THE INVASION POTENTIAL AND COMPETITIVE ABILITY OF *CAMELINA SATIVA* (L.) CRANTZ (CAMELINA) IN RANGELAND ECOSYSTEMS

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ABSTRACT

Camelina sativa (L.) Crantz (large-seeded false-flax) is a recently introduced oilseed crop in Montana and has potential for large scale production for the biofuel market. However, due to weedy characteristics, the invasive potential of this species is of concern. A need exists to create a pre-entry protocol to accurately assess risk to minimize uncertainty inherent in qualitative weed risk assessment approaches. We assessed the probability of *C. sativa* to invade rangeland ecosystems of southwest Montana to address this concern. The objectives of this study were to 1) quantitatively assess the invasion potential of *C. sativa* by collecting demographic data over two years and developing a population dynamics model, 2) compare experimental results and modeling outcomes to predictions suggested by a qualitative weed risk assessment system, and 3) assess the impact of growing conditions on the relative competitiveness of *C. sativa* and *Brassica napus* (L.) (canola).

Objective 1 was carried out in two contrasting rangeland ecosystems to assess the effects of disturbance and seeding season on emergence, survival, and fecundity rates of *C. sativa*. Population growth (λ) was forecasted by developing a population dynamics model. Resulting λ values from simulations using observed data never exceeded 0.03 and the maximum time to extinction was six years. The low λ values indicate that the threat of invasion by this species in the studied ecosystems is low.

Objective 2 compared quantitative results to predictions from the Australian weed risk assessment (WRA) model. In contrast to experimental results, outcomes from the WRA suggested that this species should not be allowed entry into the region. These opposing results highlight the need for a more comprehensive approach to weed risk assessment.

Objective 3 was conducted over three trials in two greenhouses. A replacement series design was used to estimate the effects of soil conditions and the presence of an invasive weed, *Bromus tectorum* (L.) (cheatgrass, downy brome), on the competitive outcomes between *C. sativa* and canola. Replacement series diagrams determined that competition occurred and that canola was the superior competitor in all treatments, thus providing further evidence that the invasion potential of *C. sativa* is low.

CHAPTER 1

PROJECT BACKGROUND AND OBJECTIVES

Introduction

Over the past few decades, the doctrine of creating a system by which the USA can produce its own fuel has steadily gained momentum. Central to this theory is the hypothesis that doing so will increase national security, as it will reduce reliance on unstable countries to supply oil. However, easily extractable fossil fuel supplies are finite, and society must eventually implement the use of alternative fuel sources (Deffeyes 2001). By developing new technologies that reduce our consumption of fossil fuels, additional benefits will be gained beyond that of increased national security. For example, alternative fuel sources such as biofuels may help in reducing emissions of CO_2 and other greenhouse gases (Bernardo et al. 2003; Fargione et al. 2008; Low and Booth 2007; Rosenzweig 2007; Zhou et al. 2003). In turn, this could help to reduce negative effects predicted to occur with global climate change (King 2004).

In addition to the benefits of decreased dependence on foreign oil and the potential greenhouse gas reductions that biofuels can provide, they are a logical first step in the selection of alternative energy sources because, unlike other fossil fuel alternatives, biofuels can be utilized without modification to existing fuel infrastructure (Carere et al. 2008; Walter et al. 2008). However, three conditions must be met for biofuels to become a viable option. First, a net energy gain must be achieved for all production processes relative to the energy provided from the final product (Hill et al. 2006). Second, biofuels must be economically viable and produced in consistently sufficient quantities (Hill et al. 2006). Third, environmental sustainability is needed in the production of biofuels if we are to meet industry goals and government requirements (Robertson et al. 2008).

The majority of biofuels in the United States currently are produced from major crops such as *Zea mays* L. (corn) (Walter et al. 2008) and *Glycine max* L. Merr. (soybean) (Hill et al. 2006). In conventional farming, these crops rely heavily on synthetic inputs that are derived from fossil fuels. Therefore, this practice might be viewed as unsustainable and only marginally helpful in mitigating GHG emissions. In fact, Hill et al. (2006) determined that ethanol produced from *Z. mays* provided a net gain of only 25% of the energy that was consumed during production. They further estimated that if all of the land that was planted in the USA to *G. max* and *Z. mays* in 2005 were used as biofuel feedstock, it would account for only 6% of the diesel demand and 12% of the gasoline demand, respectively.

New technologies including cellulosic ethanol are rapidly developing because they have the potential for increasing the conversion process efficiency from biomass to biofuel (Walter et al. 2008). Another benefit of cellulosic ethanol is that many of the species being considered for production such as *Panicum virgatum* L. (switchgrass) and *Arundo donax* L. (giant reed), can be produced on land with little water availability, as compared to biofuel produced from *Z. mays* and *G. max* (DiTomaso et al. 2007; Low and Booth 2007). Moreover, these perennial crops are tolerant of low fertility situations, which may reduce environmental pollutants as compared to *Z. mays* ethanol (Tilman et al. 2006). Despite these benefits, cellulosic ethanol has yet to overcome various technological and biological knowledge gaps (Himmel et al. 2007).

Oilseed crops are currently the most efficient way to produce biofuel, with a net energy gain of up to 93% after all production processes are completed (Hill et al. 2006). Oilseeds that are currently grown in the Northern Great Plains and can be used for biofuel production include *Brassica* spp. (canola), *Carthamus tinctorius* L. (safflower), *Linum* spp. (flax) , and most recently, *Camelina sativa* L. Crantz (gold of pleasure, large-seeded false flax). *Camelina sativa* is of particular agronomic interest in the Northern Great Plains because it grows well on marginal land with minimal inputs (Frohlich and Rice 2005; McVay and Lamb 2008; Putnam et al. 1993; Saucke and Ackermann 2006; Shukla et al. 2002); however, many questions remain unanswered relative to its potential to establish and persist in non-agronomic ecosystems. For example, under what biotic and abiotic conditions can one expect *C. sativa* to establish? If it can establish, what amount of propagule pressure is necessary for persistence in the environment? What level of competitiveness relative to regionally common plant species does *C. sativa* possess? The overall goal of this research is to answer these questions and to gain an understanding of the biological characteristics of *C. sativa* and thus allow prediction of any unintended impacts in the Northern Great Plains.

Literature Review

Historical Uses of *Camelina sativa*

Camelina sativa (gold of pleasure, large-seeded false-flax, linseed dodder) is a member of the Brassicaceae family (Frohlich and Rice 2005; McVay and Lamb 2008;

Putnam et al. 1993; Shukla et al. 2002; Zubr 1997, 2003b). Its history dates back to the Bronze (1500-400 B.C.) and Iron (400 B.C.-500 A.D.) Ages of Scandinavia and Western Europe where it was cultivated for human consumption (Zubr 1997, 2003b). In the 19th century *C. sativa* was widely cultivated in France, Belgium, Holland, and Russia, but production declined through the mid 20th century (Frohlich and Rice 2005; Zubr 1997, 2003a). The decline of *C. sativa* as an agronomic crop is hypothesized to be a result of the low cost and the relative ease of hydrogenating *Brassica* spp. (rape) oil, as well as the lack of knowledge about the health benefits of polyunsaturated fats present in *C. sativa* oil (Crowley 1999). Others argue that the crop lost popularity because of difficulty harvesting it due to its small seed size (Zubr 1997). Currently, small amounts of *C. sativa* are produced in Russia, Poland, and Germany (McVay and Lamb 2008).

Camelina sativa was introduced to the Americas as a crop contaminant (Putnam et al. 1993) where it has remained a weed in the production of flax. It has been a weed in Montana since the early 20th century (Blankenship 1901). There has recently been a resurgence of interest in the crop potential of *C. sativa* mainly due to its unique oil properties (Eidhin et al. 2003; Putnam et al. 1993; Shukla et al. 2002; Urbaniak et al. 2008; Vollmann et al. 2001; Vollmann et al. 2007; Zubr 1997).

Biology, Ecology, and Management of *Camelina sativa*

Camelina sativa is a drought tolerant annual herbaceous oil-seed species that exhibits perfect sexual reproduction. It is a short season crop with a life cycle of 85-100 d, and can be planted early in the year because it is frost tolerant (Putnam et al. 1993). Initially, *C. sativa* grows as a rosette (Zubr 1997), then bolts to heights of up to 90 cm by the end of the growing season (McVay and Lamb 2008; Putnam et al. 1993). The leaves are lanceolate, 5-8 cm long with slight pubescence, entire margins, and a light green color. As the plant matures, it produces a flowering stalk and the lower leaves desiccate. *Camelina sativa* produces numerous four-petaled yellow flowers on a raceme (USFS-ILPIN 2002). The seed pods are 6-14 mm long (Putnam et al. 1993) and contain an average of 15 seeds per pod, with a 1000-seed-weight of 0.8-1.8 g (Zubr 1997).

Although capable of growing in poor soils with fewer inputs than other oilseeds (Eidhin et al. 2003; Frohlich and Rice 2005; Gilbertson et al. 2007; Shukla et al. 2002), Jackson (2008) showed that *C. sativa* performs best in Montana with nitrogen levels between 78.4 and 100.7 kg ha^{-1} total N. In Germany, Agegnehu and Honermeier (1997) found that *C. sativa* yield increased as nitrogen fertilizer rate was increased, up to 120 kg ha^{-1}. However, oil levels were shown to decrease as nitrogen was increased (Agegnehu and Honermeier 1997; Jackson 2008; Urbaniak et al. 2008). *Camelina sativa* will respond to phosphorous fertilizer when ambient soil concentrations are below 12 ppm (Jackson 2008).

Researchers at Huntley, MT, in 2006 obtained their highest yields, 1093 and 1067 kg ha^{-1}, with a seeding rate of 6.6 and 8.96 kg ha^{-1}, respectively. (McVay and Lamb 2008). In Germany, Agegnehu and Honermeier (1997) reported a yield of 2057 kg ha^{-1}, at a seeding rate of 5.85 kg ha^{-1}. To ensure a consistent crop stand, a minimum of 3.38 to 5.6 kg ha^{-1} of seed is recommended (McVay and Lamb 2008). To maximize yield, *C. sativa* seeds should be sown near the soil surface, about 6 mm deep and should be planted in mid-March in Montana because yields decline as seeding is delayed (McVay and Lamb 2008).

Camelina sativa requires little to no chemical protection against pests (Putnam et al. 1993). This may be due to the presence of anti-herbivore phytochemicals (Lovett and Jackson 1980). *Phyllotreta cruciferae* Goeze (flea beetle) does not seem to have the affinity for *C. sativa* as it does for *B. napus* (Putnam et al. 1993); however, *Peronospora camelinae* Gaum (downy mildew) has been observed as a problem west of the continental divide in Montana (McVay and Lamb 2008).

It has been claimed that *C. sativa* is competitive against weeds (Putnam et al. 1993; Zubr 1997); however to my knowledge, only twice has its competitive ability been quantified. Crowley (1997) found that spring sown *C. sativa* could be grown successfully without the use of herbicides, but a winter sown crop was much more susceptible to significant weed impacts on yield. Saucke and Ackerman (2006) showed a significant suppressive effect against annual weeds when *C. sativa* was intercropped with a spring-sown *Pisum sativum* L. (field pea) crop, versus when *P. sativum* was grown in monoculture. Although they did not attribute this to allelopathy, Lovett and Jackson (1980) provided evidence of reduced growth of *Linum usitatissimum* L. (flax) seedlings due to *C. sativa* and they hypothesized that allelopathic phytochemicals were the cause.

Market Potential of *Camelina sativa*

With society's heightened concern about global climate change and unpredictable oil prices, the reduction of greenhouse gas emissions and fossil fuel independence are the main drivers of the *C. sativa* market. By 2010, two separate companies in Montana plan to build refineries capable of producing 378.5 mil l of biodiesel per year, with *C. sativa* as the primary feedstock (Schumacher and Smith 2007). Assuming an average yield of 1666 kg ha^{-1} and 40% oil content, this would require a minimum of 1 mil ha of cultivation for the two refineries (Schumacher and Smith 2007).

Camelina sativa biodiesel has been shown to provide an increase in engine power, but a decrease in fuel economy in comparison to mineral diesel (Bernardo et al. 2003; Frohlich and Rice 2005). Additionally, emissions from N_2O, an important greenhouse gas (McTaggart et al. 1997), are greater when using oil from *C. sativa*, but CO and CO_2 emissions are less than mineral diesel fuel (Bernardo et al. 2003). Frohlich and Rice (2005) showed that using biodiesel from *C. sativa* may be problematic in cold climates, which would be of concern in Montana.

In addition to its use as a biofuel, *C. sativa* has potential in the human health food market. The seeds possess a total oil content of > 40% (Shukla et al. 2002; Zubr 2003a). Of this 40%, there is a high level of OMEGA-3 fatty acids (α-linolenic acid, ALA) compared to other commonly used plant oils. ALA is an essential nutrient in human health, and most commonly associated with a diet that includes fish (Zubr 2003a). A unique property of the ALA in *C. sativa* is that it is more oxidatively stable than other plant-based ALA sources, giving it a longer shelf life and therefore, better suitability as a food source (Eidhin et al. 2003).

After the oil is extracted from the seed, the waste product called the ‘meal’ contains about 5% ALA (McVay and Lamb 2008). Because of this, the meal shows promise as a feed for swine, beef, fish, and poultry to create ALA-enhanced food products. The U.S. Food and Drug Administration recently approved the use of *C. sativa* meal, up to 10% by weight of the total ration, for feedlot cattle and broiler chickens (USDA-MT 2009).

Benefits of Including *Camelina sativa* into a Crop Rotation

Diversifying a cereal crop production by including *C. sativa* in the rotation could have many environmental and economic benefits. Weed and disease cycles can be broken (Derksen et al. 2002; Krupinsky et al. 2002; Zentner et al. 2002) and production risk due to uncertain markets and inconsistent precipitation can be lessened (Miller and Holmes 2005; Zentner et al. 2002). Other potential rotational benefits include reduction of soil erosion and an increase in soil moisture and nutrients (Zentner et al. 2002). Diversifying a cereal crop system with the inclusion of an oilseed crop may increase revenue as wheat yields have been shown to be greater following an oilseed (Johnston et al. 2002; Zentner et al. 2002). Finally, oilseed markets operate independent of the cereal grain market, helping to provide a stable income for farmers (Miller and Holmes 2005; Zentner et al. 2002).

Weed communities in a diverse cropping system could become more diverse and could limit the prevalence of any one weed (Derksen et al. 2002). Consequently, this could decrease the reliance of herbicides with one particular mode of action, reducing production costs and the selective pressure towards herbicide resistance (Derksen et al. 2002). In accordance, Blackshaw (1994) found that diversifying a *Triticum hybernum* L. (winter wheat) rotation with spring canola effectively suppressed *Bromus tectorum* L. (downy brome) populations over the course of a six-year period in comparison to continuous winter wheat. This result could be due, in part, to the fact that oilseed crops have been shown to be good competitors against weeds early in the growing season because they have a canopy that closes early (Hakansson 2003).

In addition to rotational effects, a spatially or temporally diverse weed community may provide other benefits (Landis et al. 2005; Norris and Kogan 2005; Pollnac et al. 2009). Whole-field biodiversity and the concomitant prevalence of beneficial organisms may be increased as a result of increasing weed diversity. This, in turn, may enhance natural pest-regulation processes, because weeds can provide habitat for predatory insects (Landis et al. 2005). Additionally, ecosystem services such as nutrient cycling and plant-pollinator interactions can be enhanced through increased weed biodiversity (Altieri 1999; Swanton and Murphy 1996). Although benefits can be realized, it should be noted that these effects vary widely relative to growth habit and rate, time of emergence, length of life cycle, total density, and relative proportions of both weed and crop (Hakansson 2003; Radosevich 1987).

Biology and Ecology of *Camelina microcarpa*

Camelina microcarpa Andrz. ex. DC. (littlepod false flax) is a non-native weed that is closely related to *C. sativa*. It was most likely introduced from Europe as a contaminant of flax (Stubbendieck et al. 1994) and has become a naturalized weed throughout North America (ARS 1971). It is commonly found in disturbed sites across the United States, except in the southernmost portions, and throughout Canada (ARS 1971). It has been present in Gallatin County, Montana since 1897 (Rice 1997).

