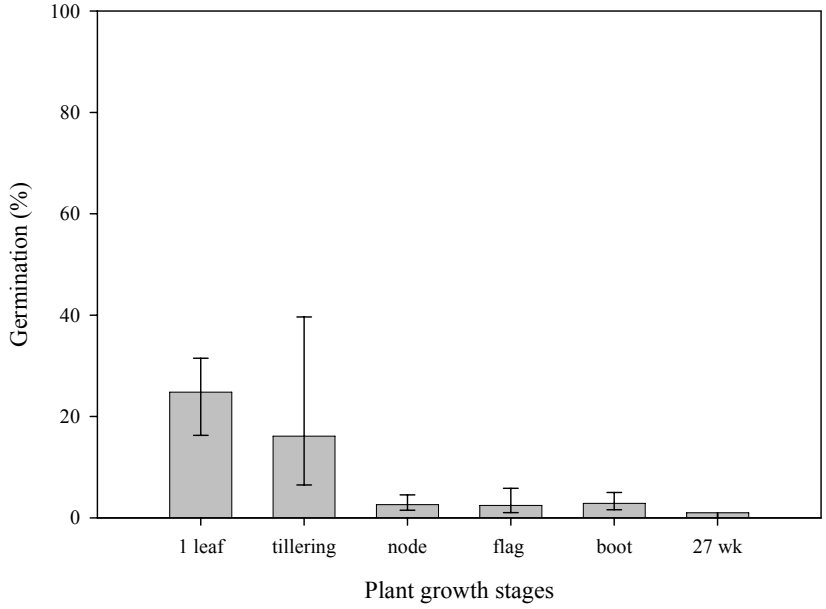

Figure 4.2: Relationship between wheat growth stages and small broomrape seed germination. Bars indicate 95% confidence limits.

In our studies, wheat induced up to 25% germination of small broomrape seeds. This result is in agreement with Ross et al. (2004) who reported that wheat induced 25% to 40% small broomrape germination. Lins et al. (2005) found that wheat induced 20% to 70% germination of small broomrape seeds. However, our results indicated that wheat can stimulate germination of small broomrape seeds only at the early growth stages (1-leaf to tillering stage). This result is in agreement with other researchers. Sunderland (1960) concluded that the synthesis of Striga and small broomrape germination stimulants decreased with maturity of maize roots. Hameed et al. (1973) reported that root exudates of marigold (*Tagetes erecta*) and peanut

(*Arachis hypogaea* L.) exhibit qualitative and quantitative changes in their composition with the increasing age of the plant.

Crop rotation with false hosts has long been proposed and practiced as control measure for parasitic weeds. It has been shown in various studies that intercropping cereals with legumes leads to reduced Striga infestation in the following cereal crop (Carsky et al. 1994; Odhiambo and Ransom 1994). Kleifeld et al. (1994) reported that the ability of a false host species to deplete broomrape from the soil seed bank increased when the false host species was long lived with an extensive root system. Repeated production of false host crops would be necessary to deplete the soil seed bank given that small broomrape can produce millions of seeds per hectare. Therefore, wheat exudates can not entirely deplete the small broomrape soil seed bank, but this strategy can be integrated into a small broomrape management system.

In conclusion, red clover exudates stimulated more small broomrape germination than did wheat exudates. Red clover produced germination stimulant at all growth stages, while wheat produced germination stimulant only at early stages of development**.**

SOURCES OF MATERIALS

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VWR filter qualitative filter paper. VWR Scientific Product, West Chester, PA 19380.

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CHAPTER 5

Effect of wheat (*Triticum aestivum*) rotation cycles on small broomrape (*Orobanche minor*) infestation in red clover (*Trifolium pratense*)

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ABSTRACT

Broomrapes (*Orobanche* spp.) are root holoparasitic plants that cause severe damage to economically important crops. False hosts, plants that induce germination but not further development, can be used to deplete small broomrape (*Orobanche minor* Sm.) soil seed banks. Wheat is a false host for small broomrape. A study was conducted to investigate the number of winter wheat rotation life cycles (WRC) that are required to reduce parasitism of subsequent red clover crops. The greatest number of small broomrape attachments and the greatest small broomrape biomass were observed with 0-wheat rotation cycles (0-WRC), and both were reduced if wheat was grown. There were no differences among the number of wheat life cycles, although there was a trend for more reduction with repeated wheat cycles. Red clover flower number increased with 4-WRC compared to all other treatments. Above ground red clover biomass was less with 0-WRC treatment than any of the other treatments, and there were no differences among any of the other treatments. Including wheat in rotation with red clover has the potential to reduce small broomrape impact on red clover.

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

Key words: False host, germination stimulant, integrated weed management, parasitic plant, red clover, seed germination, wheat.

INTRODUCTION

Broomrapes (*Orobanche* spp.) are obligate, chlorophyll-lacking root parasites of many dicotyledonous species and cause severe damage to vegetable and field crops worldwide (Parker and Riches 1993). Small broomrape is an annual species that in the state of Oregon in the USA germinates in early spring and produces seed in early summer (Lins et al. 2005). Small broomrape is a federally listed noxious weed in the USA. Red clover is the first seed crop in the USA reported to be contaminated with small broomrape (Colquhoun et al. 2001, 2002).

