Effects of Biological Control and Precipitation on Spotted Knapweed (Centaurea stoebe) Population Dynamics

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Effects of Biological Control and Precipitation on Spotted Knapweed (Centaurea stoebe) Population Dynamics

by

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B.A., University of Colorado Boulder, 2009
M.A., University of Colorado Boulder, 2011

A thesis submitted to the
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This thesis entitled:
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Population Dynamics
written by Anastasia Phyllis Maines
has been approved for the Department of Ecology and Evolutionary Biology

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The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.
Maines, Anastasia Phyllis (M.A., Ecology and Evolutionary Biology)
Effects of Biological Control and Precipitation on Spotted Knapweed (Centaurea stoebe)
Population Dynamics

Thesis directed by Professor Timothy R. Seastedt

ABSTRACT

Biological control insects can be a sustainable management strategy to control negative effects of invasive plants. However, population-level effects of spotted knapweed biological control insects are debated. I used a combination of field manipulations and modeling to examine how herbivory and precipitation effect population dynamics. Field studies demonstrated that recruitment rates were reduced by herbivory and plant competition, but varied by site. The best model to explain observed population declines included the effects of biological control agents. The results also show that the ability to achieve control of knapweed (i.e., conditions where $\lambda \leq 1$) depend on high densities or large per capita impact of biological controls, both of which are known to vary at this field site and elsewhere. In certain habitats (e.g., disturbed riparian areas) insect herbivory alone is unlikely to control densities, but knapweed densities in most other habitats can be reduced by biological control agents.
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The sustainable control of regionally abundant invasive plants is crucial to mitigate negative impacts of introduced plants on ecosystem function. Management of plant populations requires both reducing established invasive populations and protecting vulnerable landscapes from invasion (Shea & Kelly, 1998; Pokorny et al., 2005). Many plant species are introduced but do not become invasive (Davis, 2009). By understanding how invasive plants become established in introduced ecosystems we can better understand how to manage established populations to reduce or eliminate negative impacts (Davis et al., 2000; MacDougall and Turkington, 2005; Hansen, 2007; Eckberg et al., 2012). Several hypotheses have been proposed to explain how plants become invasive. Plant species may be invasive because they are superior competitors to the background plant community. Funk and Vitousek (2007) showed that invasive plants were able to more efficiently use resources than the native vegetation in a resource-poor environment. Alternatively, invasive plants may have an advantage over native vegetation due to escape from specialists in the introduced range (the Enemy Release Hypothesis; Keane and Crawley, 2002) or ability to exploit novel ecosystems (MacDougall and Turkinton, 2005; Davis et al., 2000). Enemy release may lead to increased competitive ability against native plants. For example, invasive plants that have a greater seed rain than native community (DiVittorio et al., 2007). In the case that these two mechanisms of dominance work in concert, a question arises of whether introduced biological controls can sufficiently overcome advantages to control the invasive plant.

For invasive plants that are released from herbivory in the introduced range, biological control presents a sustainable prospect for controlling established populations. Herbivory can
reduce plant reproduction through direct and indirect effects (Underwood and Halpern, 2012; Louda and Potvin, 1995; Eckberg et al., 2012; Rose et al., 2005; Knochel et al., 2010b; Wooley et al., 2011). Herbivores can directly control plant reproduction through direct effects on reproductive output (such as seed predation; Louda and Potvin, 1995; Wooley et al., 2011). Indirect effects on reproduction can occur through effects such as reduced adult lifespan or reduced plant size, which can result in fewer flowers being produced (Knochel et al., 2010b; Underwood and Halpern, 2012). Herbivory can control populations even when plants have competitive advantages, such as stronger seedling establishment than natives (Suwa and Louda, 2012).

Biological control has the advantage of sustainability if the biological control agent becomes established on the host plant (Seastedt et al., 2007; Story et al., 2008; Surles and Kok, 1978). However, the environmental context can influence the effectiveness of biological control. Biological controls may not be sufficient to control an invasive populations at all sites. Introduced biological control weevil Rhinocyllus conicus was sufficient to control invasive nodding thistle (Carduus nutans) in the eastern United States, insufficient to control the New Zealand population, and has become itself invasive on the native Platte thistle (Cirsium canescens) (Surles and Kok, 1978; Groenteman et al., 2011; Rose et al., 2005). Multiple releases do not always have additive effects if species directly compete or indirectly interfere, such as altering plant phenology through its herbivory (Milbrath & Nechols, 2004). Furthermore, eradication may rarely be an outcome with biological control alone.