Camelina microcarpa is an annual (Gilkey 1957) or winter annual herbaceous forb (ARS 1971; Stubbendieck et al. 1994). It has an erect growth habit (Gilkey 1957) and can grow up to 80 cm tall (ARS 1971; Stubbendieck et al. 1994). The leaves are alternate, linear to lanceolate (ARS 1971), with entire or slightly wavy margins (Gilkey 1957), 2-8 cm long (Stubbendieck et al. 1994). The lower leaves exhibit dense simple or stellate pubescence 1-2 mm long (ARS 1971), while the upper leaves are generally smooth (Gilkey 1957). The inflorescence is a raceme that is often greater than 20 cm in length, and contains many four-petaled yellow to pale yellow flowers, 3-4 mm in length (ARS 1971; Gilkey 1957; Stubbendieck et al. 1994). The fruit is a silicle, 4-7 mm long and 4-5 mm in diameter (ARS 1971; Stubbendieck et al. 1994). The seeds are reddish – dark brown and are less than 1 mm long (ARS 1971; Gilkey 1957).

In general, *C. microcarpa* grows shorter than *C. sativa*. Additionally, while *C. microcarpa* is densely hairy on the lower leaves, *C. sativa* is glaborous or only slightly hairy on the lower leaves (Dorn 1984; Gilkey 1957). Also, the pedicels of *C. microcarpa* are rarely greater than 17 mm long, while the pedicels of *C. sativa* usually longer than 17 mm (Dorn 1984). The seeds of *C. microcarpa* are about half the size of *C. sativa (Gilkey 1957)*, and the pods are 2-4 mm smaller (Dorn 1984).

Invasion Potential of Biofuel Crops

Although current biofuel crops such as *Z. mays* and *G. max* are unable to survive outside of cultivation, the current generation of biofuel candidates consists of plants that require minimal human intervention (DiTomaso et al. 2007). These crops are often non-native species selected based on attributes such as drought and low-fertility tolerance, short life-cycles, and rapid accumulation of biomass (Barney and DiTomaso 2008; DiTomaso et al. 2007; Low and Booth 2007). These plants exhibit increased competitive ability (Barney and DiTomaso 2008) which is further enhanced when their primary method of reproduction is vegetative (Radosevich et al. 2007), as is often the case for many perennial plants (Noble et al. 1979; Radosevich et al. 2007). These characteristics, while beneficial for biofuel production, are also traits that many of our most problematic plant invaders possess (Low and Booth 2007). In fact, some of these proposed biofuel cultivars including *Miscanthus* x *giganteus* (miscanthus) come from weedy relatives such as *Miscanthus sinensis* Anderss., and are invasive in parts of the United States (DiTomaso et al. 2007; Raghu et al. 2006). Although *C. sativa* does not reproduce vegetatively, nor is it a perennial plant, some of its attributes (e.g., rapid life cycle, prolific seed production, tolerance to poor soils) could be considered invasive (Radosevich et al. 2007; Reichard 2001). Also, the fact that both *C. sativa* and *C. microcarpa* have been weeds in Montana since the early 20th century (Blankenship 1901), lends credence to my research.

Risk Assessment

Risk can be defined as a probabilistic description of the likelihood that an adverse effect will occur as a result of exposure to a hazard (Wilkinson et al. 2003). A hazard refers to the ability of an agent, such as a chemical, activity, or biological organism, to cause environmental and/or economic harm. Exposure relates to the probability that a hazard will occur (Wilkinson et al. 2003).

Risk analysis is a multi-faceted approach used to characterize risk. Its integration of science, politics, economics, and societal and cultural concerns make the process dynamic and challenging. There are three integral parts of the risk analysis process: risk assessment, risk management, and risk communication (NRC 1983). Although each is able to be defined as a discrete category, in reality there is overlap among the three entities. Risk assessment is the science-based portion of the process, while risk management and communication seek to interpret the science and incorporate societal, cultural, economic, and political considerations into the decision (Peterson and Arntzen 2004).

Risk assessment as a discipline began by attempting to characterize risk, in terms of dose-response relationships, posed by potential environmental hazards such as pesticides on human health. The process proceeds in a logical, step-wise fashion that is recursive, objective, and transparent and provides a quantitative and/or qualitative evaluation of risk based on probability (NRC 1983). An important characteristic of the risk assessment process is that risk is constantly being refined based on new science and the monitoring of past decisions (White and Schwarz 1998; Wolt and Peterson 2000).

There are five essential steps in the risk assessment process: problem formulation, hazard identification, dose-response assessment, exposure assessment, and risk characterization (Peterson and Arntzen 2004). The problem formulation step clearly defines the scope of the assessment (Peterson and Arntzen 2004; Powell 2004). Hazard identification traditionally seeks to determine if an agent is capable of producing adverse effects on humans (NRC 1983). The third step, dose-response assessment, identifies the relationship between the doses of an agent to the outcome on an individual, usually an animal. The data are then extrapolated to the target population, with uncertainties and limitations clearly identified (NRC 1983). Fourth, the exposure assessment estimates the frequency, duration, and intensity of exposure to a hazard relative to the individual (NRC 1983). Finally, risk characterization estimates the probability that a certain health effect will occur under different scenarios (NRC 1983).

Weed Risk Assessment

Although traditional risk assessment was developed to quantify the effect of chemical and radiation stressors on people and the environment, it has recently been expanded to include risk assessment of invasive species (Powell 2004). Although difficulties certainly exist in traditional risk assessment, chemicals and other toxins typically can be estimated in terms of the precise amount necessary to cause a human, plant, or animal chronic or acute harm. Exposure assessments of invasive species, on the other hand, are difficult to quantify because living organisms can multiply and spread throughout the environment (Stohlgren and Schnase 2006). Additionally, public perception of what constitutes damage is highly subjective, thus making risk analysis of invasive species even more difficult. For example, how much biodiversity loss in an area is acceptable? What type and how much alteration of ecosystem function should be allowed? Therefore, invasive species policies are driven not only by science, but by social aspects as well.

Many introduced terrestrial and aquatic species have caused unintended environmental side effects with direct implications to ecological biodiversity (Groves et al. 2001). Specifically, invasive species have been implicated in altering disturbance regimes, hydrologic processes, nutrient cycling, and community structure (Gordon 1998). Furthermore, annual economic losses due to invasions have been estimated at nearly $120 billion (Pimentel et al. 2005). Examples of noxious weeds commonly found in Montana that were intentionally introduced include *Potentilla recta* L. (sulphur cinquefoil), *Leucanthemum vulgare* Lam. (oxeye daisy), *Lythrum salicaria* L. (purple loosestrife), and *Tamarix* spp (saltcedar). Although the majority of these invasions could have been prevented if a comprehensive weed risk assessment (WRA) protocol had been in place at the time of introduction, little knowledge exists on how to conduct a thorough evaluation of the invasion potential of a plant species.

Due to the complexities of ecosystems, successful prediction of potentially invasive species is difficult. Although WRA is in its infancy as a profession, there are principles from invasion ecology and traditional risk assessment that can be applied to assess and decrease risk of environmental or economic damage due to invasive species. Current WRA techniques are qualitatively based (Powell 2004), focusing on plant traits such as seed size, competitive ability in the native habitat, and weed status elsewhere (Cousens 2008; Rejmanek 2001). After these biological characteristics have been identified, a score is assigned that corresponds to a rating of low, medium, or high (USDA-APHIS 2004). A major limitation of this qualitative method of assessment is that it is based largely on the subjectivity of the assessor (Powell 2004).

Although there are a handful of predictive WRA methods currently in use, the system that has been most widely adopted was developed in Australia in 1997 (Cousens

2008) and is used by Australian and New Zealand inspection and quarantine personnel to screen any plants under consideration for introduction. The protocol utilizes available information about various characteristics of the plant including biological, ecological, biogeographical, and historical aspects to develop an overall numerical score that assigns the species to a category of 'accept', 'reject', or 'evaluate further' (Pheloung et al. 1999). The single best predictor of invasiveness is a plant's history of invading elsewhere (Reichard 2001). Therefore, that question in the WRA model carries the heaviest weight out of the overall score. The remaining questions seek to identify other characteristics that increase its invasion potential. Assuming that the necessary biological information about the plant is readily available, the system can operate in a relatively objective manner (Pheloung et al. 1999). Though not without problems, the Australian system has been regarded as successful (Caley et al. 2006) and therefore, has been adapted for use in Hawaii, the Czech Republic, and Florida (Cousens 2008).

Another example of a qualitative WRA system has been developed by the Invaders Database System (http://invader.dbs.umt.edu). This system attempts to predict potential noxious weeds from all exotic species present in Montana and Idaho that were introduced after 1951. Using a set of 21 invasive attributes they screened 120 exotic species. Exotics that arrived in Montana and Idaho before 1951 were also screened for cross-validation of the model. Accuracy of the model was 83.3%. Of the 120 species selected to be screened, 15 were already classified as noxious weeds in Montana and/or Idaho. The model correctly identified 11 of 15 species as being potentially invasive. The model did not evaluate *C. sativa*, because it was introduced before 1951.

Although the above methods have been accepted as an effective system based on qualitative information, we propose that any plant species that is to be introduced to a region outside of its native range and utilized on a large scale be subjected to more rigorous, in-field, quantitative testing. This quantitative approach should illustrate life cycle dynamics of the species in the event of escape from cultivation and subsequent establishment in various environments (Barney and DiTomaso 2008; Mack 2008; Raghu et al. 2006). Cousens (2008) states that a quantitative WRA method that balances qualitative accuracy with limited resources is useful after a plant has already been introduced (*post-entry*). However, we are proposing to develop a quantitative WRA method to be used in *pre-entry* situations, in accordance with DiTomaso (2007).

Project Justification and Objectives

Camelina sativa has been embraced by many across Montana and the Northern Great Plains in hopes of starting a renewable energy industry and providing economic benefits for rural areas. If the amount of land devoted to *C. sativa* production increases drastically, as industry goals suggest, it will be beneficial to have some knowledge about possible non-target ecosystem effects attributable to this species. With this research, I hope to provide relevant information, in terms of the ability to forecast the dynamics of *C. sativa*, based on its biological and ecological characteristics. There are two main objectives to this study:

Objective 1: Assess the population dynamics of *Camelina sativa* in rangeland environments adjacent to disturbed areas that are likely to receive escaped propagules.

1.1. Assess *C. sativa* emergence, survival, and reproduction rates in response to different disturbances and seeding seasons to provide a quantitative prediction of its invasion potential.

1.2. Compare and contrast quantitative evidence of *C. sativa* invasion potential gained from field experiments to results suggested by a currently used qualitative weed risk assessment system.

Objective 2: Determine the relative competitive abilities of *C. sativa* and canola under different growing conditions in a controlled environment.

On a local scale, a goal of this research is to provide society with insight into the likelihood of *C. sativa* populations becoming naturalized in areas other than croplands in Gallatin County, Montana. As an overarching goal, we hope that the quantitative weed risk assessment protocol developed from this project can be adapted and utilized in other geographic regions to screen potential plant introductions that are proposed to be cultivated on a large scale.

CHAPTER 2

ASSESSMENT OF THE INVASION POTENTIAL AND DEMOGRAPHY OF A PROPOSED BIOFUEL CROP IN RANGELAND ECOSYSTEMS

Introduction

Reducing greenhouse gas emissions to ameliorate the effects of global climate change and decreasing USA dependence on foreign oil are two potential benefits of large-scale biofuel production (Buddenhagen et al. 2009; Carere et al. 2008; Hill et al. 2006; Walter et al. 2008). For the biofuel industry to be economically and environmentally sustainable however, the benefits must outweigh the costs (Mack 2008). Most plants that are touted as ideal biofuel candidates are non-native species that are being deliberately introduced into ecosystems outside of their native range (Barney and DiTomaso 2008) and the traits inherent in these species are often considered to be weed characteristics (DiTomaso et al. 2007; Raghu et al. 2006). Also, many of these plants are selected, bred, and sometimes genetically engineered in hopes of further increasing tolerance to adverse growing conditions, have the ability for rapid growth, and can survive to produce high yields with minimal human intervention (Low and Booth 2007). Therefore, escape from cultivation and subsequent invasion into other ecosystems may occur, creating a situation in which the costs may outweigh the benefits.

Weed invasions have been implicated in reducing biodiversity (Belcher and Wilson 1989), altering nutrient and hydrologic cycles (Gordon 1998; Mack et al. 2000), impacting native plant-pollinator interactions (Kremen and Ricketts 2000), and displacing wildlife (Thompson 1996). Additionally, extensive economic damage can manifest in the form of weed control costs (Pimentel et al. 2000) and reduction of rangeland forage quality (DiTomaso 2000). Evaluating the probability of biofuel feedstocks to become weeds could help minimize these risks (Buddenhagen et al. 2009; Cousens 2008; Raghu et al. 2006).

The essence of invasion biology lies in the ability to make accurate predictions about the probability of species to invade (Davis 2009). The use of species traits to forecast invasions has sometimes been successful and remains as one of the best predictors (Reichard 2001). In fact, the tools currently available to detect potential plant invaders rely on qualitatively based models that seek to make generalized predictions based on these traits (Powell 2004). There are four major problems with this approach. First, inconsistencies arise due to the complex interplay between characteristics of the invader and the ecosystem in question, as the traits that make a species prone to invade in one ecosystem may not lead to its success elsewhere (Davis 2009). Furthermore, a trait that leads to success in one stage of an invasion does not guarantee its success in subsequent stages (Kolar and Lodge 2002). Second, propagule pressure and its spatial dynamics greatly influence the probability of invasion (Buddenhagen et al. 2009; Davis 2009; Lockwood et al. 2005) and to incorporate this into a prediction model is difficult, as the mechanisms of dispersal for many species are not entirely understood (Wilson et al. 2009). Third, temporal stochasticity in biotic and abiotic factors makes accurate prediction even more difficult (White and Schwarz 1998). Lastly, because these are qualitative assessments, there is inevitable subjectivity when answering questions of these models (Kolar and Lodge 2002; Powell 2004; Simberloff 2005).

The most widely used plant invasion prediction model is the Australian Weed Risk Assessment (hereafter referred to as 'WRA'), officially adopted by the Australian Quarantine and Inspection Service in 1997 as a tool to screen non-native potential plant introductions (Daehler et al. 2004; Walton 2001). The WRA characterizes risk based on answers to questions that pertain to a plant's biological, ecological, and biogeographical characteristics (Pheloung et al. 1999). A score is then assigned to that plant and it is placed in one of three categories: 'accept' for introduction, 'evaluate' further, or 'reject'. This system has been regarded as successful (Caley et al. 2006; Cousens 2008; Pheloung et al. 1999; Weber et al. 2009) and it has been adopted and modified for use in other parts of the world such as Hawaii and other Pacific Islands (Daehler et al. 2004; Gordon et al. 2008). However, because the WRA cannot accurately characterize all of the important interactions mentioned earlier and leaves most of the biological aspects of assessment up to expert opinion because of the lack of data, its outcomes can be applied only in a very general way and therefore will always be limited in its power to predict invasion potential of species (Caley et al. 2008; Cousens 2008; Pheloung 2001; Pheloung et al. 1999; Powell 2004).

Terminology throughout the invasion biology literature has been inconsistent (Daehler 2001; Davis and Thompson 2002, 2000, 2001). The term 'invasion' itself is amongst the most ambiguous (Colautti and MacIsaac 2004), with definitions ranging from 'non-agricultural weeds that establish and spread to new habitats' (Daehler 2001) to 'non-native plants that produce measurable impacts on ecosystem function or community distribution' (Parker et al. 1999). In this research, our definition of 'invasion' is in line with the first example, as we made no attempt to quantify impacts on ecosystem structure or function. In terms of predicting invasions, Davis (2009) used the term 'invasion pressure' to describe the dynamic relationships among species and ecosystem attributes, and the role of propagule pressure. The term 'invasion potential', as used in this paper is a simplification of Davis' invasion potential concept. Because this is only a first step in assessing the invasion risk of *Camelina sativa* (L.) Crantz (gold of pleasure, large-seeded false flax), we do not integrate spatial dynamics, nor do we try to predict the way in which the probability of establishment will change in response to environments different from our field sites. Therefore, the term 'invasion potential', as used in this thesis, refers to the probability that *C. sativa* will exhibit positive population growth, as this can indicate the possibility of negative ecosystem impacts in the future (Davis et al. 2000). To do this, we explored the use of population dynamics modeling as a quantitative approach to risk assessment to minimize the subjectivity and uncertainty inherent in qualitatively based prediction models.

Several methods have been used to model population dynamics of organisms (Caswell 2001; Freckleton et al. 2008). The goal of these modeling approaches is to capture the rates of births, deaths, immigration, and emigration, and their effects on population size and distribution among life history stages. In a weed-specific context, modeling can aid in developing effective management strategies, formulating hypotheses, and directing research priorities (Maxwell et al. 1988). One commonly used modeling method to characterize population dynamics is through the use of difference equations, an approach that provides several advantages. First, difference equations are simple to use and understand. Second, difference equations provide the ability to calculate the population growth rate (λ), determine the stable age distribution, and perform sensitivity analyses. Finally, difference equations are useful for populations in which individuals progress through life as a cohort, as is often the case in agriculture where annual plants are bred to emerge and develop through life in a predictable manner (Case 2000; Crawley 1997; May 1974, 1976). As such, this is a biologically reasonable approach to model the population dynamics of non-perennial plant species.