Broomrapes parasitize members of many plant families including Asteraceae (Romanova et al. 2001), Fabaceae (Goldwasser et al. 1997), and Solanaceae (Romanova et al. 2001). Plants species are classified as hosts, false hosts, or non-hosts. False host plants produce chemical exudates that promote small broomrape germination, but not attachment (Joel et al. 1995). False hosts crops have been identified for various broomrape species. Sorghum (*Sorghum bicolor* L.), corn (*Zea mays* L.), cucumber (*Cucumis sativus* L.) and flax (*Linum usitatissimum* L.) have been used as false host crops for branched broomrape (*O. ramose*) (Abu-Irmaileh 1984; Parker and Riches 1993), while sorghum, barley *(Hordeum vulgare* L.), and hairy fruit vetch (*Vicia dasycarpa* spp.) have been used as false hosts for crenate broomrape (*O. crenata*) (Kasasian 1973; Linke et al. 1991a). Yoneyama et al. (2001) reported basil

(*Ocimum basilicum* L.), carrot (*Daucus carota* L.), cucumber, corn, onion (*Allium cepa* L.), and soybean (*Glycine max* (L.) Merr.), as false hosts for small broomrape (Yoneyama et al. 2001). Ross et al. (2004) identified wheat to be a false host that induced 25 to 40% small broomrape seed germination, and Lins et al. (2006) reported that wheat induced 20 to 70% small broomrape seed germination.

Crop rotation with false hosts has been practiced as a control measure for parasitic weeds. False host crops decreased the crenate broomrape seed bank by 30% in one cropping cycle (Linke et al. 1993). Kleifeld et al. (1994) tested false hosts crops to decrease infestation of Egyptian broomrape (*O. aegyptiaca* Pers.), and reported that growing flax in two successive winter seasons or one summer cropping with mung beans (*Phaseolus aureus* Roxbg.) reduced early infestation of the parasite and significantly increased tomato (*Lycopersicum esculentum* Mill.) vigor and production. In other research, intercropping cereals with legumes reduced infestation by the parasitic weed, Striga, in the following cereal crop (Carsky et al. 1994; Odhiambo and Ransom 1994).

Small broomrape management through rotation to non-host or false host crops is critical as other control measures are developed and implemented in red clover seed production. Therefore, the current research was conducted to demonstrate the potential for cultural, non-chemical small broomrape control in a wheat-red clover rotational system. The number of winter wheat life cycles required to reduce parasitism of subsequent red clover crops was studied in controlled conditions in a greenhouse.

MATERIALS AND METHODS

Small broomrape seed was collected from small broomrape inflorescences parasitizing red clover in a field in Oregon. The inflorescences were dried at room temperature and seeds were cleaned using 100-µm sieves and stored in the dark at room temperature.

'Kenland' red clover and 'Foote' wheat varieties were used for this study. Experiments were conducted in 10 L plastic pots containing commercial potting mix artificially infested with 35 mg of small broomrape seed. Pots were placed in a greenhouse where the temperature was approximately 22 C and supplemental lighting was provided for 10 h per day. Five red clover or wheat seeds were planted at a depth of 2 cm. Three wk after germination, plants were thinned to 1 per pot. The rotation cycle treatments (WRC) included: clover (0-WRC), wheat-clover (1-WRC), wheat-wheat-clover (2-WRC), wheat-wheat-wheat-clover (3-WRC), and wheat-wheat-wheat-wheat-clover (4-WRC). The pots were watered and fertilized as needed. Red clover plants were cut once before initial flowering to stimulate forage production that is practiced by growers. Experiments were harvested at red clover full bloom and wheat at full heading.

At the end of the red clover rotation in each cycle, red clover roots were washed and separated from attached broomrape. Small broomrape attachments were counted. Small broomrape biomass, red clover total above ground biomass from both

harvest times and red clover root biomass were determined after drying for 48 h at 70 C. Red clover flowers were counted.

The experiment was arranged in a completely randomized design with six replications for each treatment, and the experiment was repeated. Statistical analysis was performed using SAS and ANOVA was conducted (SAS 2002). Small broomrape attachment number, small broomrape biomass, small broomrape flower number, and red clover root biomass were log transformed to improve normality but the back transformed data are presented. Treatment means were separated using 95% confidence limits ($P \leq 0.05$).

RESULTS AND DISCUSSION

There was no experiment by treatment interaction; therefore, experimental results were combined.

Differences existed among treatments for the number of small broomrape attachments ($P < 0.0001$). The greatest number of attachments was observed in the 0-WRC treatment. However, there were no statistical differences among rotations when wheat was included but the trend was for fewer attachments with more wheat cycles (Figure 5.1).