1.2 Spotted Knapweed (Centaurea stoebe)

The mechanisms for the dominance of spotted knapweed (Centaurea stoebe), a perennial plant that is invasive in North America, have been debated (Figure 1.1; Knochel and Seastedt,
2009). It has characteristics that suggest that it could be a strong competitor. Established populations can form dense monocultures, which limit the growth of native plants (Fraser & Carlyle, 2011). However, other evidence suggests that spotted knapweed is an opportunistic plant, taking advantage of disturbances and reduced herbivory in the introduced range. Field and greenhouse studies have demonstrated that plant competition can reduce spotted knapweed recruitment, suggesting that it requires a disturbance to become established in some areas (Pokorny et al., 2005; Knochel et al., 2010a). It is also less palatable to native insect herbivores, resulting in fewer top-down controls for spotted knapweed in comparison to herbivores limiting native plants (Sheley et al., 1998; Schaffner et al., 2011). Thus, established spotted knapweed populations in the introduced range have a competitive advantage over native plants due to fewer top-down controls in the introduced range and are a prime candidate for biological control.

Figure 1.1. Single flowering stem of C. stoebe. (Photo taken July 27, 2011)

A suite of biological control insects have been released for the control of C. stoebe including the root feeding weevil Cyphocleonus achates, the seed head weevil Larinus minutus, and seed head predator flies Urophora spp. (Story et al., 2006, 2008; Figure 1.2). These three
biological control agents released for the control of *C. stoebe* have been shown to have additive effects in experimental manipulations (Knochel et al., 2010b; Crowe and Bourchier, 2006). The results of these releases have been monitored for more than 10 years across Western North America and declines have been observed in Montana, Colorado, and British Columbia since the introduction of biological controls (Story et al., 2006, 2008; Knochel et al., 2010b; Gayton and Miller, 2012). Recently, there is some debate over whether observed declines are due to the controls or to other factors (Corn et al., 2007; Ortega et al., 2012).

There is some debate whether biological controls can reduce survival and seed production enough to control populations, suggesting that drought has been a contributing factor for observed declines in spotted knapweed populations (Corn et al., 2007; Story et al., 2008; Ortega et al., 2012). Water addition did not impact rosette survival (Corn et al., 2007), however native competition may be less effective competition for spotted knapweed under high precipitation regimes (Maron and Marler, 2008). Complicating our understanding of how seed predators and other herbivores impact populations is the variability in observed seedling recruitment (Sheley et al., 1998; Knochel et al., 2010a).

Figure 1.2. Specialist herbivores for *C. stoebe*. Top: *Larinus minutus* (smaller weevil) and *Cyphoclenous achates* adults on spotted knapweed flower. Bottom: *Cyphoclenous achates* pupae and adult in spotted knapweed root. (Photos taken July 12, 2012).
1.3 Matrix Models

Modeling can be useful whether the concern is preserving threatened species (Louda and Potvin, 1995) or managing invasive ones (Shea & Kelly, 1998; Hansen, 2007). Matrix models can be used to separate observed effects by comparing the weight of evidence for different models (Hilborn and Mangel, 1997; Sticker and Stiling, 2012). Ecological models can be used to translate short-term population measures (such reproduction and growth probabilities) into long-term forecasts of treatment effects (Sticker and Stiling, 2012). The benefit of this approach is a more complete picture of population estimates from 1-2 years of monitoring than these measures alone provide (Emery & Gross, 2005).

A matrix model requires only a few population measurements over 2-3 years, but can suggest which segment of the life cycle contributes the most to the invasion (Shea & Kelly, 1998). Models allow comparisons of populations in different situations, thus increasing the accuracy of predictions about population control. Modeling approaches demonstrated that differences between the responses of Australian and New Zealand *C. nutans* populations to biological control were due to differences in population dynamics at the different sites (Shea et al., 2005). These approaches also demonstrated that the apparent variability in the effect of herbivory on population dynamics was due to site differences (Eckberg et al., 2012).

1.4 Thesis Summary

Spotted knapweed is a prime candidate for incorporating known effects into a matrix model to determine how these effects impact population dynamics. Numerous field and greenhouse studies have been conducted on spotted knapweed; much is known about the response of individual stages to both biological control and precipitation manipulations. The goal of my thesis work was to create a population model for spotted knapweed to determine how
biological controls impact population growth. I examined the effectiveness of the suite of biological controls present at the field suite to control spotted knapweed populations. I compared the effect of biological control with the alternative hypotheses that drought was causing the observed decline. The advantage of using matrix models is that these two effects can be separated and examined individually and in combination. I summarized research findings from my own and other field studies to parameterize several alternative models to explain the observed decline at the field site. I conducted field studies to supplement the data already obtained.

In chapter two I discuss the results of field studies on the effects of established plant competition and precipitation on seedling establishment. I also examined the effect of herbivory on seedlings and small rosettes. These field studies provide information about germination and survival of seedlings and survival and growth of rosettes.