It is possible that the amount of land allocated to *C. sativa* production as a biofuel crop in Montana could increase substantially in the next few years (Pilgeram et al. 2007). The characteristics that make this an attractive crop to produce (tolerance to drought, frost, and low fertility, low incidence of pests and disease, ability to be seeded early) may also increase the probability that it will be able to survive outside of cultivation. The purpose of this study was to assess the risk of *C. sativa* to invade disturbed rangeland ecosystems in southwest Montana along roads where crop seeds are likely to be dispersed. Specifically, the objectives of this study were to (*i*) quantitatively estimate *C. sativa* emergence, survival, and reproduction rates in response to different disturbances and seeding times in two contrasting rangeland ecosystems and (*ii*), compare and contrast quantitative evidence of *C. sativa* invasion potential gained from field experiments to qualitative results suggested by the WRA.

Materials and Methods

Site Description

This study was conducted in two rangeland ecosystems in Gallatin Co., MT. The first was located at the Montana State University Fort Ellis Research Center (45°40.25'N, 110°57.48'W), 1506 m elevation, and the second was at the Montana State University Red Bluff Research Ranch (45°35.45'N, 111°36.95'W), 1420 m elevation. Both sites were south-facing with minimal slope, but with differences in annual precipitation, temperature, soil characteristics (Table 2.1), and plant communities (Tables 2.2, A.1). Both sites were chosen because they represent historically disturbed rangeland sites that are likely to receive unintentionally dispersed seed (i.e. along a highway and adjacent to a highway gravel pit).

Experimental Design, Application of Disturbances, and Seeding

This experiment was conducted twice, during the 2008 and 2009 growing seasons. The experimental design was a randomized split-plot arrangement (Fig. 2.1). Ten replications at Fort Ellis and eight replications at Red Bluff were established in 2008. Due to space constraints, nine replications were established at Fort Ellis and six at Red Bluff in 2009. Main plots measured 3.5 m by 1 m and subplots were 1.5 m by 1 m. A buffer of 1 m between main plots helped reduce herbicide drift and a 0.5 m buffer between subplots minimized seed movement.

Plots were randomly assigned to one disturbance type (herbicide, mechanical, or no disturbance) applied before seeding. Mechanical disturbance was applied on 29 October, 2007 and 9 October, 2008 by using a shovel to overturn the top 15 cm of soil.

Two herbicide treatments, metsulfuron + chlorsulfuron (Cimarron Plus) (DuPont, Wilmington, DE) and imazipic + glyphosate (Journey) (BASF Corp., Research Triangle Park, NC) were applied with a backpack sprayer with a fan nozzle at rates of 91.35 ml ha^{-1} and 1.177 l ha^{-1}, respectively, on 19 November, 2007 and 26 October, 2008. Two plots in each replication received no disturbance. One of these plots received *C. sativa* seeds and was used to test the ability of this species to emerge, survive, and reproduce in the presence of pre-existing vegetation without disturbance. The other plot was used as a control, receiving no *C. sativa* seeds to determine the presence of any pre-existing seed. *Camelina sativa* can be sown either in the fall, as a dormant seeding, or in the spring (Putnam et al. 1993). Consequently, each main plot was split (referred to as 'subplots' hereafter) and assigned either a fall or spring seeding.

Before fall seeding in 2007, *C. sativa* var. 'Celine' seed germinability was evaluated in a Conviron CMP 3244 growth chamber (Conviron, Winnipeg, Canada) at 20° C and 24 h photoperiod. Four subsamples of 25 seeds each were placed on hydrated blotter paper in clear boxes. After five days, *C. sativa* percent germination was 98% ± 1% (mean ± SD). *Camelina sativa* seeds were distributed onto the soil surface at the field sites at a rate of approximately 6,666 m^{-2} (10,000 seeds per subplot) and stepped on lightly to reduce movement by wind or water. Fall seeding for the first trial occurred on 6 and 7 December, 2007 at Red Bluff and Fort Ellis, respectively. The spring seeding for the first trial occurred on 15 and 24 March, 2008 at Red Bluff and Ft. Ellis, respectively. The second trial fall seeding took place on 2 November, 2008 at both sites and spring seeding occurred on 21 March, 2009 at both sites.

Camelina sativa Demographic Data Collection

Emergence and Seedling Survival: *Camelina sativa* seedling emergence rates were estimated using five 100 cm^2 rings randomly placed within each subplot. The rings were constructed from 2.5 mm wire and were permanently placed for the duration of the study. Beginning in March of each year, weekly counts of *C. sativa* seedlings in each ring were conducted to estimate the number of seedlings m^{-2}. A seedling was considered to have emerged when cotyledons became visible. The emergence rate was estimated by summing the numbers of seedlings in the rings, scaling to 1 m^2, and dividing by the number of seeds sown m^{-2} (6,666). *Camelina sativa* survival rates were estimated by dividing the number plants harvested from each subplot at the end of the growing season by the number of estimated emerged seedlings per subplot.

Plant Biomass and Fecundity: At maturity (early – late August), all *C. sativa* plants were harvested by cutting the stem at the soil surface. For each subplot, all plants were placed in one paper bag and stored at room temperature until samples were processed (up to 1 mo). Plant biomass and seed weight were measured to the nearest 0.001 g for each subplot. The number of seeds produced per subplot was estimated from the relationship of seed biomass to seed number obtained from the data of the individually-marked plants (see below). The r^2 values for the relationship between seed number and seed biomass collected at Fort Ellis in 2008 and 2009 were 0.91 ($p < 0.001$) and 0.93 ($p < 0.001$), respectively. Lack of seed survival and subsequent populations to produce seed at Red Bluff in 2008 precluded quantifying the seed number to seed biomass relationship. However, in 2009 the r^2 value was 0.81 ($p < 0.001$).

Data Analysis

Few consistent patterns of emergence were observed across sites and years. Consequently, emergence analyses were conducted separately for each site-year. To minimize problems associated with non-constant variance and non-normality, emergence rates were $\sin^{-1}$ (square root)-transformed and subjected to analysis of variance using the mixed model (PROC MIXED) procedure of SAS version 9.1 for UNIX (SAS Institute, Inc., Cary, NC). Results of this analysis indicated significant seeding season × disturbance type interaction and further comparisons were made using the SLICE option in SAS version 9.1.

Differences in biomass of harvested *C. sativa* plants were evaluated relative to seeding season. A Type III ANOVA model was fit to the data using R statistical software (version 2.8.1). The model included seeding season and site and was blocked by year. This analysis did not include disturbance as a variable because plants that survived to be harvested occurred only in the mechanical disturbance treatment.

Camelina sativa Population Dynamics Model

A model was constructed with Microsoft Excel and Microsoft Visual Basic 2007 to investigate *C. sativa* population dynamics. Due to lack of plant survival in other treatments, only the mechanically disturbed plots and associated vital rates were included in the model. *Camelina sativa* population dynamics in response to a mechanical disturbance exhibited stark differences, particularly in plant survival, between the two field seasons. Therefore, the model was constructed with the option to simulate data using three approaches for each site: using 2008 data only, using 2009 data only, or using the two years combined. Variability was incorporated by randomly selecting sets of vital rates from a plot across one or two field seasons. All vital rates included in the model were from values observed in the field in spring-seeded plots except the fall emergence rate which was calculated from plant counts in the fall-seeded plots.

All simulations began at time t = 0 under the assumption that propagules escaped in spring just before the growing season, for an initial spring seedbank (SBs) density of 6,666 m^{-2}. The state variables in the model were seedlings (Sdl), seed producing adults (A), seeds produced (SP), and fall seedbank (SBf), and are linked by the vital rates: emergence from SBs (S_{emrg}), Sdl survival to A (S_t), seed produced per A (S_{pp}), and seed survival from fall to fall (S_s) (Table 2.3; Fig. 2.2). All seed that was produced was returned to SB_f, as we assumed that immigration was equal to emigration. Although the model included an option to specify a value for S_s, our data suggest this vital rate was 0 eliminating the potential for the population to become established and become invasive (see Appendix A). As such, for all t > 0, seedlings emerged from SB_f in the fall and remained dormant as winter annuals until the following spring. Those fall seedlings that survived the winter were represented by the vital rate, F_{emrg}.

The numbers of individuals at each life stage subsequent to initial seeding of SBs were determined by randomly selecting one set of vital rates from a plot. Multiplication of vital rates and the number of individuals in each life stage provided the population size of the next life stage (Table 2.4). At the end of each life cycle, the population growth rate, λ, was calculated as:

$$\lambda = \frac{N(t)}{N(t-1)} \qquad \text{Eq. 2.1}$$

where λ is the population growth rate, $N_{(t)}$ is the number of seedlings at the present time step, and $N_{(t\text{-}1)}$ is the number of seedlings at the previous time step.

In instances where population growth was declining, *C. sativa* populations were simulated until the population reached a quasi-extinction threshold of 1 seed km^{-2}. The quasi-extinction threshold is commonly used in modeling population dynamics and is useful in situations where the population is exponentially declining in a constant environment, yet will never reach extinction (Caswell 2001). When this threshold was reached, a geometric mean value of λ was then calculated. Using the geometric mean to determine the average value of lambda is preferred over the arithmetic mean because using the latter tends to overestimate population growth rates (Case 2000; Freckleton and Watkinson 1998).

<u>Simulation Scenarios:</u> The model was run separately for each field site under different scenarios to explore its characteristics and to determine factors governing the population dynamics of *C. sativa* in the presence of a mechanical disturbance. First, the ‘default’ scenario projected the population using observed values and provided a representation of what might happen under actual conditions. Each set of vital rates had an equal probability of being selected and the number available to choose from ranged from 6-19, depending on how many replications (plots) were used for each site and year(s). The simulations were run 1000 times, each time projecting the population until

the quasi-extinction threshold was reached. After the last run the geometric mean λ and times to extinction for each run were averaged. The second modeling scenario was a 'worst case' situation where *C. sativa* population dynamics were modeled separately for each site by using the highest value of each vital rate that was observed over the two field seasons. There was no variability under this scenario and therefore required only one run. Each simulation was run until the quasi-extinction threshold was reached, and the geometric mean λ and the time to extinction were calculated. The third modeling scenario was a sensitivity analysis of seed survival (S_s) and propagule pressure. This was conducted separately for each site using vital rate values averaged across the two field seasons and an initial seeding rate of 6,666 seeds m^{-2} (Table A.2). Based on hypothetical changes in S_s, as well as propagule pressure (number of seeds m^{-2} added for all $t > 0$), we determined which combinations of these two parameters would result in a geometric mean $\lambda > 1$ over 21 years. As with the second scenario, there was no variability and therefore these simulations were run only once.

Australian Weed Risk Assessment

Because it has been suggested that the WRA can be successfully used in other parts of the world with very little modification (Daehler et al. 2004; Gordon et al. 2008), the invasion potential of *C. sativa* was further evaluated using this system. This model uses a spreadsheet-based approach and consists of 49 questions divided into three categories: biogeography, undesirable attributes, and biology/ecology. A minimum of two questions must be answered in each of the first two sections and six must be answered from the third section (Pheloung et al. 1999). Most questions are given equal weight, and although the user has the ability to change this, all evaluations for *C. sativa* were done using default settings. Potential scores range from -14 to 29, with a higher score indicating the plant has a higher risk of becoming a weed. A score below one corresponds to 'accept' for introduction, while scores of 1-6, and 7+ placed the plant in the 'evaluate' further category and the 'reject' category, respectively.

The WRA model was run four times, each time altering the responses of five questions to which definitive answers in the literature or from this research could not be obtained. Two of these questions pertained to the ability of seeds to remain viable as they pass through birds and other animals. The third question evaluated the ability of the plant to hybridize naturally. The fourth question to which the answer was unknown was about the similarity of the climate between the plant's native range and its introduced range, while the last question is about the quality of the data used to answer the previous question. It is suggested that if no climate matching data are available, a '2' be assigned (Pheloung et al. 1999). This corresponds to 'high' species climate suitability to Australia and 'high' climate match data quality. We varied the answer to this question from '0' ('low') to '2' in different runs.

Results

Seedling Emergence

Different patterns of *C. sativa* seedling emergence were observed between Fort Ellis and Red Bluff. Irrespective of disturbance type or year, emergence of fall-seeded *C. sativa* at Red Bluff was generally lower than when it was seeded in the spring (Fig. 2.3). Emergence rates of *C. sativa* in the mechanical disturbance was the lowest of all treatments at Red Bluff in 2008, averaging 0.014 ± 0.009 (mean ± SD) when seeded in the fall, and 0.021 ± 0.013 when seeded in the spring. In 2009 however, this trend was not apparent as there was little difference among treatments regardless of seeding season.

Similar to Red Bluff, *C. sativa* emergence rates in 2008 and 2009 at Fort Ellis were lower when seeded in the fall than when seeded in the spring (Fig. 2.4). Although not always significantly different in comparison to other disturbances, the lowest emergence rates were observed in the metsulfuron + chlorsulfuron treatment. In contrast to the patterns observed at Red Bluff, the mechanically disturbed plots at Fort Ellis produced the highest emergence rates of all the treatments, especially when seeded in the spring. However, due to high variability in 2009 this emergence rate was not different than the glyphosate + imazipic or the undisturbed plots (0.14 ± 0.05 and 0.18 ± 0.07, respectively).

Seedling Survival

The only *C. sativa* plants that survived beyond emergence at both sites and in both years were found in the mechanically disturbed plots. As with emergence, *C. sativa* seedling survival to maturity at Fort Ellis and Red Bluff over two seasons displayed no consistent trends (Table 2.5). For example, spring seeded subplots in 2008 produced only three survivors at Red Bluff, but a total of 3,325 mature plants were harvested at Fort Ellis. The difference between sites was not as drastic in 2009, but the opposite trend was noticed, with more plants from spring seeded subplots surviving at Red Bluff than at Fort Ellis (Table 2.5).

Camelina sativa Biomass and Fecundity

Biomass of mature *C. sativa* plants was greater in fall-seeded plots than in spring-seeded plots at both Red Bluff and Fort Ellis ($p < 0.001$; Fig.2.5). *Camelina sativa* biomass and seed production were positively correlated ($r^2 = 0.82$, $p < 0.001$), with fall sown plants producing more seeds per plant than spring sown plants (Fig A.1).

With the exception of spring-seeded plots in 2009, total seed production was always lower at Red Bluff than at Fort Ellis. Plants from three spring-seeded subplots at Fort Ellis in 2008 produced seed in excess of the original 6,666 m^{-2}. However, when averaged across subplots, seed production was never greater than the amount that was originally sown (Table 2.6).

Population Dynamics Model

Using the observed values under the 'default' scenario for Fort Ellis and Red Bluff, the average λ value for 1000 simulations never exceeded 0.03 and the maximum time to extinction was six years (Table 2.7). Data simulation under the 'worst case' scenario, where vital rates were assigned the highest observed values over the two field seasons, resulted in λ values of 0.35 and 0.03 and extinction times of 22 yr and 7 yr for Fort Ellis and Red Bluff, respectively. The third modeling scenario revealed that, using the average vital rate values across the two field seasons and varying the annual propagule pressure rates for all $t > 0$ from 100 to 10,000 seeds m^{-2} and the seed survival rates (S_s) from 0 to 0.8, extremely high rates of each parameter were necessary to obtain increasing populations ($\lambda > 1.0$) at both sites (Fig. 2.6). Fort Ellis populations required 6,500 seeds m^{-2} to be added annually and seed survival (S_s) to be increased from 0 (observed) to 0.8 to produce increasing populations. Increasing populations were not obtained at Red Bluff until annual seed additions exceeded 100,000 seeds m^{-2} when S_s = 0.8.

Australian Weed Risk Assessment

The outcome from the WRA varied depending on how questions were answered but under no circumstances was *C. sativa* accepted for introduction. In scenario one, the questions about animal dispersal of propagules and hybridization with weedy relatives were answered ‘yes’ and the climate questions were answered with a score of 2 (high climate suitability). The resulting score for this ‘worst case’ scenario was 7 (‘reject’). In scenario two, when the model was run with climate matching still set at 2, but the other answers changed to ‘no’, the WRA score was 1 (‘evaluate’). The model was run two more times; during each run the answers to the climate questions were set to 0 (low), while the questions about dispersal and hybridization were answered ‘yes’ and ‘no’ for runs one and two, respectively. The resulting scores were the same as when climate matching was set to ‘2’, with a score of 1 when the dispersal and hybridization questions were answered ‘no’ and a score of 7 when they were answered ‘yes’. Thus, the outcomes of ‘reject’ and ‘evaluate’ were achieved, indicating that *C. sativa* should not be allowed entry into the region based on our current knowledge of this species.