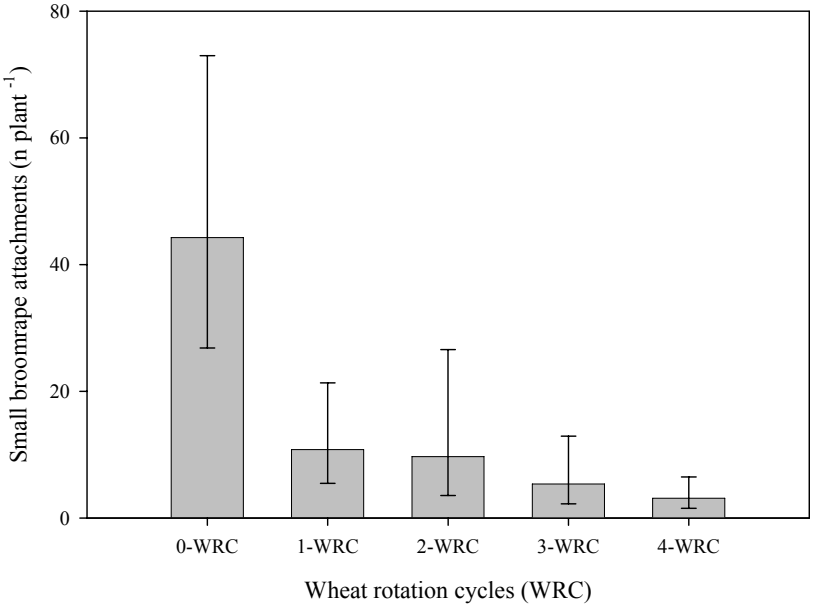

Figure 5.1: Relationship between wheat rotational cycles and small broomrape number of attachments. Bars indicate 95% confidence limits.

Differences existed among treatments for small broomrape biomass ($P < 0.0004$). Small broomrape biomass was greater in the 0-WRC treatment than in all treatments where wheat was included. There were no differences among the number of wheat rotations when wheat was included (Figure 5.2).

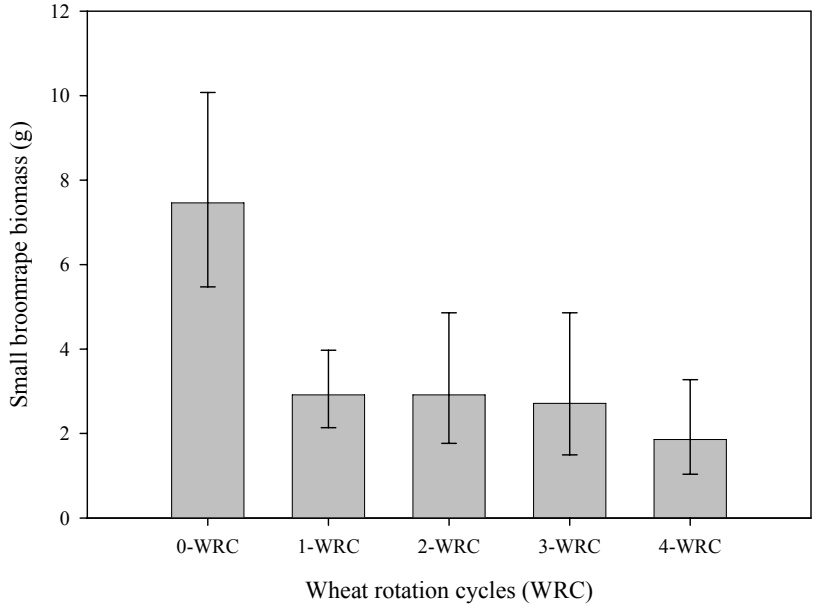

Figure 5.2: Relationship between wheat rotational cycles and small broomrape biomass. Bars indicate 95% confidence limits.

Differences existed among treatments for the number of red clover flowers ($P < 0.012$). There was a difference between 0-WRC and 4-WRC treatment but not the other WRC treatments, and there were no differences among WRC treatments that included at least 1 cycle of wheat (Figure 5.3).

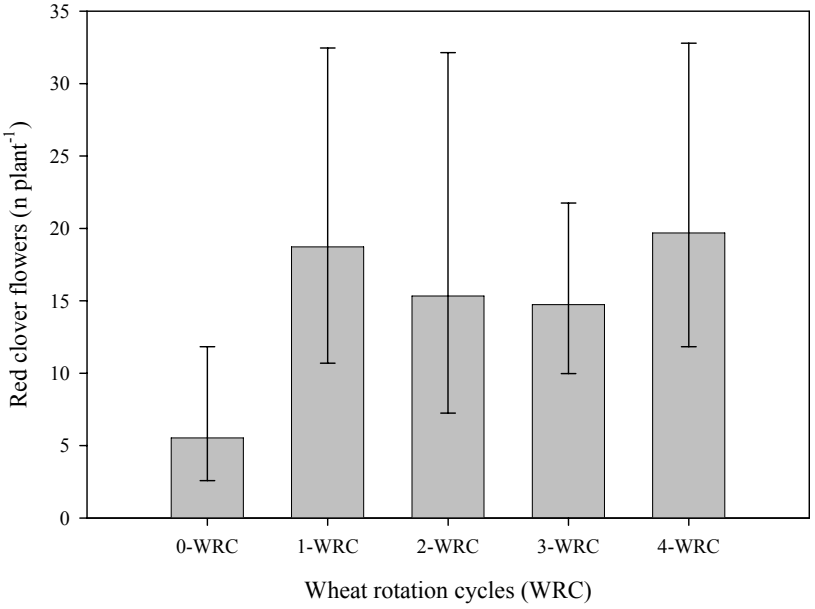

Figure 5.3: Relationship between wheat rotational cycles and number of red clover flowers. Bars indicate 95% confidence limits.

There were no differences in root biomass among treatments ($P < 0.0618$) (Figure 5.4).