In chapter three, I present the result of matrix model comparisons of predicted population-level outcomes of biological controls and precipitation effects. I parameterized models with reported results of effects, including newly reported results on the interaction between effects of precipitation and biological controls on seed production. Finally, I compared model predictions to observed population densities from the permanent transect at the field site.
CHAPTER 2: FACTORS AFFECTING SEEDLING ESTABLISHMENT RATES OF SPOTTED KNAPEWEE (CENTAUREA STOEBE)

A. P. Maines

2.1 ABSTRACT

Predicting site vulnerability to non-native establishment remains a difficult goal. Seedling recruitment is an important component of plant establishment and can affect the success of control strategies. I conducted field studies to determine the effects of plant competition and differing precipitation regimes on spotted knapweed recruitment. I also examined the effect of herbivory on rosette survival and growth. Seeds were sown into plots with vegetation intact or removed at three sites that differed in plant composition, slope, and aspect. Seeds were also sown into plots where plant competition and precipitation were manipulated in a factorial design at a single site. Field studies demonstrated that site impacted germination rate, while site, herbivory, and plant competition affected recruitment rates. There was a wide range in germination and establishment, with site averages ranging from 13.1% to 32.5% germination of seeds and establishment rates ranging from 0% to 11.2 ± 1.6% of seeds were established plants. Rosette survival was significantly higher when herbivores were excluded from plots. Establishment rates increased with supplemented precipitation. Of the 8000 seeds added to plots in one study, only 8 had become plants by autumn: seven of which survived in watered plots with intact vegetation. Collectively, these results show that seedling establishment is a critical phase in spotted knapweed population dynamics. My study site also showed the variation among habitats based upon site and climatic conditions previously reported across study sites. Furthermore, herbivory impacts all stages of the lifecycle from the seedling onward. The observed differences explain the reported variability in recruitment seen in the literature and inform efforts to control spotted knapweed with plant competition and biological controls.
Key words: Seedling recruitment; Spotted knapweed; Plant competition, Invasive plants, Herbivory.

2.2 INTRODUCTION

Established invasive plants are a management concern due to potential negative impacts on ecosystems services. Possible negative ecological consequences include reduced native plant diversity (Hejda et al., 2009) and altered species interactions (Seastedt & Pysek, 2011). When invasive plants are unpalatable to livestock and wildlife, they can also become a financial concern for rangeland managers (Watson & Renney, 1974; Moore et al., 1989; DiTomaso, 2000). Due to these concerns, finding sustainable controls that reduce the negative effects of invasive plants is important to conservationists, land managers, and weed scientists. Yet despite the importance of controlling invasive plants, predicting where non-native plants will establish and dominate the landscape remains challenging (Seastedt & Pysek, 2011). A critical stage in the population dynamics of invasion is recruitment: seed germination and seedling establishment. Prior to becoming dominant in the landscape, plants must gain a foothold in the environment through recruitment of individuals, and site characteristics are clearly important factors in this success (Evans et al., 2012; Ruprecht et al., 2013). Establishment rates affect the degree to which seed production must be reduced in order for biological controls to be effective (Knochel et al., 2010a). Thus understanding how environmental variation affects spotted knapweed seedling establishment may help explain how to control this invasive plant.

Highly variable seedling recruitment rates complicate our understanding of how seed predators and other herbivores impact populations, with establishment rates ranging from 0.06% to 36% of seeds becoming new plants (Sheley et al., 1998; Pokorny et al., 2005; Knochel et al.,
Field and greenhouse studies have demonstrated that plant competition can reduce spotted knapweed recruitment, suggesting that it requires a disturbance to become established in some areas (Pokorny et al., 2005; Knochel et al., 2010a). Yet, other results show that spotted knapweed compensates for increased mortality with increased recruitment from rosettes to adults in some conditions, thereby resulting in no net change in adult population size (Ortega et al., 2012).

Some evidence suggests that spotted knapweed (*Centaurea stoebe*) dominates opportunistically, taking advantage of disturbances and reduced herbivory in the introduced range. Introduced to North America from Eurasia, established populations can form dense monocultures limiting the growth of native plants (Fraser and Carlyle, 2011). Spotted knapweed is often unpalatable to native insect herbivores, resulting in fewer top-down controls for spotted knapweed relative to native plants (Sheley et al., 1998; Schaffner et al., 2011). Such a release from herbivory may allow established populations in the introduced range to have a competitive advantage over native plants.

In the decades following the introduction of biological controls aimed at reducing seed production and plant survival, spotted knapweed populations have declined in some cases (Story et al., 2006, 2008; Seastedt et al., 2007; Knochel et al., 2010b; Gayton and Miller, 2012). These declines give support to the enemy escape hypothesis explaining the dominance of spotted knapweed.