Discussion

Population dynamics models have been used successfully for many purposes throughout ecology (Case 2000; Caswell 2001). In a plant-specific context, models have been used to assess likely effects of management tactics in terms of conservation of endangered plants (e.g. Griffith and Forseth 2005; Menges 1990; Schemske et al. 1994), or control of weedy species in both natural and agricultural systems (e.g. Davis et al. 2006; Forcella 1992; Freckleton et al. 2008; Maxwell et al. 1988; Parker 2000). In this study, we used a population dynamics model not to assess the effects of management scenarios, but as a way of evaluating the risk of establishment of *C. sativa* in representative rangeland ecosystems within southwest Montana. We successfully showed that the invasion potential of *C. sativa* into these rangelands is low. Furthermore, we illustrated that data-driven population models as an invasion prediction tool can offer valuable site-specific information that is otherwise overlooked by qualitative prediction models.

Previous studies (e.g. Davis et al. 2004; Jordan et al. 1995) provided evidence that seed survival is an important parameter in annual weed population dynamics. Furthermore, it is a significant component in plant invasions (Radosevich et al. 2007), as the most successful invaders possess high levels of seed dormancy and hence, seed survival (Timmins and Owen 2001). In accordance with the low invasion potential of *C. sativa* into the evaluated rangelands, evidence suggests that seeds do not survive overwinter, thus negating the possibility for continuity between generations to sustain a population (see Appendix A). This is probably because seeds sown in fall were exposed to a higher risk of predation and natural decay versus seeds that were sown in the spring. Supporting this, Crawley (1992) observed seed predation rates as high as 60% day^{-1} in non-agricultural systems.

The third modeling scenario investigated the likely outcomes that could result from the combined manipulations of seed survival and propagule pressure. In light of the evidence suggesting low *C. sativa* seed survival, it may seem moot to investigate changes in this parameter. However, this scenario is realistic because *Camelina microcarpa* Andrz. ex. DC. (little pod false flax), a non-native congener of *C. sativa*, is a weed that possesses seed dormancy and is common throughout most of Montana (USDA-NRCS 2010). It is possible that hybridization could occur between these two species, thus transferring the dormancy trait and increasing the invasive potential to *C. sativa*, as intra-genera gene transfer between species has been previously documented (Vila et al. 2000). In our simulations however, manipulating seed survival alone, even increasing to biologically improbable rates, did not result in population growth. For this, we needed to add seeds each spring in combination with increased seed survival. This is also a likely scenario because the amount of land devoted to the production of *C. sativa* is projected to increase in Montana (Schumacher and Smith 2007), thus creating a situation of sustained propagule pressure (Wilson et al. 2009) and increased likelihood of invasion (Leung and Mandrak 2007; Lockwood et al. 2005; Martínez-Ghersa and Ghersa 2006; Wilson et al. 2009). However, and further validating our argument of low risk of invasion into rangelands by *C. sativa*, results of the modeling exercise indicated that it was necessary to drastically increase seed survival and propagule pressure to obtain positive population growth.

Disturbance regimes have long been recognized as an integral force on ecosystem structure and function, helping to maintain species richness and diversity (Grime 1977;

Hobbs and Huenneke 1992; Larson 2003; Larson et al. 2001). However, anthropogenic disturbances that drastically depart from the frequency and severity of naturally occurring disturbance regimes can facilitate non-native plant invasions (Alpert et al. 2000; D'Antonio and Vitousek 1992; Hobbs and Huenneke 1992). In this experiment, *C. sativa* survival beyond emergence occurred only in plots subjected to mechanical disturbance. There are two potential reasons for this observation. First, lack of survival in all treatments except mechanical disturbance could be due to the presence of plant litter from previous years' growth. Although litter has been shown as being positively associated with seedling emergence (Evans and Young 1970), it can preclude subsequent survival (Fowler 1988; Kubo et al. 2004), as seedlings of small-seeded species such as *C. sativa* may not have enough stored energy to penetrate the litter layer (Leishman and Westoby 1994; Seiwa and Kikuzawa 1996). The second explanation is that the lack of interspecific competitors and plant litter in mechanically disturbed plots probably provided resources, including sunlight, that were unavailable in other treatments. Small-seeded species are dependent on high sunlight for successful establishment (Crawley 1997; Milberg et al. 2000), and lack of litter in the mechanically disturbed plots enhanced the amount of light received by *C. sativa* seedlings. Also, fewer neighboring plants reduced the intensity of interspecific competition and therefore increased the availability of other resources such as soil nutrients and water. These results show that *C. sativa* acts as a ruderal species, as it is dependent on high levels of disturbance for successful colonization (Grime 1977; Radosevich et al. 2007).

C. sativa survival occurred in both years only in the mechanical disturbance, but with a high temporal and spatial variability at both Fort Ellis and Red Bluff. Temporal variability in abiotic factors, namely temperature and precipitation, has been recognized as a driver of weed population dynamics (Cousens and Mortimer 1995; Forcella 1992; Freckleton and Stephens 2009; Freckleton et al. 2008) and could have affected the survival of *C. sativa*, as 2009 had higher than average early season precipitation. However, the fact that the survival rates at Red Bluff and Fort Ellis displayed opposite trends in 2009 leads to the need for additional research on the factors driving *C. sativa* survivorship.

At Red Bluff in 2009, *C. sativa* survival past the flowering stage was limited by *Odocoileus virginianus* Zimmerman (white-tailed deer) and grasshopper herbivory (personal observation; see Appendix A for specific mortality rates). Supporting these observations, Ehrlen (1995) determined that vertebrate grazers caused significant reductions in the number of medium (231-772 mm^3) to large (> 772 mm^3) *Lathyrus vernus* (L.) Bernh. (spring vetch) plants. He further observed that that these plants failed to produce seed after grazing and concluded that herbivory by vertebrates can significantly impact plant population dynamics. Parker (1985) observed that defoliation due to *Hesperotettix viridis* Thomas (grasshopper) resulted in a 400% decrease in mean seed production of the shrub *Gutierrezia microcephala* (DC.) A. Gray (threadleaf snakeweed). In Montana, previous studies documented that *O. virginianus, Centrocercus urophasianus* Bonaparte (sage grouse), and *Antilocapra americana* Ord (pronghorn antelope) incorporate *Camelina* spp. into their diet (Allen 1968; Peterson 1970; Pilgeram et al. 2007).

Although *C. sativa* seeds do not survive over winter, we observed spring emergence from fall-seeded subplots, suggesting that *C. sativa* plants can act as winter annuals, a characteristic that has been investigated agronomically with mixed results (Crowley 1999; Gilbertson et al. 2007; Putnam et al. 1993; Zubr 1997). In this study, plants that survived to maturity in fall- seeded subplots had significantly higher biomass and seed production versus plants harvested from spring-sown plots. This is because they germinated in the late fall or winter, established a root system, and remained dormant until conditions permitted continued growth in the spring. This head start in growth may be why fall-seeded plants were larger and more reproductively successful than their spring-seeded counterparts.

Camelina sativa Population Dynamics Model: Limitations and Assumptions

The population dynamics model developed to assess the invasion potential of *C. sativa* in rangeland ecosystems is based on data collected over the course of only two years. As such, the lack of data about long term vital rate variability means this model is limited in its predictive power (Freckleton and Watkinson 1998). Also, data were collected at only two field sites, both of which are infested by non-natives. Therefore, the inference space is limited to other semi-degraded ecosystems with similar biotic and abiotic conditions. Finally, data were collected after a freshly created mechanical disturbance and this model operates under the assumption that the system is being disturbed annually. In a real-world situation, this frequency of disturbance is unlikely, as the ground that was disturbed would soon begin to be re-colonized by plants. Therefore, the already low population growth rates from the modeling exercises may indeed be over estimating what would be observed in reality.

This model assumed no spatial dynamics among *C. sativa* populations. However, primary and secondary seed dispersal are important components of a comprehensive population dynamics model (Case 2000). If we had found the invasion potential of *C. sativa* to be high, an effective second step would be to refine this model by collecting data about dispersal to consider spatial aspects of population dynamics.

The Necessity of a Comprehensive Approach to Assess Weed Risk

Species proposed for introduction to areas outside of their native range should be subjected to a regulatory framework by which to detect possible invaders (Ewel et al. 1999). However, opinions differ as to the most efficient way to accomplish this task with limited resources. Qualitative model-based approaches, although useful, are limited in that they can offer only general predictions (Davis 2009). Conversely, quantitative mechanistic models are limited in their ability to provide useful knowledge outside of the specific site in which data were collected (Kareiva et al. 1996). Both approaches have uncertainty relative to the predictions they produce (Caley et al. 2006; Freckleton and Stephens 2009).

A comprehensive approach to weed risk assessment needs to be implemented such that both qualitative and quantitative approaches are incorporated (Cousens 2008). This multi-step system of assessing risk would greatly enhance predictability by reducing the uncertainty inherent in a single-step approach. This method could be used to assess economically important species exhibiting weedy characteristics, such as biofuels, that are proposed to be planted on a large scale (Cousens 2008). Not only will this approach result in greater effectiveness in screening out invasive species, it will also decrease the risk of rejecting an economically valuable non-weed that could be mislabeled by using purely qualitative methods such as the WRA.

Overall Conclusions and Implications

This risk assessment of *C. sativa* supports the theory that a quantitative, in-field approach to assessing weed risk is a viable second step to a qualitative model-based system such as the WRA. The implementation of a multi-step approach similar to the one we used is efficient, realistic, and capable of providing crucial knowledge that would otherwise be unknown about a plant. We recommend that potential biofuel feedstocks which possess weedy attributes be subjected to in-field testing to determine invasion potential.

On a local level, this experiment helped to answer some unknown questions about the biology and ecology of *C. sativa*. This species is being heavily promoted in Montana by private industry, yet it has a congener (*C. microcarpa*) that has been an established weed in Montana for more than 100 years (Rice 1997). Clearly, it was necessary to evaluate this species for its invasion potential, as the amount of land devoted to *C. sativa* is projected to increase. We provided preliminary evidence that, in the evaluated environments and experimental conditions, *C. sativa* poses no significant invasion threat to undisturbed and anthropogenically disturbed rangelands adjacent to crop fields or roads.

Table 2.1: Annual precipitation, temperature, and soil properties at Red Bluff and Fort Ellis, MT.

	Annual Precipitation-30 yr avg. (cm)	Annual Mean Temperature-30 yr avg. (°C)	Soil Organic Matter (%)	Soil NO_3 (kg/ha)	Soil pH
Red Bluff	40.41	6.5	1.4	3.36	7.1
Fort Ellis	49.02	6.2	4.6	7.86	6.6

Soil samples were taken on 29 September, 2008 at Red Bluff and Fort Ellis with a hand-held soil probe. Soil samples to a depth of 15 cm were taken from within undisturbed subplots and aggregated. Analysis was conducted by Agvise Laboratories (Northwood, ND) based on a subsample of the aggregated soil cores.

Table 2.2: Species richness and percent canopy cover by functional group and most prevalent species at Red Bluff and Fort Ellis.

Vegetation Attributes - Percent Cover and Species Richness		
	Red Bluff	Fort Ellis
% Grass	31	52
% Forbs	7	36
% Litter	55	10
% Bareground	7	2
Bromus tectorum	27	x
Calamogrostis canadensis	4	x
Alyssum simplex	3	x
Poa pratensis	x	27
Bromus inermis	x	20
Artemesia ludoviciana	x	10
Hypericum perforatum	x	8
Lupinus spp.	x	6
Total species richness	11	26

Canopy cover was assessed on 30 June, 2009 at Fort Ellis and 2 July, 2009 at Red Bluff using visual estimation within 1 m^{-2} quadrats.
x = species not present at that field site. See Table A.1 for complete species list.

Table 2.3: Notation used to describe the population dynamics model of *Camelina sativa*.

Life Stages	Definition
SBs	Spring seedbank
Sdl	Seedlings
A	Mature seed producing adults
SP	Seed produced
SBf	Fall seedbank
Vital Rates	
S_{emrg}	Seedling emergence from spring seedbank
S_t	Survival from seedling to seed producing adults
S_{pp}	Seeds produced per plant
F_{emrg}	Seedling emergence from fall seedbank
S_s	Seed survival

Table 2.4: Life stages and equations used in the *Camelina sativa* population dynamics model.

Life Stage	Equation
SBs*	-
Sdl	$(SBs * S_{emrg}) + (SBf * F_{emrg})$
A	$Sdl * S_t$
SP	$A * S_{pp}$
SBf	$SP + (SBf_{t-1} - Sdl_t) * S_s$

* = initial seedbank density of 6,666 seeds m^{-2} at time $t = 0$ for all modeling scenarios. Also refers to the amount of seeds supplied to the system (propagule pressure) for all $t > 0$ in modeling scenario three (sensitivity analysis).

Table 2.5: *Camelina sativa* survival rates (mean ± SD) from emergence to maturity (S_t) and total number of plants harvested from Red Bluff and Fort Ellis in 2008 and 2009.

Site	Year	Seeding Season	S_t	Number of Plants Harvested
Red Bluff	2008	Fall	0.0	0
		Spring	0.01±0.01	3
	2009	Fall	0.15±0.07	93
		Spring	0.02±0.02	88
Fort Ellis	2008	Fall	0.16±0.15	145
		Spring	0.11±0.06	3325
	2009	Fall	0.13±0.09	347
		Spring	0.001±0.004	32

S_t = survival rate of plants to maturity (harvest) relative to estimated emergence rate.

Table 2.6: Estimated *Camelina sativa* seed production (mean ± SD) averaged across replications at Red Bluff and Fort Ellis in 2008 and 2009.

Site	Year	Seeding Season	Number of Seed Produced	% of Amount Originally Sown
Red Bluff	2008	Fall	0	0.0
		Spring	58±116	0.8±1.7
	2009	Fall	395±237	5.9±3.5
		Spring	162±169	2.4±2.5
Fort Ellis	2008	Fall	1595±1953	23.9±29.3
		Spring	5107±3693	76.6±55.4
	2009	Fall	1087±1810	16.3±27.1
		Spring	38±79	0.5±1.2

Table 2.7: Average population growth rate (λ) values and maximum time to extinction results of the *Camelina sativa* population dynamics model after 1000 simulations using observed values from 2008, 2009, and 08/09 combined.

Site	Year	λ (mean±SD)	Max. Yrs. to Extinction
Red Bluff	2008	0.010 ± 0.025	4
	2009	0.002 ± 0.002	4
	08/09	0.005 ± 0.014	4
Fort Ellis	2008	0.017 ± 0.023	6
	2009	0.001 ± 0.002	4
	08/09	0.030 ± 0.064	4

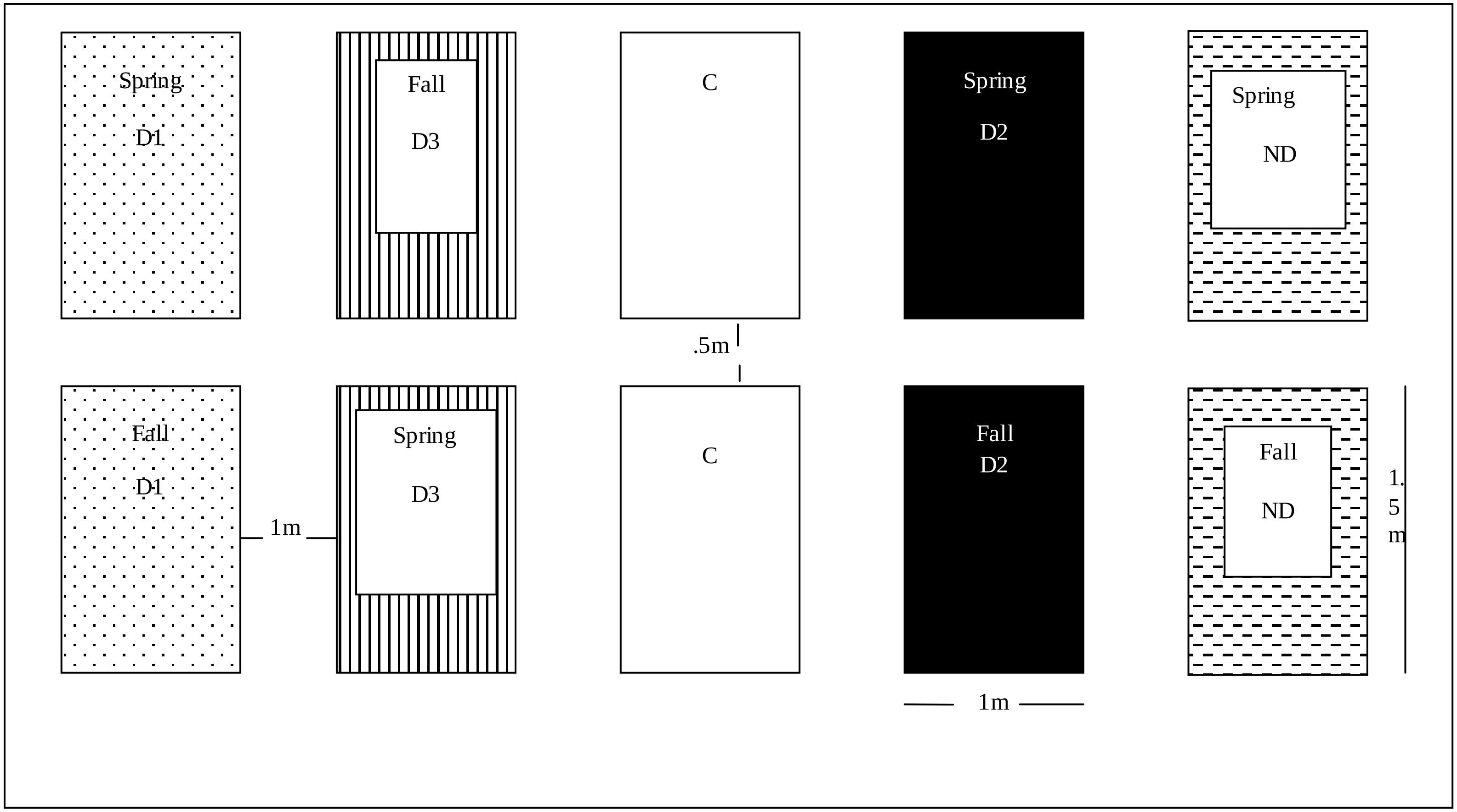

Figure 2.1: Example of a replicate used to assess the invasion potential of *Camelina sativa* into rangeland ecosystems. Disturbances 'D1' (glyphosate + imazipic), 'D2' (metsulfuron + chlorsulfuron), 'D3' (mechanical), 'ND' (no disturbance), and 'C' (control) were applied to main plots. Seasonal seeding (spring/fall) was assigned to each subplot with the exception of 'C', where no seeds were added.