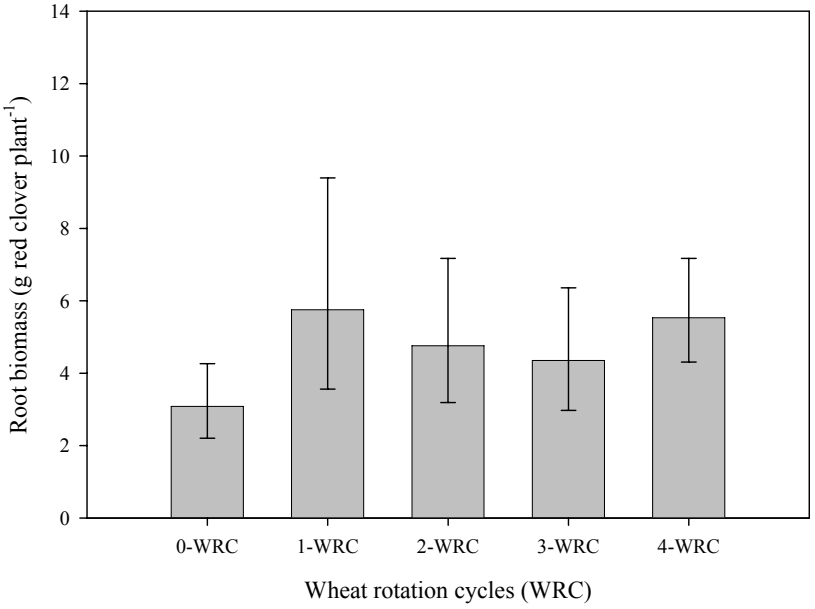

Figure 5.4: Relationship between wheat rotational cycles and red clover root biomass. Bars indicate 95% confidence limits.

Above ground biomass differed among treatments ($P < 0.0001$). Above ground biomass in the 0-WRC treatment was less than any of the other treatments, and there were no differences among any of the other treatments (Figure 5.5).

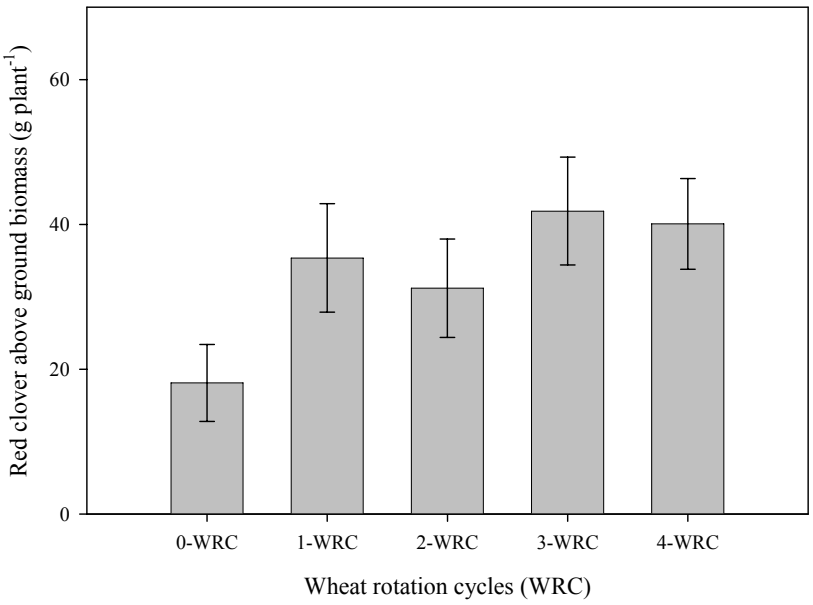

Figure 5.5: Relationship between wheat rotational cycles and red clover total above ground biomass. Bars indicate 95% confidence limits.

We found that small broomrape biomass and number of attachments were reduced after 1-WRC, and we found also that there were no differences among the number of wheat rotations when wheat was included. Similar results were observed by Lins et al. (2006) who reported that the small broomrape attachment number was reduced on red clover plants grown in pots previously planted to wheat. Kleifeld et al. (1994) reported that growing flax in two successive winter seasons or one summer cropping with mung beans (*Phaseolus aureus* Roxbg.) reduced early infestation of Egyptian broomrape (*O. aegyptiaca* Pers.), and significantly increased tomato (*Lycopersicum esculentum* Mill.) vigor and production. Gbehounou and Adango

(2003) showed reduced incidence of Striga after one maize cowpea (*Vigna unguiculata* L.) (Variety TVX1850-01F) rotation cycle. Khan et al. (2006) also reported that intercropping maize and the fodder legume (*Desmodium* spp.) reduced the number of Striga up to 99.2% in one cropping season (Khan et al. 2006). However, other researchers reported the need for more than one rotation cycle. Al-Menoufi (1991) reported the need for 3 to 5 years of false crops to reduce broomrape infestations. In addition, he reported a 3 and 1% crenate broomrape infestation in faba bean following 3 and 4 crops of Egyptian clover (*Trifolium alexandrinum* L.), respectively, compared with 67% infestation when faba bean was grown continuously. Kebreab and Murdoch (2001) predicted that sustainable control of the parasite can only be achieved by reducing the soil seed bank to levels of 1000 to 2000 seeds m^{-2}. In addition, it was predicted that it would take 3 to 4 years to reduce the infestation from 13000 to 2000 seeds m^{-2}.

Small broomrape infection influenced several measures of red clover growth and productivity. In our study, the number of red clover flowers in the 0-WRC treatment was less than in the 1- to 4-WRC treatments. The reduced number of flowers in the 0-WRC treatment was associated with the higher number of small broomrape attachments. Grenz et al. (2005) reported that parasitism by crenate broomrape reduced faba bean pod number.

Our results indicate that wheat rotational cycles had no effect on red clover root biomass. Aflakpui et al. (2002) reported that infection of maize by Striga did not

affect maize root biomass but led to reduced shoot biomass. Frost et al. (1997) showed that Striga affected sorghum shoot biomass more than root biomass. However, other researchers reported increased host crop root biomass with broomrape parasitism (Grave at al. 1990; Manschadi et al. 2001).

In our study, red clover above ground total biomass increased with wheat in the rotation cycles and with decrease in small broomrape number of attachments. Oswald and Ransom (2001) reported the effect of the different rotation crops on maize yield depended on the extent they decreased Striga infestation.

This study demonstrated that the marked reduction of small broomrape population in red clover planted after wheat is one factor contributing to the increased red clover biomass production. This study also indicated that a wheat rotation cycle reduced the number of broomrape attachments. In addition, 1-WRC was equal to 4-WRC which suggests that one wheat rotation cycle can reduce small broomrape infestation and increase red clover biomass production.

Wheat rotations cycles decreased the small broomrape seed bank in the soil; therefore, wheat can be used as a false host crop as part of small broomrape management. This cultural, non-chemical control measure of small broomrape can be implemented easily in red clover seed production. Red clover is commonly intercropped with a cereal crop in western Oregon. In this system, winter wheat could be planted in the infested area in the fall, act as a false host during the winter and early spring, and then red clover could be interseeded in the spring. Wheat is harvested for

seed in the summer, allowing for the regrowth of an established red clover crop below the harvested wheat canopy. The subsequent small broomrape infection level on the red clover could be eliminated or reduced due to the reduction in the small broomrape seed bank caused by the false host. Furthermore, multiple control tactics may ensure that red clover seed production is free of small broomrape.

SOURCE OF MATERIALS

Red clover cultivar ‘Kenland’, Tangent Seed Lab Int'l., 33731 Highway 99E, P.O. Box 331, Tangent, OR 97389.

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

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CHAPTER 6

GENERAL CONCLUSION

This study was conducted to gain a better understanding of small broomrape (*Orobanche minor* Sm.) biology in order to develop and implement an integrated, biologically based small broomrape management option using wheat (*Triticum aestivum L.*) as a false host to reduce the soil seed bank. Temperature had a substantial effect on small broomrape germination response. High temperature accelerated small broomrape germination, whereas cold temperature slowed small broomrape germination. The results of this study can be used to describe small broomrape germination over a wide temperature range. In addition, a growing degree day model for small broomrape parasitism of red clover has been developed (Eizenberg et al. 2004, 2005); this model provided a descriptive of the relationship between small broomrape attachment, tubercle production and shoot emergence. However, in this model, small broomrape germination rate was not measured. Including small broomrape germination in this model will increase the predictive resolution for the individual parasitism stages and enable us to precisely predict the individual parasitism stages.

Our results revealed the effect of temperature on small broomrape germination stimulant production by red clover (*Trifolium pratense* L.), a host, and, wheat, a false

host. Long exposure to low temperature reduced germination stimulant production in red clover, which would lead to reducing parasitism in the winter. This result partly explains the low small broomrape infection in red clover fields with the low temperatures in early fall and winter. In our study, wheat exudates were much less effective at inducing small broomrape germination; in addition, wheat and red clover produced stimulants differently in response to different temperatures. Temperature may influence the synchronization of development between the host and the parasite; therefore, altering the planting date of the host crop species may affect the parasitism level due to changing the microclimate. Moreover, the ability to predict temperature conditions which allow maximum small broomrape germination can be used to determine when to plant the false host.

Red clover produced germination stimulant at all growth stages, while wheat produced germination stimulant only at early stages of development. This result suggests that wheat should be planted so that the early growth stages of wheat will be synchronized with optimum small broomrape seed germination so that wheat can induce as much small broomrape germination as possible. In addition, this result may serve as a basis for developing a decision support system for optimal small broomrape management.

In our study on the number of winter wheat rotation life cycles (WRC) that are required to reduce parasitism of subsequent red clover crops, small broomrape emergence and density in red clover were reduced through the use of wheat rotation

cycles. The marked reduction of small broomrape population in red clover planted after wheat was one factor contributing to the increased red clover biomass production. In addition, one wheat rotation cycle was equal to four wheat rotation cycles which suggests that one wheat rotation cycle can reduce small broomrape infestation and increase red clover biomass production, although there was a trend for more reduction in small broomrape number and biomass with more wheat cycles.

Management of small broomrape early in its life cycle is critical for the prevention of small broomrape seed production and red clover seed yield loss. Integrated management strategies of small broomrape must reduce the small broomrape soil seed bank and limit the growth of the parasite on host plants. Including wheat in rotation with red clover has the potential to reduce small broomrape impact on red clover. However, wheat probably cannot eliminate the small broomrape soil seed bank in a single life cycle. The herbicide imazamox prevented both emergence and seed production when applied to parasitized red clover before small broomrape emergence (Lins et al., 2005). The use of imazamox, in combination with wheat as a false host, could limit the early stages of parasite attachment and growth and deplete the small broomrape soil seed bank on infested sites.

Future research on small broomrape management should include extraction and analysis of the wheat germination stimulant quantity and quality relative to red clover germination stimulant. Research also is needed for locating the wheat genes that are responsible for regulating the production of the small broomrape germination

stimulant. In addition, research is needed for locating quantitative trait loci associated with small broomrape resistance in white clover.