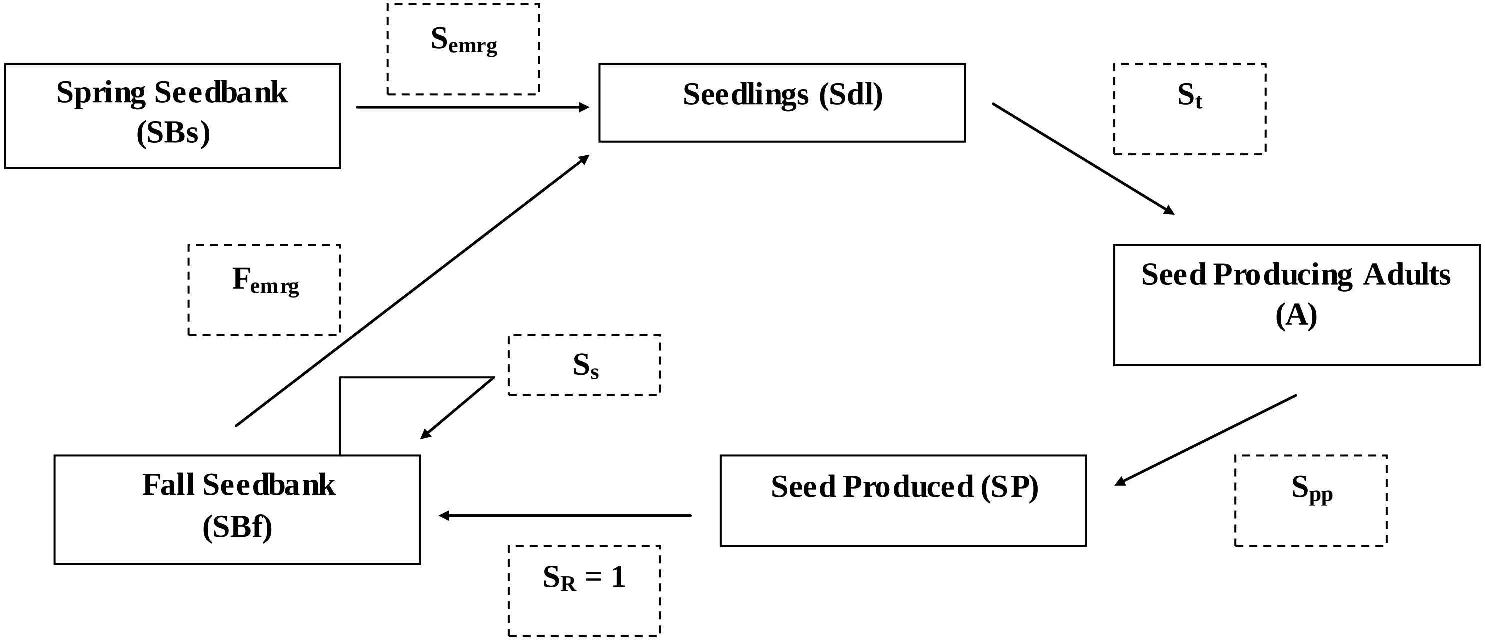

Figure 2.2: A life cycle diagram illustrating the model structure used to assess the population dynamics of *Camelina sativa*. Solid boxes and dashed boxes represent measurable state variables and vital rates, respectively. Vital rates are denoted as follows: S_{emrg} = seedling emergence from spring seedbank, S_t = survival from emergence to maturity, S_{pp} = seeds produced per plant, S_R = seed rain (proportion of seed that is returned to the seedbank; always = 1), S_s = seed survival, F_{emrg} = seedling emergence in spring from fall seed bank. Simulations always began under the assumption that propagules escaped in spring just prior to the growing season at time t = 0. With the exception of modeling scenario three, subsequent years ($t > 0$) did not include S_{emrg} from SBs, as the population transition rate to seedlings between years was regulated by F_{emrg} from SBf.

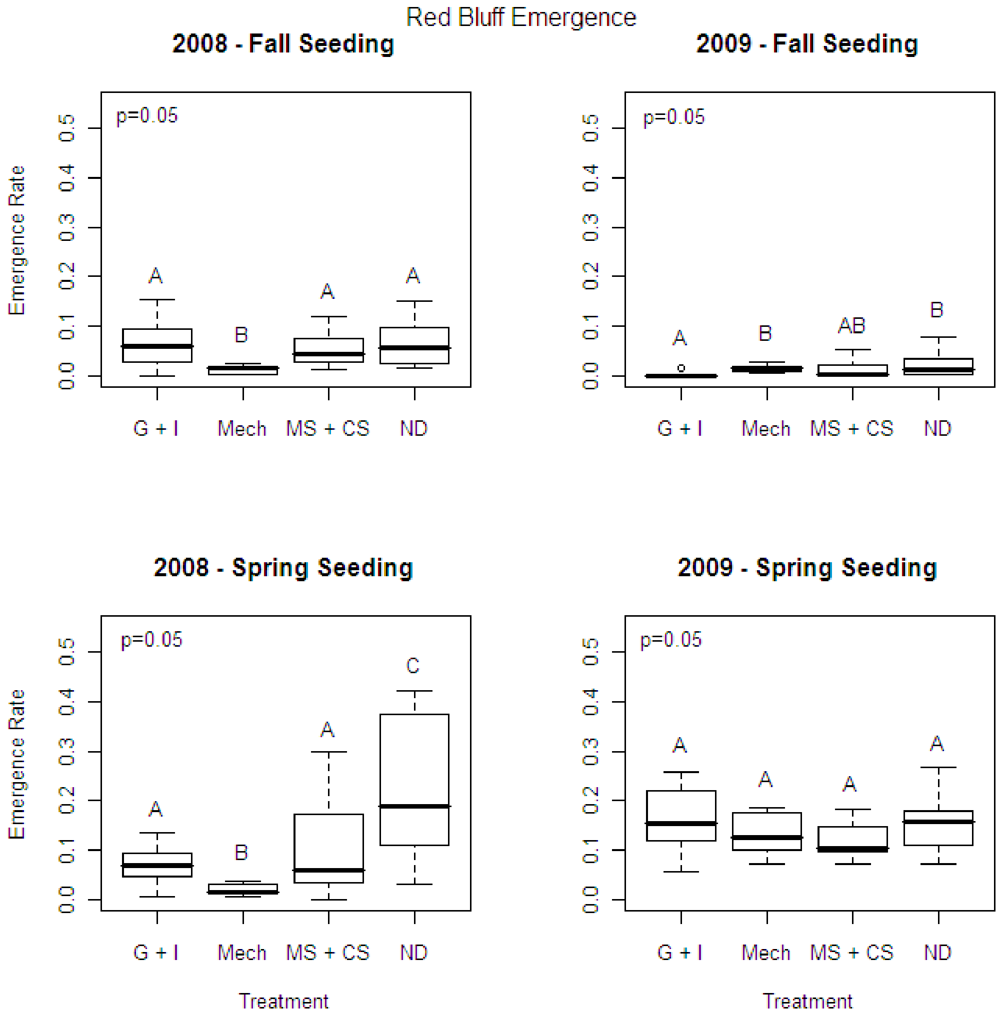

Figure 2.3: *Camelina sativa* emergence values (untransformed) in response to different rangeland management practices conducted at the Red Bluff Research Ranch, MT. 'G+I'= glyphosate + imazipic; 'Mech' = mechanical disturbance; 'MS + CS' = metsulfuron + chlorsulfuron; 'ND' = no disturbance. Letters within the plots represent significant differences at p = 0.05. The bold horizontal line in the boxes shows the median value and the middle 50% of the data is within the box. The ends of the dashed lines (whiskers) show the minimum and maximum values unless there are outliers, in which case ○ signifies an outlier.

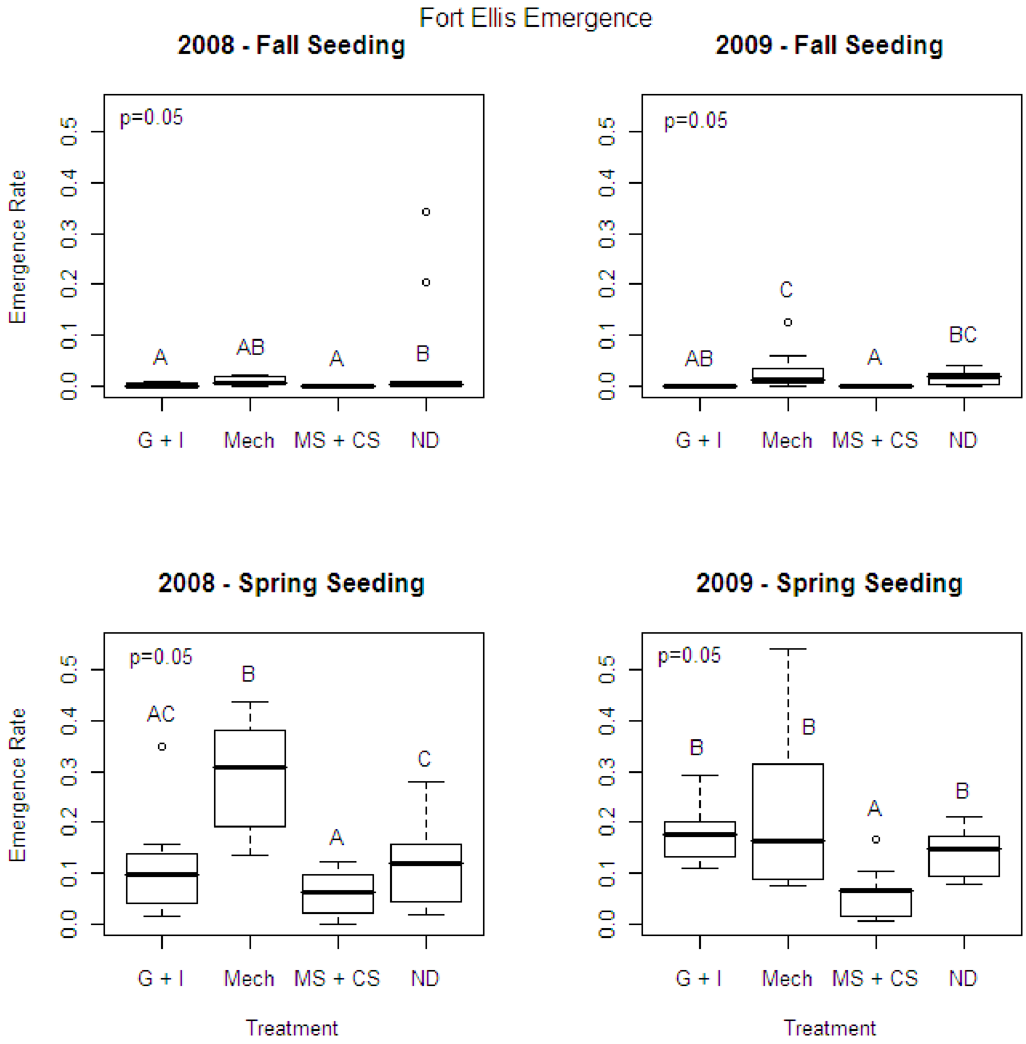

Figure 2.4: *Camelina sativa* emergence values (untransformed) in response to different rangeland management practices at the Fort Ellis Research Center, Bozeman, MT. 'G+I' = glyphosate + imazipic; 'Mech' = mechanical disturbance; 'MS + CS' = metsulfuron + chlorsulfuron; 'ND' = no disturbance. Letters within the plots represent significant at p = 0.05. The bold horizontal line in the boxes shows the median value and the middle 50% of the data is within the box. The ends of the dashed lines (whiskers) show the minimum and maximum values unless there are outliers, in which case '○' signifies an outlier.

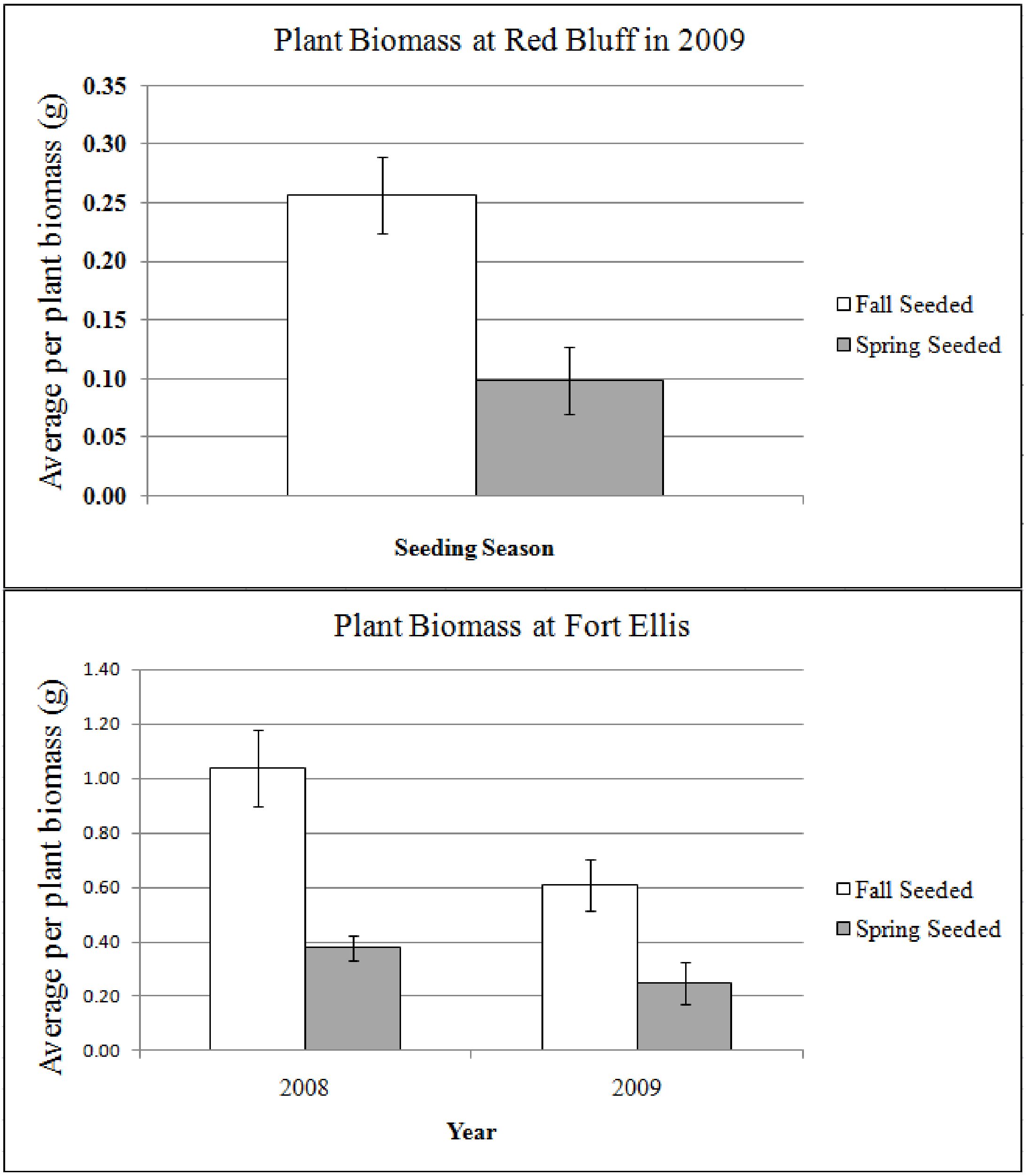

Figure 2.5: Average *Camelina sativa* plant biomass (g) at Fort Ellis and at Red Bluff, MT. 2008 is omitted from Red Bluff due to lack of plant survival. Error bars represent ± 1 SEM. Biomass of plants from spring seeded plots was significantly less than biomass of plants from fall seeded plots ($p < 0.001$).