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APPENDIX

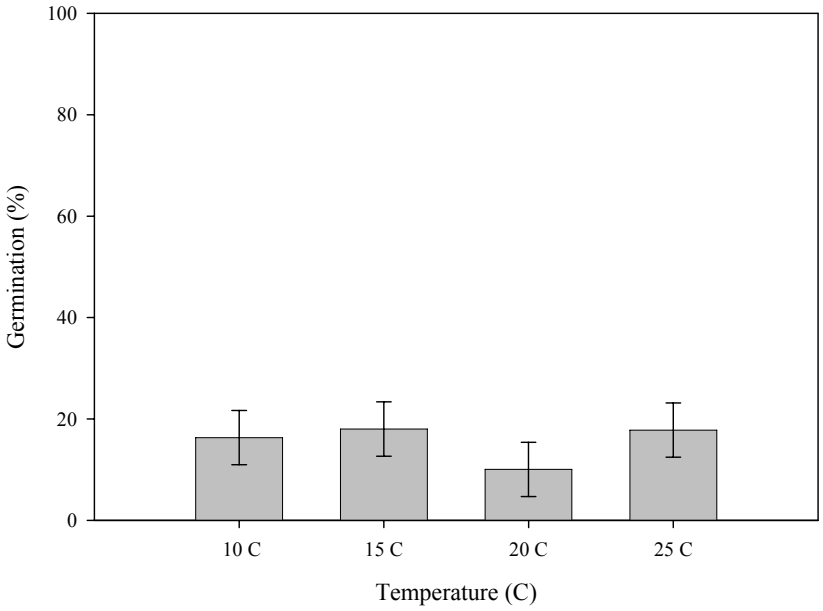

Figure A.3.1: Relationship between temperature and red clover germination stimulant production 4 WAP on small broomrape seed germination 7 DAT. Bars indicate 95% confidence limits.

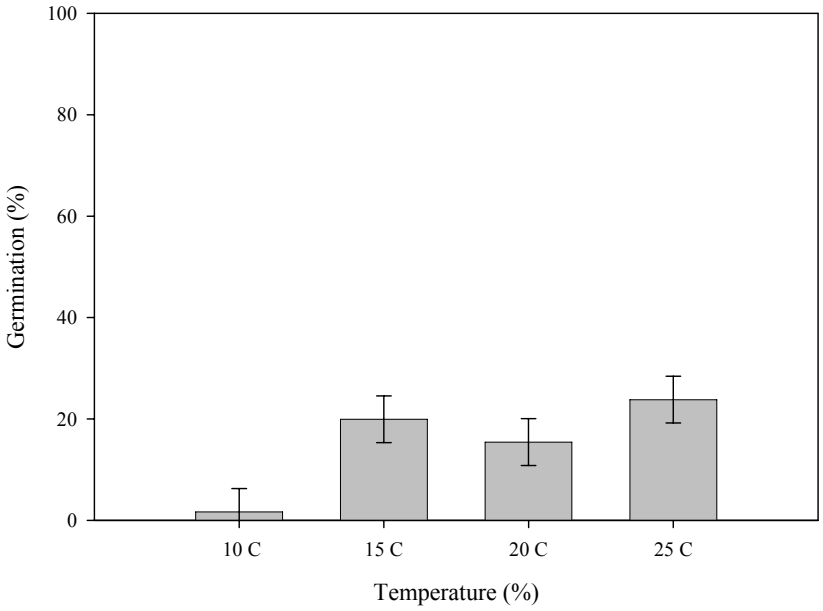

Figure A.3.2: Relationship between temperature and red clover germination stimulant production 8 WAP on small broomrape seed germination 7 DAT. Bars indicate 95% confidence limits.

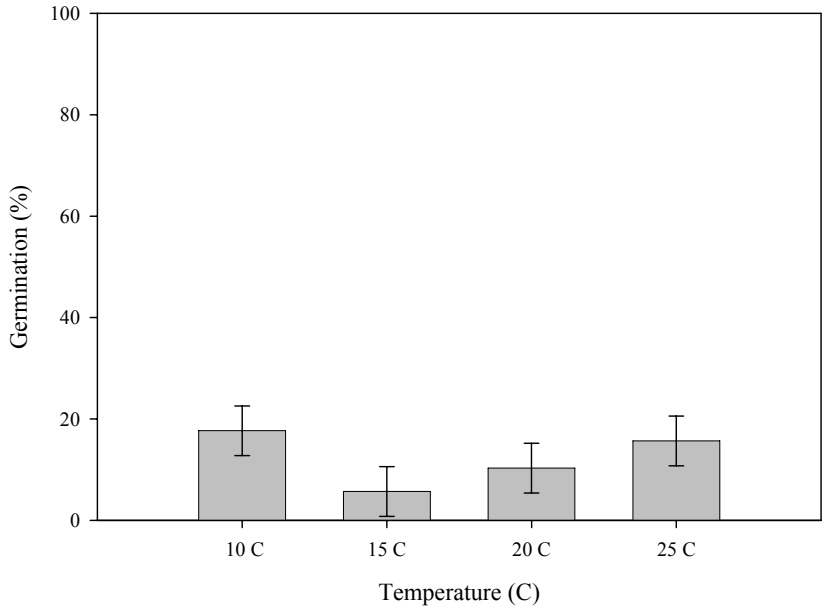

Figure A.3.3: Relationship between temperature and wheat germination stimulant production 4 WAP on small broomrape seed germination 7 DAT. Bars indicate 95% confidence limits.

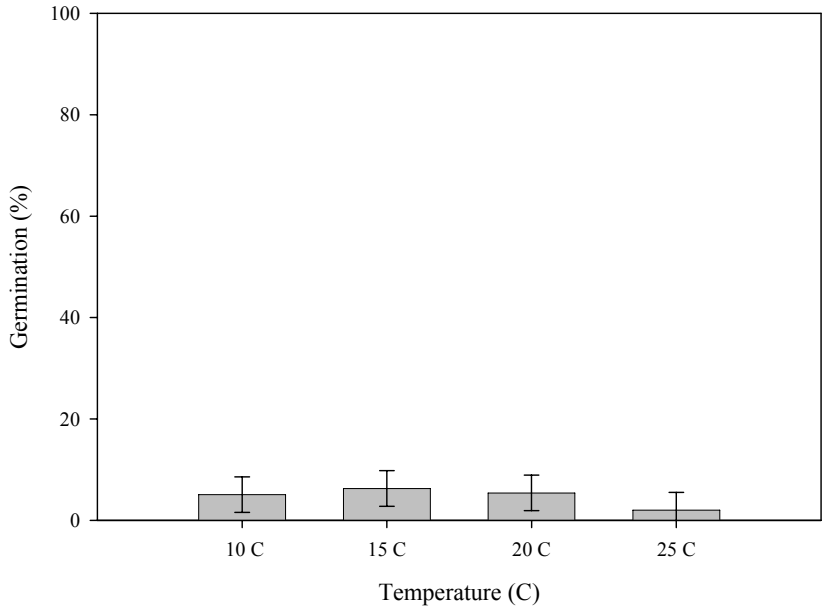

Figure A.3.4: Relationship between temperature and wheat germination stimulant production 8 WAP on small broomrape seed germination 7 DAT. Bars indicate 95% confidence limits.

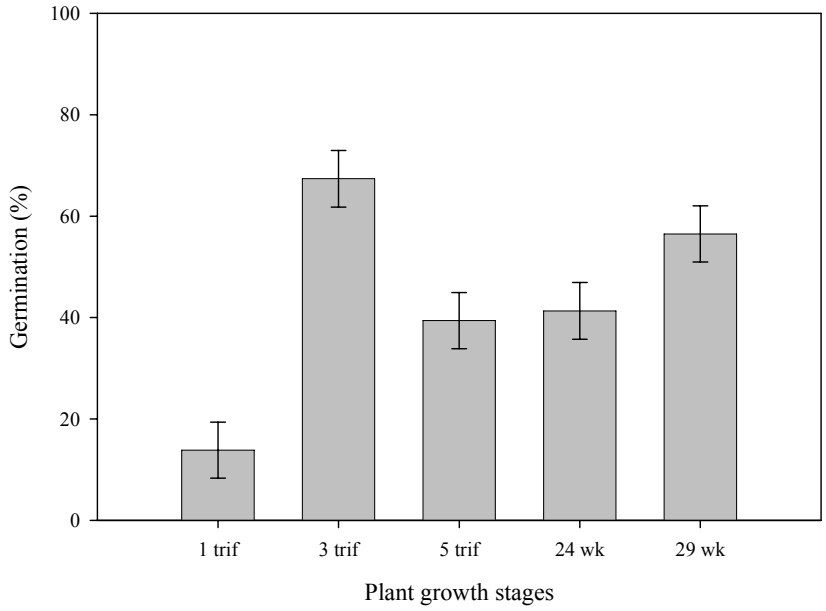

Figure A.4.1: Relationship between red clover growth stages and small broomrape seed germination 7 DAT. Bars indicate 95% confidence limits.

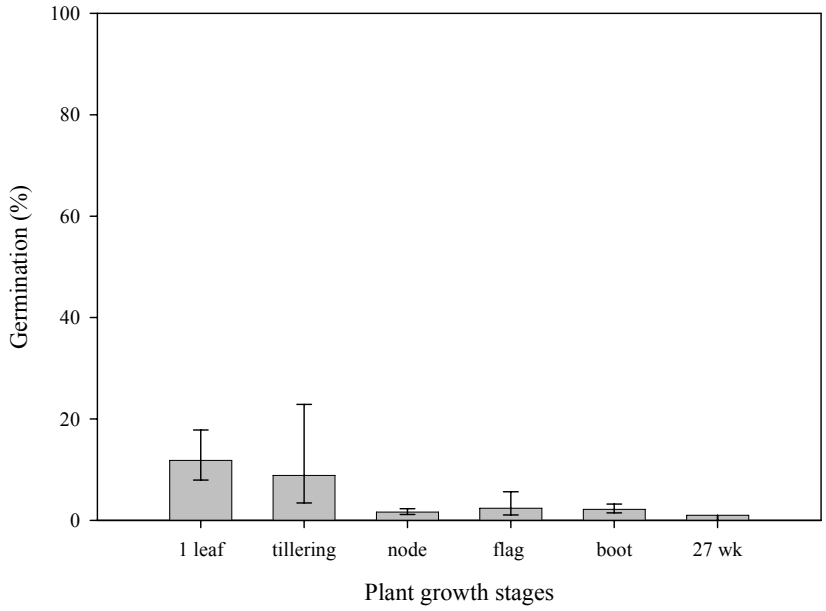

Figure A.4.2: Relationship between wheat growth stages and small broomrape seed germination 7 DAT. Bars indicate 95% confidence limits.