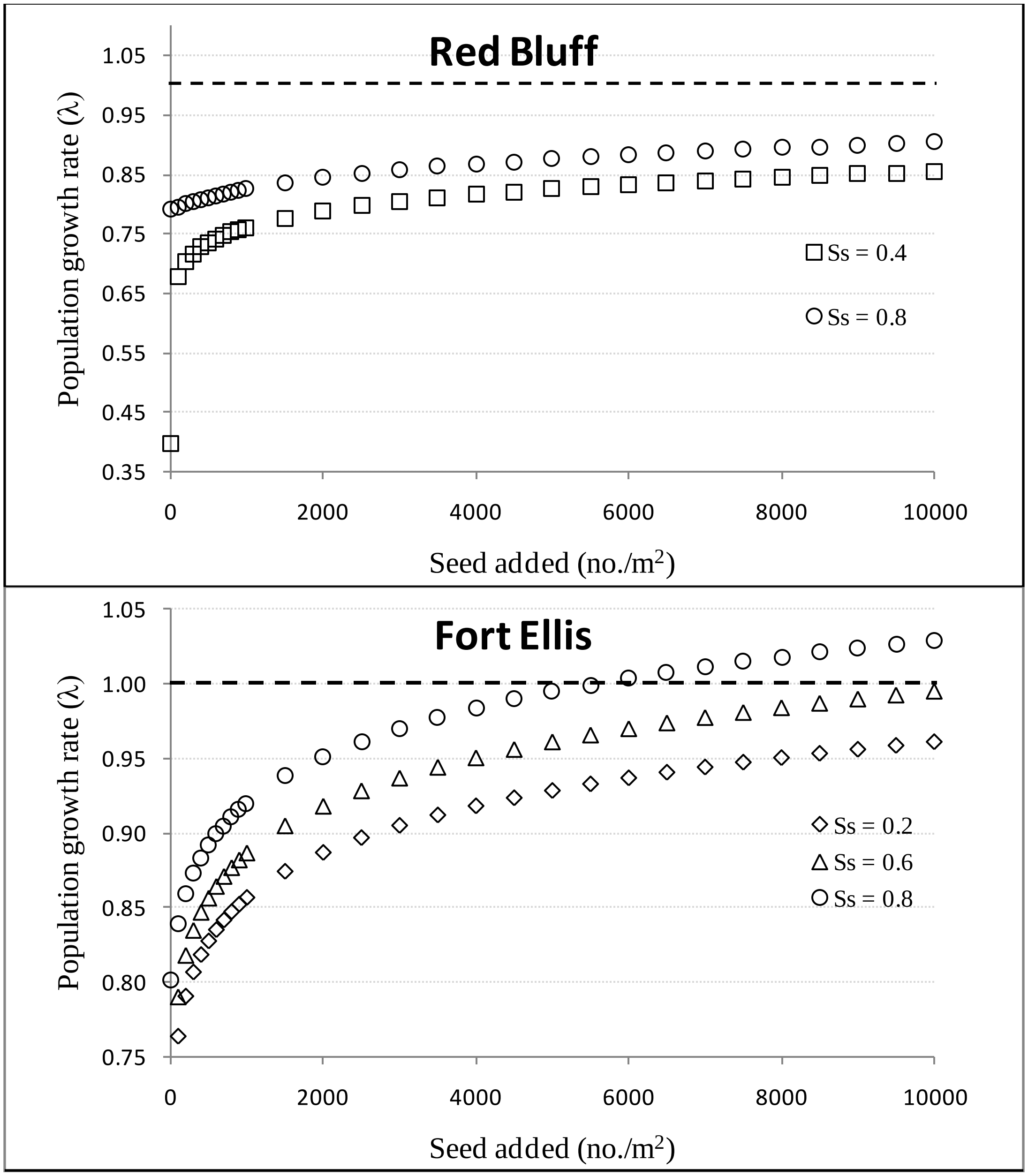

Figure 2.6: Results of the combined manipulations of seed survival (S_s) and propagule pressure, while other vital rates were held at the average across two field seasons, on the population growth rate (λ) of *Camelina sativa*. The bold dashed line indicates the point at which $\lambda = 1$. The original amount of seed provided was 6,666 m^{-2} at $t = 0$; the x-axis refers to the amount of seed added for all $t > 0$. The point at which $\lambda > 1$ for Red Bluff is not shown because it was not obtained until $S_s = 0.8$ and propagule pressure was 100,000 seeds $year^{-1}$ for all $t > 0$. For clarity, Ss rates of 0.2 and 0.6 were not plotted for Red Bluff, as they overlapped with Ss = 0.4.

CHAPTER 3

IMPACT OF GROWING CONDITIONS ON THE COMPETITIVE ABILITY OF *CAMELINA SATIVA* (L.) CRANTZ (CAMELINA), A PROPOSED BIOFUEL CROP

Introduction

Invasive plant species are a threat to natural and agronomic systems alike and have been implicated in the alteration of ecosystem processes and disturbance regimes, as well as in reducing biodiversity of native species (D'Antonio and Vitousek 1992; Gordon 1998; Mack 1981; Mack et al. 2000; Wilcove et al. 1998). Economically, invasive species are estimated to cause $27 billion dollars annually in environmental damage and control costs (Pimentel et al. 2005). Despite these concerns, the number of plant species proposed for introduction into the USA has increased greatly in the past decade as a result of the growing interest in producing plant-based biofuels to decrease greenhouse gas emissions and dependency on foreign oil (Hill et al. 2006; Ragauskas et al. 2006).

Many, if not all of the proposed biofuel species, are selected based on characteristics that make them easy to produce such as vegetative reproduction, rapid life cycle, and few natural enemies in the introduced habitat. Although these traits are beneficial in terms of the ease and efficiency of growing, they represent significant threats to non-target ecosystems should they escape cultivation (Keane and Crawley 2002; Rejmanek and Richardson 1996; Sutherland 2004). Assessing the invasion potential and competitive ability of these species before widespread cultivation is an important first step to protect the biodiversity and ecosystem functions of natural areas

(Barney and DiTomaso 2008; Cook et al. 2007; Cousens 2008; Davis 2008; Raghu et al. 2006).

Camelina sativa (L.) Crantz (gold of pleasure, large seeded false flax), a proposed biofuel species, is an oilseed crop and a member of the *Brassicaceae* family. It has been recently introduced into Montana and has the potential to become widely cultivated across the Northern Great Plains, as it is aptly suited for adverse environmental conditions. First, *C. sativa* is tolerant to frost and can therefore be planted as early as mid-March (Putnam et al. 1993). Second, it is capable of withstanding drought and can tolerate low quality, nutrient-poor soils (Gilbertson et al. 2007; Putnam et al. 1993). Third, it has been claimed that *C. sativa* is highly competitive against weeds (McVay and Lamb 2008; Putnam et al. 1993). Finally, *C. sativa* is a short-season crop (85-100 d), so it can escape the late summer heat that frequently causes yield reductions in other crops (Angadi et al. 2000; McVay and Lamb 2008). Because of these characteristics, along with the invasive attributes of prolific production of very small seeds, there is a concern about this species' weedy potential (Baker 1974; Basu et al. 2004; DiTomaso et al. 2007; Lockwood et al. 2005; Reichard 2001; Rejmanek and Richardson 1996). Unfortunately, knowledge of these biological characteristics alone is not sufficient in predicting the invasion potential of a plant (DiTomaso et al. 2007). If the amount of land devoted to *C. sativa* increases in the coming years, it will be beneficial to understand the competitive ability of this species.

Canola (*Brassica* spp.) is a widely cultivated crop in the Northern Great Plains and can be an important component of diverse crop rotations because it can increase the following year's wheat yields, break pest and disease cycles, and help to mitigate economic risks associated with monocropping (Johnston et al. 2002; Zentner et al. 2002). However, canola's tolerance to heat is low and high temperatures during flowering can reduce seed yield (Angadi et al. 2000). Also, canola requires relatively high amounts of soil water (Johnston et al. 2002) and water stress at critical growth stages can negatively affect yield (Nielsen 1997). Canola has not been recognized as naturalized or invasive in non-cropland ecosystems of the Northern Great Plains and therefore, represents a good candidate with which to compare the competitive characteristics of *C. sativa*.

Competition experiments conducted in controlled environments are a beneficial first step in predicting plant invasions (Fredshavn et al. 1995) and replacement series experiments have been widely used to assess interactions between species (Cousens 1991; Jolliffe 2000; Jolliffe et al. 1984; Radosevich 1987; Rejmanek et al. 1989; Snaydon 1991). Although this experimental design has been criticized (see Connolly 1986, 1988; Law and Watkinson 1987; Snaydon 1991), it is valid if the research objective is to determine the type and direction of interference between two species (Cousens 1991). The objective of this experiment was to assess the relative importance of growing conditions on the competitive ability of *C. sativa*. Specifically, we evaluated how soil characteristics and the presence of an invasive weed, *Bromus tectorum* (L.) (downy brome, cheatgrass) modify the competitive interactions between *C. sativa* and spring canola (*Brassica napus* L.) as a first approximation of assessing the invasion potential of *C. sativa*.

Materials and Methods

The relative competitiveness of *C. sativa* var. 'Celine' against glyphosate-tolerant spring canola was assessed in a full factorial experiment under greenhouse conditions in summer 2009 in Bozeman, MT. Three trials were conducted in two greenhouses with a 16 h photoperiod under natural sunlight. Trials 1 and 3 were conducted in the same greenhouse and had average day and night temperatures of 26 and 19 C, respectively. Trial 2 was conducted in a second greenhouse with average day and night temperatures of 22.5 and 20.5 C, respectively.

Two explanatory variables (soil type and presence of *B. tectorum*), each with two levels, were included in this study. Each soil type contained a mixture of washed concrete sand, loam soil, and Canadian Sphagnum peat moss. The first mixture, 'MSU', was used in the ratio of 1:1:1 and had AquaGro 2000 G (Aquatrols, Paulsboro, NJ) wetting agent blended in at .59 kg m^{-3}. The second soil treatment, 'sandy', was the same as the 'MSU' except with 2x sand, for a ratio of 2:1:1. Soils were steam pasteurized at 80 C for 45 min. *Bromus tectorum* was included in the experiment as either present at the density of 12 plants pot^{-1}or absent. Treatments of *B. tectorum* (with, without) and soil mix ('MSU', 'sandy') were randomly assigned to each pot (see Table 3.1 for treatment combinations and abbreviations used hereafter).

Camelina sativa and canola were grown at a constant density of 12 plants in a replacement series design (de Wit 1960), planted at five ratios (0:1, 25:75, 50:50, 75:25, 1:0), in 14 x 18 cm (ht × diam) pots (Fig. 3.1). After pots were filled with soil, 24 *B. tectorum* seeds were randomly scattered on the soil surface. Next, *C. sativa* and canola were sown using cardboard templates with holes to ensure a consistent planting arrangement. Three seeds of either canola or *C. sativa* were placed in each hole and covered with approximately 1 cm of soil. Each treatment combination was replicated six times (120 pots per trial) and pots were placed approximately 10 cm apart in a completely randomized design.

Camelina sativa, canola, and *B. tectorum* plants were thinned 1 wk after planting to obtain desired densities. *Camelina sativa* and canola were removed to one plant per designated spot by cutting the seedlings at the base to avoid disturbing neighbors. *Bromus tectorum* was thinned to 12 plants per pot by pulling the entire seedling, thereby precluding subsequent emergence. After thinning, some canola or *C. sativa* positions were empty due to lack of germination and/or seedling establishment and seedlings of the respective species were transplanted to fill the vacancies. No transplanting was needed for *B. tectorum*, as emergence was adequate. Within each trial, all pots were watered uniformly and rotated weekly for the duration of the experiment.

Plants were harvested 7 wk after planting. Phenologically, this was near the end of the flowering stage of *C. sativa* and canola as pods were beginning to develop, while *B. tectorum* was still in its vegetative phase. *Camelina sativa*, canola, and *B. tectorum* aboveground parts were cut at the soil surface, separated by species, and placed in paper bags. Plant material was dried at 50 C for 4 d in a forced-air oven. Plant species dry weight was recorded to the nearest 0.01 g on a per pot basis.

Data Analysis

Following Radosevich et al. (2007), *C. sativa* and canola relative yield (RY) and relative yield total (RYT) were calculated for all treatment combinations at each planting ratio as:

$$RY_A = Y_{AB} / Y_A \qquad \text{(Eq. 3.1)}$$

and

$$RYT = RY_A + RY_B \qquad \text{(Eq. 3.2)}$$

where RY_A is the relative yield of species A, Y_{AB} is the yield of species A in mixture with species B, and Y_A is the yield of species A in monoculture. RYT is the sum of the relative yields for species A and B.

Evidence for competitive interactions was investigated with regression analysis using R statistical software (version 2.8.1) to determine if RY data were described more accurately with a quadratic trendline than with a linear trendline, as the shapes of the curves can indicate the type of interference that occurred between species (Cousens 1991; Rejmanek et al. 1989). Briefly, if the RY curve for one species is convex and the RY curve for the other species is concave, it suggests that the interaction is competitive. Alternatively, if both species' RY curves are convex or if both are concave, the type of interference is that of mutual stimulation or mutual antagonism, respectively. If the RY trends of both species displays a linear relationship (i.e., slope of the RY curve = 1) then it is assumed that equal competition or no competition occurred, but differentiation between the two is not possible with replacement series experiments (Cousens 1991; Jolliffe 2000).

In this study, analyses were performed separately for each species, trial, and treatment combination, where RY was the response variable and planting proportion was the explanatory variable. Using ANOVA in R statistical software program (version 2.8.1), competing linear models were subjected to a partial F-test to determine if the inclusion of quadratic term provided a significantly improved fit over the linear model ($p = 0.05$) (Crawley 2007; Kleinbaum et al. 1988; Verzani 2005). Additionally, *C. sativa* and canola RY and RYT were plotted in replacement series diagrams to qualitatively determine the competitive outcomes (Harper 1977).

RYT was tested to determine if any of the combined relative yields for *C. sativa* and canola were significantly different from 1, as this indicates if over-yielding (RYT > 1), mutual antagonism (RYT < 1), or competition for the same resources (RYT = 1) occurred (Williams and McCarthy 2001). This test was performed using R statistical software (version 2.8.1) by calculating 95% confidence intervals of RYT values at each planting ratio (except for monoculture) for each treatment combination, with trials pooled.

The impact of growing conditions on *C. sativa* and canola yield was tested by comparing dry biomass (g pot^{-1}) of each species when planted alone or with *B. tectorum* using R statistical software (version 2.8.1). An appropriate ANOVA model was fit to determine if interactions were present among trial and treatment. Significant interactions were further analyzed with multiple comparison tests and p values were adjusted using the Holm method (Holm 1979).

Results

For all trials and treatments of *C. sativa*, the model with a quadratic term provided significantly improved fit compared to a straight line, indicating that there is evidence of curvature in the RY data of each species ($p < 0.01$). For canola, there were three exceptions where a quadratic model did not more accurately describe the data than a straight line. Specifically, in the 0B-SS treatment in trial one, the quadratic trend was marginally not different from the linear model ($p = 0.053$) and in the WB-SS treatment of trials two and three, the linear trend more accurately described the data ($p = 0.06$ and $p = 0.15$, respectively). The fact that RY curves of *C. sativa* were all significantly concave (quadratic regression coefficient was positive) and the majority of canola RY curves were convex (quadratic regression coefficient was negative), indicates that these two species competitively interact for resources at the total plant density that we used (Harper 1977). Specifically, *C. sativa* was shown to be the inferior competitor (Fig. 3.2).