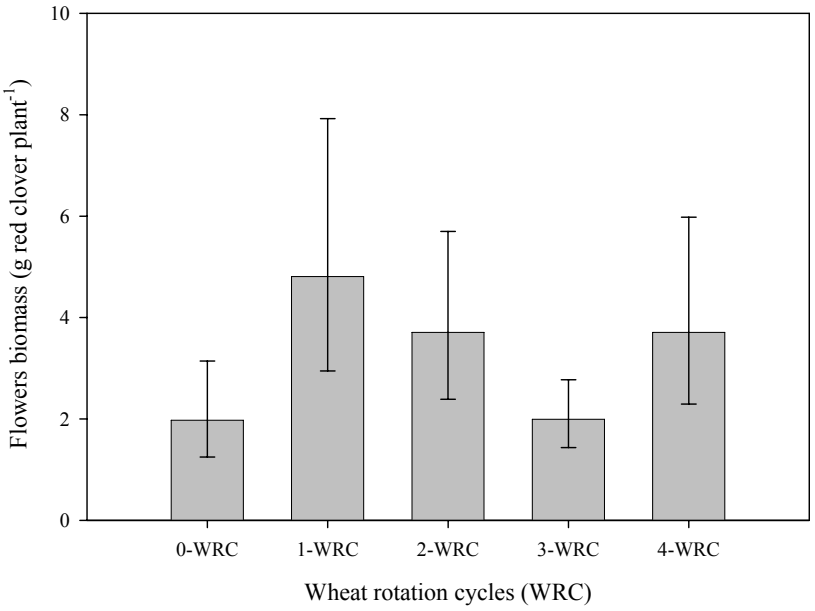

Figure A.5.1: Relationship between wheat rotational cycles and red clover flower biomass. Bars indicate 95% confidence limits.

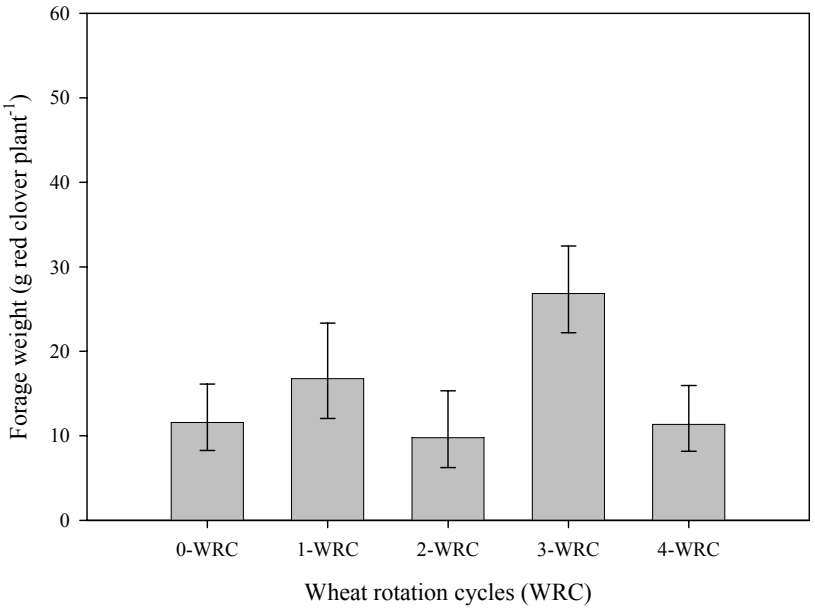

Figure A.5.2: Relationship between wheat rotational cycles and red clover forage biomass. Bars indicate 95% confidence limits.

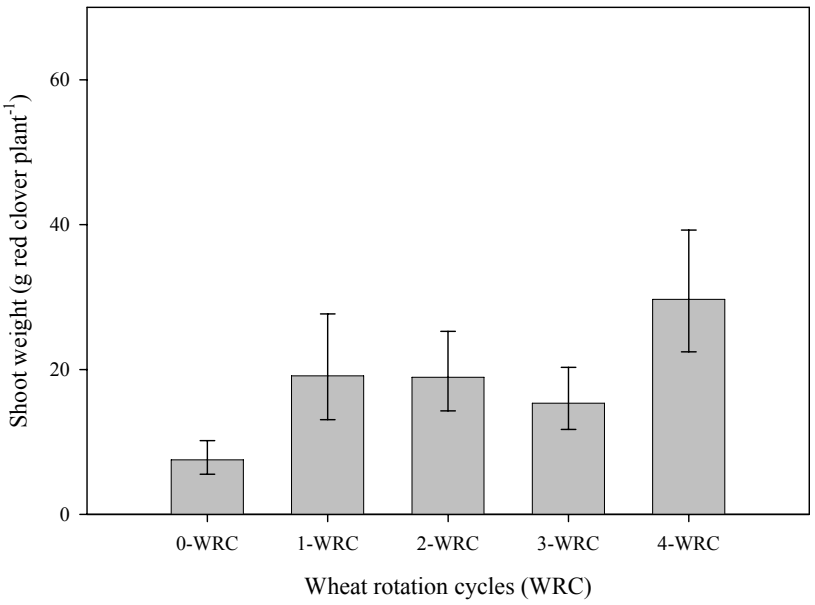

Figure A.5.3: Relationship between wheat rotational cycles and red clover shoot biomass. Bars indicate 95% confidence limits.