In general, RYT values were not different than 1, based on 95% confidence intervals, indicating that there was no evidence of over- or under-yielding by *C. sativa* and canola when grown together. The only situation where over-yielding occurred was observed in the 0B-MS treatment at the 50:50 planting proportion, where the average RYT across trials was 1.12 ± 0.11 (mean ± SD). Evidence for under-yielding (mutual antagonism) was noticed in 0B-SS treatment at the planting proportions of 25:75 (canola

: *C. sativa*) and 50:50, where RYT values averaged across trials were 0.82 ± 0.12 and 0.84 ± 0.03, respectively.

The comparison of canola and *C. sativa* dry biomass (g pot^{-1}) among treatments when planted alone or in presence of *B. tectorum* indicated a significant trial × treatment interaction for both species ($p < 0.01$). For *C. sativa*, the impact of soil type and presence of *B. tectorum* on yield was significant in all trials ($p < 0.01$) (Table 3.2). Specifically, when grown in the 'MSU' mix, aboveground dry biomass of *C. sativa* was reduced in the presence of *B. tectorum* in trials one and two ($p = 0.03$ and $p = 0.04$, respectively), but no difference was observed in the third ($p = 0.64$). When grown in the 'sandy' soil, *C. sativa* yields were lower when *B. tectorum* was included in one of the three trials ($p < 0.001$). When comparing *C. sativa* yield relative to soil treatment in the absence of *B. tectorum*, no consistent patterns were observed. In trial one, yield was higher in the 'MSU' mix than in the 'sandy' mix ($p =0.02$), in trial two the opposite result occurred ($p = 0.04$), and in trial three there was no difference in yield ($p = 0.13$).

Analysis of aboveground dry canola biomass response relative to soil type and presence/absence of *B. tectorum* indicated no treatment differences in trials two and three ($p = 0.97$ and $p = 0.40$, respectively), but differences were seen in trial one ($p < 0.001$; Table 3.3). In trial one the highest yield was found in the 0B-MS treatment combination and the lowest yield was in response to the WB-WS treatment. Similar to *C. sativa*, the presence of *B. tectorum* decreased canola yields in trial one. When grown in the 'MSU' soil mix, canola aboveground dry biomass was less when *B. tectorum* was included (p <

0.01). In the ‘sandy’ soil, canola yield was also decreased due to the presence of *B. tectorum* (p = 0.02).

Discussion

The RY curves on the replacement series diagrams suggest that *C. sativa* is an inferior competitor against canola and this competitive balance does not change relative to sand content of soil or with the presence of *B. tectorum* (Fig. 3.2). This result contrasts with the widely accepted notion that *C. sativa* is competitive against agronomic weeds (McVay and Lamb 2008; Pilgeram et al. 2007; Putnam et al. 1993; Saucke and Ackermann 2006; Zubr 1997). Although this experiment was not performed in an agronomic setting, the fact that *C. sativa* growth was constantly suppressed across a variety of growing conditions indicates that this crop may not be as competitive as previously indicated.

In addition to agronomic implications, the results of this experiment have ecological importance regarding the invasion potential of *C. sativa.* Although limited in the capability to be extrapolated from the greenhouse, Fredshavn and Poulsen (1996) suggested that competition experiments conducted in controlled environments provide a way of identifying potentially hazardous plant species before releasing them into the environment. Therefore, these findings lend further credence to the results obtained from a quantitative weed risk assessment, in which *C. sativa* invasion potential into rangelands was determined to be low (see Chapter 2).

A limitation of the replacement series design is that the difference in the initial size of competing species is not accounted for (Connolly 1986; Jolliffe 2000). In particular, the RY index is particularly susceptible to size bias compared with other commonly used indices of plant competition (Grace et al. 1992). However, out of the many indices that have been developed to interpret replacement series experiments, RY and RYT were used because they are clear in their meaning and can be easily interpreted (Williams and McCarthy 2001). It could be that the competitive outcomes in this experiment were because canola seeds are larger than *C. sativa* seeds. This difference was further realized upon emergence, as canola seedlings were larger and appeared more vigorous than *C. sativa* seedlings (personal observation; no data available). This initial size discrepancy could have influenced the results because plant growth is self-compounding, particularly early in the life cycle (Weiner and Thomas 1986). It has been shown that the competitive relationship due to initial size may even out as the duration of an experiment increases (see Fredshavn et al. 1995; Grace et al. 1992), however this is not widespread in the literature, because most competition studies are relatively short term.

A second limitation of the replacement series design is that the overall planting density chosen must be great enough so that interspecific competition occurs, and failing to do so may lead to erroneous results. Typically, replacement series outcomes will be independent of total density as long as the overall density chosen is at the point where the law of constant final yield in monoculture is realized (Radosevich 1987; Taylor and Aarssen 1989). Even if the density is great enough that the results are valid, it is not advised to extrapolate beyond those conditions.

Our results suggest that *C. sativa* and canola competed for the same resources, as is illustrated by the fact that RYT values were rarely different from 1 (Fowler 1982; Radosevich 1987; Radosevich et al. 2007). However, a similar experiment implemented under field conditions might produce different results, as the root zone would increase and the ability for one species to avoid the other in the vertical soil profile would be possible. For example, when comparing interference among species in greenhouse conditions versus field conditions using a replacement series design, Fowler (1982) observed differences in both the intensity and direction of competitive interactions among species in response to environment. She concluded that this result could have been due to partitioning of soil resources in the field and the ability of species to access those resources differentially.

In conclusion, this study showed that canola can consistently out-compete *C. sativa* across growing conditions in greenhouse settings. These results, along with those from the assessment of the invasion potential of *C. sativa* (Chapter 2) suggest that, under the studied conditions, *C. sativa* is a seemingly benign plant. However, to confirm the validity of the competitive balance between *C. sativa* and canola observed in this study, it would be necessary to complement our results with those obtained in a similar experiment performed under field conditions.

Table 3.1: Experimental factors and their abbreviations included in a replacement series competition experiment conducted to assess the impact of soil characteristics and the presence of an invasive weed, *Bromus tectorum*, on the competitive interactions between *Camelina sativa* and glyphosate-tolerant spring canola (*Brassica napus*).

Soil Mixture	*Bromus tectorum*	
	Without *Bromus tectorum*	With *Bromus tectorum*
'MSU'	0B-MS	WB-MS
'Sandy'	0B-SS	WB-SS

All soil mixes included sand, loam soil and Canadian Sphagnum peat moss and were blended at ratios of 1:1:1 and 2:1:1 for 'MSU' and 'sandy', respectively. *Bromus tectorum* was seeded at a rate of 24 seeds pot^{-1} to reach a target density of 12 plants pot^{-1}.

Table 3.2: *Camelina sativa* dry biomass (g pot^{-1}) (Average (SD)) in response to treatment when grown in the absence of canola.

	Treatment			
Trial	0B-MS	0B-SS	WB-MS	WB-SS
1	6.49 (1.61)^{a}	4.05 (0.92)^{bc}	4.50 (0.70)^{b}	2.32 (0.30)^{c}
2	5.45 (1.00)^{a}	6.87 (0.92)^{b}	4.13 (0.66)^{c}	3.81 (0.81)^{c}
3	4.67 (0.92)^{a}	3.38 (0.75)^{ac}	4.05 (0.43)^{ac}	2.26 (0.58)^{c}

All trials were analyzed separately because of significant trial × treatment interaction ($p < 0.001$). Treatments within the same trial (same row) with the same letters are not significantly different based on multiple comparison tests ($p = 0.05$); p-values adjusted using the Holm method. See Table 3.1 for treatment abbreviations.

Table 3.3: Canola dry biomass (g pot^{-1}) (Average (SD)) in response to treatment when grown in the absence of *Camelina sativa*.

	Treatment			
Trial	0B-MS	0B-SS	WB-MS	WB-SS
1	11.18 (0.82)^{a}	7.59 (0.99)^{b}	8.70 (1.38)^{bc}	5.82 (0.90)^{d}
2*	7.25 (0.96)	5.77 (1.42)	5.23 (0.93)	7.40 (0.95)
3*	6.37 (0.10)	5.56 (1.39)	7.61 (1.23)	4.93 (1.13)

All trials were analyzed separately because of significant trial × treatment interaction ($p < 0.01$). Treatments within trial one with the same letters are not significantly different based on multiple comparison tests ($p = 0.05$); p-values adjusted using the Holm method. * = multiple comparisons not conducted because one-way ANOVA indicated no significant treatment differences for trials two and three ($p = 0.97$ and $p = 0.40$, respectively). See Table 3.1 for treatment abbreviations.

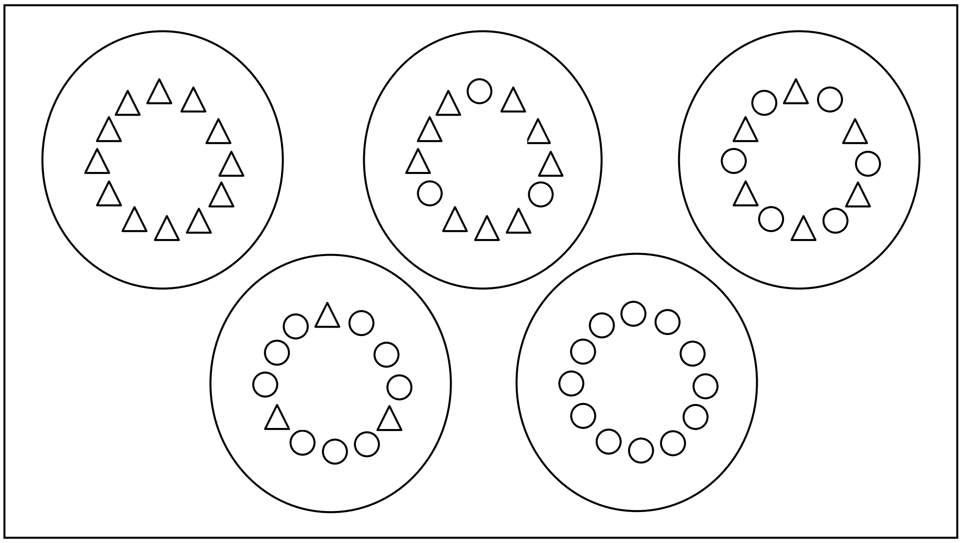

Figure 3.1: Illustration of replacement series planting arrangements used to assess how soil characteristics and the presence of an invasive weed, *Bromus tectorum* modify the competitive interactions between *Camelina sativa* (△) and spring canola (○).

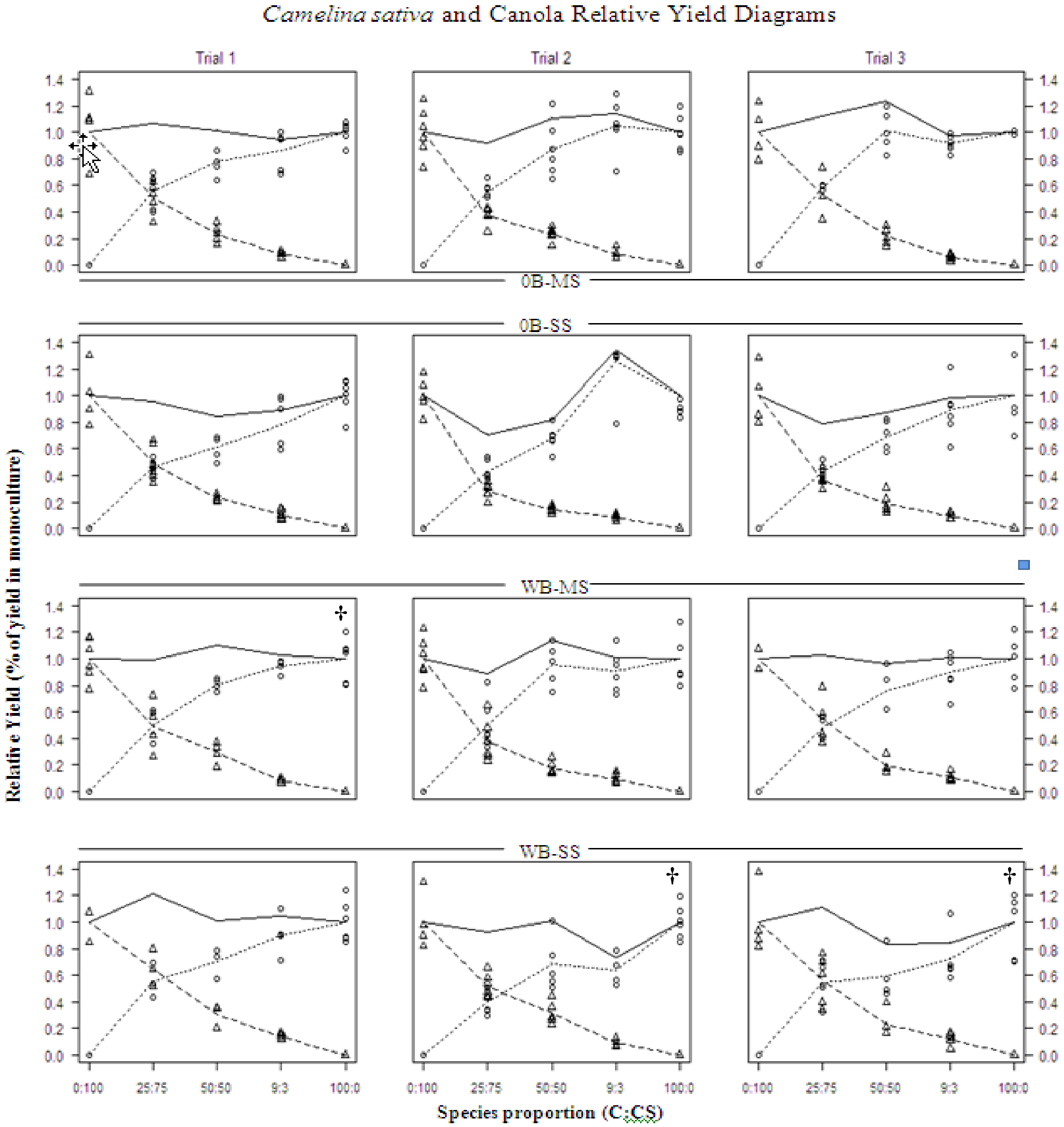

Figure 3.2: Replacement series diagrams of each trial × treatment combination depicting the impact of soil type and presence of *Bromus tectorum* on the competitive interactions between canola (C) (○) and *Camelina sativa* (CS) (Δ). Each column of plots represents a trial and the rows represent a treatment (see Table 3.1 for treatment abbreviations). Relative yield (RY) and relative yield total (RYT) values represent aboveground dry biomass at the various proportions relative to the yield of that species when planted alone (except for the presence of *B. tectorum* in two treatments). Trendlines are based on average values and are represented as: (····), RY canola; (– –), RY *C. sativa*; (—), RYT. All *C. sativa* RY trendlines deviated significantly from the non-interaction line (i.e., slope = 1; not shown) at p = 0.05. All canola RY trendlines but three deviated significantly from the non-interaction line – those that are not different from a non-interaction line are denoted with † in the upper right-hand corner of the plot.

CHAPTER 4

SUMMARY OF FINDINGS AND FUTURE RESEARCH

Most of the information currently available on *C. sativa* is about its chemical content, quality of the oil, and the applicability to the biodiesel and human health/animal feed markets. Agronomically, only a handful of studies have quantified its potential to become a viable crop in Montana, and much of the knowledge circulating is mostly based on word-of-mouth from private industry. Furthermore, no studies had been conducted to assess the weedy potential of *C. sativa*. This study provided previously unknown information about biological and ecological aspects of *C. sativa* and how they relate to this species' probability to invade rangeland ecosystems. In Objective 1.1, we gained information on emergence, survival, and reproduction rates of *C. sativa* in response to different disturbances and seeding seasons, at two sites that differ in their biotic and abiotic qualities. We determined that *C. sativa* will emerge in all situations; however, survival and reproduction was limited to mechanically disturbed plots. These data were then synthesized using a population dynamics model as a predictive tool, an approach that, to our knowledge, has not used to assess potential plant introductions. Although our model was a simple representation of real conditions, it suggests that *C. sativa* populations are likely to be relegated to severely disturbed sites devoid of other vegetation, e.g. railroads, roadsides, gravel pits, etc. A major caveat of these findings, however, is that they are based on only two sites and two years of data. Thus, the results should be interpreted accordingly.

Objective 1.2 of this project involved comparing our quantitative results about the invasion potential of *C. sativa* to results suggested by the qualitative Australian Weed Risk Assessment (WRA). In light of the evidence indicating *C. sativa* poses little to no threat of invasion in the evaluated rangeland ecosystems of southwest Montana, it was surprising to find that under certain scenarios this plant would be unequivocally rejected for introduction. The discrepancy between these two approaches highlights the necessity of a more comprehensive approach to assessing weed risk. Although an in-field assessment is not practical for all species, we suggest that a multi-step system would be beneficial for high value species that are proposed to be cultivated on a large scale. Specifically, the many biofuel feedstocks that are being developed with the goal of enhancing their weedy traits to make production easier are prime candidates for this methodology. This would not only reduce the chance of cultivating a potential invader, but would also reduce the risk of rejecting an economically important non-weed, such as *C. sativa*.

By assessing the relative competitiveness of *C. sativa* with the widely cultivated non-weed, canola (*Brassica napus* L.), we provided further evidence that *C. sativa* poses little threat to invade rangelands of Montana. Under greenhouse conditions, in all treatments and planting ratios, canola outperformed *C. sativa*. This result was not anticipated, as *C. sativa* is widely claimed as being a highly competitive plant in agronomic situations and it is tolerant of poor soils and water-limiting conditions (Putnam et al. 1993). We found that the competitive balance between these two species did not change relative to the presence of the invasive weed *B. tectorum* or the sand content of soil (water-limiting conditions). The inferior competitive ability of *C. sativa* witnessed in this experiment provided additional verification that this species should be of little concern as to its invasive potential. However, as with all greenhouse studies, the results are based on conditions that are not representative of natural conditions. As such, they should be interpreted with caution and extrapolation outside of the assessed conditions is not recommended.

Although our results suggest that *C. sativa* poses a minimal threat of invasion into rangeland ecosystems of southwest Montana, avenues for further research are available. Primarily, the prospect of gene flow between this domesticated species and its wild congener, *C. microcarpa*, warrant investigation. This is because gene transfer between species, in particular between crops and closely related weeds, can create novel weedy races with enhanced competitive ability, thus increasing invasion potential (DiTomaso et al. 2007; Ellstrand and Schierenbeck 2006; Ellstrand and Hoffman 1990; Vila et al. 2000; Ward et al. 2008). There are two areas of concern regarding this topic. First, genetic modification of *C. sativa* is currently underway at Montana State University to increase the fatty acid content in the seeds (Lu and Kang 2008). Although this particular genetic modification may not contribute to increased invasiveness should it be transferred to *C. microcarpa*, Lu and Kang (2008), developed a method by which genetic modification for a variety of features in *C. sativa* can be easily accomplished. If in fact genetic modification occurs such that *C. sativa* will possess herbicide tolerance or increased competitive ability (via taller or more highly branched plants), hybridization with *C. microcarpa* may be of concern. The second area of concern regarding gene transfer involves seed dormancy. Our field results indicated that *C. sativa* does not possess dormancy or seed survival traits; however, *C. microcarpa* does (personal observation) and this trait is positively associated with invasiveness (Radosevich et al. 2007). Under the hypothetical scenario that *C. sativa* becomes genetically modified in a way that increases its competitive ability, and hybridization with *C. microcarpa* to induce seed dormancy occurs, this species might need to be re-evaluated as to its invasive potential.

This research has elucidated previously unknown aspects about the invasion potential and competitive ability in regards to the likelihood of *C. sativa* becoming a weed in Montana. The methodology that was implemented to conduct this study could be used as a starting point to develop a more thorough approach to conduct quantitative risk assessments of biofuel species that may be cultivated on a large scale. In addition to providing site specific information about the demography of a plant, this in-field approach will increase our efficacy in preventing additional non-native plant invasions by decreasing the uncertainty associated with the use of a strictly qualitative approaches to weed risk assessment.

APPENDIX A

SUPPLEMENTAL EXPERIMENTS, FIGURES AND TABLES

Assessing *Camelina sativa* Population Dynamics with Individually Marked Plants

In 2008 and 2009 at Red Bluff and Fort Ellis, individual plants (hereafter 'target plants') were identified to gain estimates of survival rates from seedling to flowering and from flowering to maturity (harvest). Also, rates of herbivory as well as biomass and seeds produced per plant were gained by using target plants. It was intended that these data would be used to model the population dynamics of *C. sativa*, but due to selection bias when identifying these plants, survival rates using this method were not included in the modeling exercise. However, tracking target plants provided accurate estimates of per plant biomass and fecundity, as well as rates of herbivory.

Methods

When *C. sativa* seedlings exhibited one pair of true leaves, individual plants within mechanically-disturbed subplots were marked with one color-coded wire ring at the base of the plant. Weekly data was collected to determine the survival rates from seedling to flowering and from flowering to harvest. Although not an explicit research objective at the outset of the experiment, it was noticed that herbivory on *C. sativa* was widespread and influenced plant survival. Therefore, during weekly site visits data were collected on the rates of plant herbivory and death due to animals. If the plant had been browsed or was dead, and if the source of this was obvious, we recorded what animal was the likely suspect.

Target plants were harvested at maturity by cutting the stem at the soil surface and placed in paper bags. Samples were stored at room temperature until processing (up to 1 mo). Biomass was weighed to the nearest 0.001 g and the exact number of seeds per plant was counted.

Data Analysis

Survival rates of seedlings to flowering plants were analyzed with R statistical software (version 2.8.1) to detect differences and interactions among site, seeding season, and year. The logit transformation was used to normalize the data and a Type III ANOVA was then fit using site, year, and seeding season as explanatory variables in the final model.

Suspected inconsistencies between using target plants to estimate survival and using the method from the main experiment to estimate survival prompted data analysis to determine differences between these two approaches. Because of many zeros in the data from Red Bluff 2008, we were unable to normalize the variances and the distribution using common transformations. Consequently, that portion of the dataset was dropped and the analysis proceeded with the logit transformation using R (version 2.8.1). Due to unbalanced data a Type III ANOVA model was used.

Results and Discussion

Survival to flowering of *C. sativa* target plants in the mechanically disturbed plots varied considerably between sites and years (Table A.1). Because of this, there was a significant site × year interaction ($p < 0.001$). For example, the survival rate from emergence to flowering of fall seeded target plants at Red Bluff increased from 0.34 ± 0.28 (mean ± SD) in 2008 to 0.82 ± 0.24 in 2009. Additionally, plant survival to flowering in spring seeded plots at Fort Ellis decreased from 0.62 ± 0.17 in 2008 to just 0.02 ± 0.04 in 2009.

Survival of the *C. sativa* target plants from flowering to maturity further reduced population sizes, with no consistent trend across sites and years (Table A.3). In the most extreme case, at Red Bluff in 2008, none of 27 fall seeded target plants that survived to the flowering stage reached maturity. Conversely, at Fort Ellis in 2008, 0.68 ± 0.35 (mean ± SD) of fall seeded target plants survived from flowering to maturity.

As suspected, the method used in the main experiment to estimate plant survival produced different results to those obtained using this approach (Table A.4). Overall, using target plants to estimate plant survival resulted in significantly higher estimates of survival rates as compared to the subplot method ($p < 0.001$). The most notable difference was at Fort Ellis in 2008, when using target plants to estimate plant survival of spring seeded plots was 0.48 ± 0.25 (mean ± SD) while the approach used in the main experiment resulted in an estimate of 0.11 ± 0.06. This disparity in seedling survival estimates between using the target plants and the method from the main experiment was likely due to target plant selection. In hindsight, it became clear that these higher values were due to choosing individuals who, more often than not, were growing without immediate neighbors. This occurred because of the need to identify individuals that could be easily relocated each week. At the time these plants were marked, many seedlings still existed in dense clusters, up to 100 individuals per 100 cm^2 wire ring. It would have been difficult to mark a plant within those clusters, both without damaging neighboring seedlings and marking in such a way that they could be easily located again.

Thus, most of the target plants that were identified may have been outside the range where intraspecific competition was occurring and therefore had higher survival rates as compared to survival using the method described in the main experiment.

Target plants were also used to assess rates of herbivory. Deer browsing appeared to influence plant survival in 2008 at both sites. This became noticeable about 6 wk into the life cycle, when most plants were beginning to develop flower buds. At Fort Ellis, 0.50 ± 0.24 (mean ± SD) of the target plants were browsed by deer in 2008. Of those, 0.90 ± 0.21 died. At Red Bluff, 0.80 ± 0.13 of target plants were browsed by deer and 0.99 ± 0.02 of those died. No one particular animal was noticed to feed on *C. sativa* plants in 2009 at Red Bluff. At Fort Ellis in 2009 however, grasshoppers were observed causing extensive defoliation of the plants. 0.87 ± 0.15 of the target plants were victims of herbivory by grasshoppers, 0.83 ± 0.24 of which died. The effects of grasshoppers became particularly noticeable about 6 wk into the lifecycle and had caused mortality of most plants by week 8.

Survival of Canola in Mechanical Disturbance

The ability of glyphosate-tolerant spring canola (*Brassica napus*) to emerge, survive, and reproduce after a mechanical disturbance was investigated at Red Bluff and Fort Ellis during the 2009 growing season. Canola has a long history of cultivation throughout Montana, yet poses no risk of invasion to non-cropland areas. The purpose of this study was to compare the performance of canola to the results obtained for *C. sativa* in the main experiment.

Methods

Three replications at each site were used, each of which consisted of two 1 m^2 subplots which were randomly assigned to receive 10,000 canola seeds m^{-2} seeds in either fall or spring. Mechanical disturbance to a depth of 15 cm was applied to all subplots with a shovel on 9 September, 2008. At both sites, fall seeding occurred on 2 November, 2008 and spring seeds were sown in mid-April 2009 following the methodology described in the main experiment.

To estimate canola emergence rates, five 100 cm^2 rings were randomly placed within each 1 m^2 subplot. Beginning in March 2009, weekly counts of canola plants in rings were conducted to estimate number of seedlings m^2. Harvesting and plant storage occurred in the same manner as described for the main experiment. Plant biomass and seed weight were quantified to the nearest 0.001 g, height was recorded to the nearest 0.5 cm, and numbers of seed produced per plant was counted.

Results

In general, emergence of canola seedlings was low and there was no emergence of fall seeded canola in the mechanically disturbed plots at Red Bluff. The average emergence rate for the plots seeded in the spring was 0.0003 ± 0.0002 (mean ± SD) and the highest number of seedlings in a plot was five. In contrast to Red Bluff, fall seeded plots at Fort Ellis did have plants that emerged (0.006 ± 0.005). The emergence rate of spring seeded canola at Fort Ellis was 0.023 ± 0.023 (Fig. A.2).

No canola seedlings survived to maturity at Fort Ellis and, because of this, there was no seed produced. Only two canola plants in one of the spring seeded plots of the 10 that emerged at Red Bluff survived to maturity. They weighed 0.252 g and 1.138 g and produced 28 and 69 seeds, respectively. This was equal to 0.32% of the total number of seeds originally sown (30,000) to three spring seeded subplots.

Effects of Animal Exclosure Cages on *Camelina sativa* Plant Survival

In 2008, it was observed that animal herbivory on *C. sativa* may have impacted the population dynamics. Deer and/or other mammals appeared to have an affinity for the plant beginning around the flowering stage. Therefore, in spring of 2009, plant survival in the absence of animal herbivory was assessed.

Methods

Circular exclosures 48 cm in diameter by 91.5 cm in height made from chicken wire with 12.7 mm square holes were created. This size excluded small to large mammals from the plants; however, insects and small mammals were able to enter through the holes and underground. Three cages were placed around the study area at each site and secured with metal stakes. The ground encompassed by the cages was mechanically disturbed with a shovel just prior to seeding, which occurred on 21 March, 2009. Seeds were sown at a density of approximately 1,200 per cage, equivalent to the 6,666 seeds m^{-2} used for the main experiment. Within each of the cages, three 100 cm^2 wire rings were randomly placed.

Emergence counts began in early March, 2009 and were conducted as described for the main experiment. Plant harvesting, storage, and data collection occurred in the same manner as described for the main experiment.

Data Analysis

Camelina sativa seedling emergence and survival rates in the cages were compared to rates from the mechanically disturbed plots from the main experiment. Comparisons were accomplished using R statistical software (version 2.8.1). Data were transformed using the logit transformation. Analysis of emergence rates proceeded with Type III ANOVA.

Results

Camelina sativa emergence in the cages at both sites produced higher rates than what was observed in the mechanically disturbed plots of the main experiment ($p < 0.05$; Fig. A.3). At Fort Ellis, the average emergence rate was 0.59 ± 0.43 (mean ± SD) and at Red Bluff it was 0.18 ± 0.08. No plants in the cages survived from the seedling to maturity stage at Fort Ellis, probably due to grasshopper herbivory (personal observation). At Red Bluff, survival was highly variable among replicates. The first and second replicates had a survival rate each of 0.02, while the third had a survival rate of 0.21. In all, 74 plants survived to maturity out of the estimated 651 seedlings that emerged, for an average survival rate of 0.09 ± 0.11. Although this average is higher than that of the mechanically disturbed plots (0.02 ± 0.02), due to high among cage variability there was no difference ($p = 0.18$).

Due to a recording error when collecting mature plants at Red Bluff, neither the number nor the weight of seeds produced is known for individual cages. However, using the combined seed weight of the three cages (4.149 g) it was estimated that a total of 4762 seeds were produced ($r^2 = .81$, $p < 0.001$), which is 132.3% of the original amount of seed that was sown. The average number of seeds produced per plant for all three cages was 64, while it was only 13 for the surviving plants from the main experiment.

Overwinter Seed Survival

Overwinter seed survival is an important facet of plant population dynamics (Jordan et al. 1995; Westerman et al. 2005). Therefore, a small-scale experiment was initiated to test the overwinter survival ability of *C. sativa* seeds. Through this, we hoped to highlight the rate and relative importance of overwinter seed survival on *C. sativa* population dynamics.

Methods and Results

Four samples of 100 *C. sativa* seeds in a sand mixture placed in a nylon stocking were buried in the ground at a depth of 15 cm at Fort Ellis and Red Bluff on 2 November, 2008. The stocking allowed for movement of air, water, and microbes into the sample, yet prevented seed predation by animals.

On 21 and 28 March, 2009, the samples were removed from the ground. Most of the seeds from Fort Ellis, ranging from 92 to 96 out of 100 seeds per stocking, had germinated while underground. The seeds from Red Bluff had not germinated. Difficulty in separating the remaining seeds from the sand resulted in planting the entire contents of each stocking in small flats (16.5 × 12 × 6 cm) by spreading the sand and seeds on top of 2.5 cm of Sunshine #1 soil mix (Sun Gro Horticulture Inc., Bellevue, WA). The flats were placed in a greenhouse under natural light, and misted and monitored daily for emergence for 10 d. After this time, no emergence had occurred. This provided evidence that overwinter seed survival of *C. sativa* was at or near zero.

Survival of *Camelina sativa* in Alternative Habitats

The survival of *C. sativa* in different habitats was evaluated in 2009 at the Red Bluff site only. Of particular interest was its ability to emerge and survive to reproduction on steeper slopes and different aspects from what was used for the main experiment.

Methods

Two hillsides were identified in a nearby gully at Red Bluff; the first one had a west-facing aspect and a slope of 30 degrees while the second was east-facing with a 25 degree slope. Two replications were used on each hillside. Each replication consisted of two 1 m^2 plots, each of which represented a fall or spring seeding. Each plot was mechanically disturbed on 9 September, 2008 with a shovel. Fall seeding occurred on 2 November, 2008 and spring seeding on 21 March, 2009 using the same method and seeding rate as described for the main experiment. To monitor emergence, five 100 cm^2 wire rings were randomly placed in subplots. Beginning in March, weekly counts of seedlings in rings were conducted to estimate emergence.

Results

Emergence was low, averaging 0.0005 ± 0.0004 (mean ± SD) and 0.01 ± 0.002 for fall and spring seeded plots, respectively. Compared with fall and spring seeded emergence rates from the main experiment (0.016 ± 0.007 and 0.13 ± 0.04, respectively), emergence on the hillsides was much lower. All seedlings failed to produce true leaves and died by the first week of June 2009.

Presence of *Camelina microcarpa* in 2009

Although not apparent in 2008, *Camelina microcarpa* Andrz. ex DC. (little pod false flax) appeared at both field sites in 2009. Delineation between *C. sativa* and *C. microcarpa* was based on seed characteristics after harvesting (ARS 1971; Gilkey 1957). In the mechanically disturbed plots from 2009, 32.6% and 11.1% of seed produced at Red Bluff and Fort Ellis, respectively, was *C. microcarpa.* Interestingly, much of the *C. microcarpa* that was present at Fort Ellis in 2009 appeared in the mechanical- and herbicide-disturbed plots that had been used in the previous year. Within those 2008 plots, 72.4% (4,441) of seed production in the mechanically disturbed plots and 100% (11,715) of seed production in the herbicide-disturbed were *C. microcarpa.*

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