An alternative hypothesis to the enemy escape hypothesis proposed for the observed spotted knapweed declines is that drought, rather than the released biocontrol insects, has contributed to declines (Corn et al., 2007; Story et al., 2008; Ortega et al., 2012). Seedling recruitment may be particularly sensitive to drought due to an underdeveloped root system,
which is less effective at obtaining water (Peters, 2000). Furthermore, native competition may be less effective competition for spotted knapweed under high precipitation regimes (Maron & Marler, 2008). Water addition did not impact rosette survival (Corn et al., 2007), but it may impact seedling recruitment since seedlings may be more sensitive to resource fluctuations (particularly available moisture) than their adult counterparts. These conflicting findings about establishment rates and population-level responses to mortality suggest that certain habitats, climatic conditions or some combination of these factors can determine the outcome of biological control efforts.

Because management strategies are conducted under the variable conditions of the natural environment, field experiments can demonstrate which factors are significant under realistic environmental conditions. In my manipulative field study I examined spotted knapweed seedling establishment with and without plant competition at various sites and under controlled precipitation regimes. Since spotted knapweed is a perennial plant that can survive as a rosette or bolt and flower (Sheley et al., 1998), I also examined the effect of biological control insects on the probability of rosette survival or transition to an adult. These experiments tested the hypothesis that spotted knapweed becomes an opportunistic invader when released from herbivory in the introduced range. I predicted that seedlings would exploit resource opportunities from reduced plant competition and examined the following questions:

1. How does plant competition affect spotted knapweed recruitment?
2. How does precipitation and the interaction between plant competition and precipitation influence spotted knapweed recruitment?
3. How does herbivory influence spotted knapweed rosette survival and growth?
2.3 METHODS

2.3.1 Field Site Description

The study area was north of Boulder, Colorado in Lefthand Canyon (40.07 N, 105.19 W, elevation 1810 to 1989 m). Average spring to early summer growing season (March to July) precipitation for Boulder is 278 mm (NOAA, 2012). Grazing has not occurred at the study area since 2007 (Knochel et al., 2010a). Spotted knapweed biological controls Larinus minutus (Gyllenhal), Cyphocleonus achates (Fahr.), Sphenoptera jugoslavica (Obenb.), and gall flies Urophora spp. have been previously released and established at the site (Seastedt et al., 2007).

The study was conducted from 2011-2012 and used five sites within 2 km of each other, which differed in slope, aspect, and vegetative composition.

2.3.2 Experiment 1: Plant Competition

In March 2011, 20 experimental plots were established in each of three sites. Site one was a south-facing aspect with dominant plant cover that included Canada bluegrass (Poa compressa L.), slimflower scurfpea (Psoralidium tenniflorua Pursh.), and spreading daisy (Erigeron flagellarius A. Gray) with a small spotted knapweed population (5 to 10% relative cover). Site two was flat in aspect and slope and primary cover was orchard grass (Dactylis glomerata L.) and smooth brome (Bromus inermus Leyss.) with a moderate spotted knapweed population (15 to 20% relative cover). Site three was north-facing. Dominant cover at site three was bluegrass (Poa spp.), snowberry (Symphoricurpos Duham.) and wild bergamot (Monarda fistulosa L.) with a moderate spotted knapweed population (15 to 20% relative cover). Existing adult and juvenile spotted knapweed plants were removed from experimental plots, but the existing seed bank may have influenced the results. Spotted knapweed seed bank viability after the first year is estimated at 5 to 8% (Jacobs and Sheley, 1998; Knochel et al, 2010a). Since seed
production at these sites has been low due to insect seed consumption (Knochel and Seastedt, 2009), the resident seed contribution to the experiment was assumed to be small relative to the number of seeds added here.

At each site, 20 0.1 m² plots separated by 0.25 m were established along two parallel transects. Plots were randomly assigned to plant competition present or removed with 10 plots per treatment per site. Established vegetation remained intact in plant competition treatments or was removed to simulate a disturbance. After initial plot establishment, plot composition was not manipulated, thus non-knapweed seedlings could colonize plots. Each plot was seeded with 200 seeds plot⁻¹ (2,000 m⁻²) following a high seed rain density observed in the field (Maron & Marler, 2008; Knochel & Seastedt, 2010). Spotted knapweed biological control agents (the seed head weevil *Larinus minutus*, gall flies *Urophora* spp., and root weevil *Cyphocleonus achates*) have been present at the study location for a decade.

Spotted knapweed seed germination and seedling survival were assessed bimonthly from May to August 2011. At each census, I marked new spotted knapweed seedlings with toothpicks to determine the total number of seedlings that germinated throughout the monitoring period. I also recorded the number of seedlings with damaged leaves to obtain estimates of the rate of herbivory on seedlings. I calculated the rate of herbivory as the number of seedlings with evidence of herbivory divided by total number of seedlings in the plot. Field densities of biological control insects were not manipulated. I defined seedling recruitment as the total number of plants plot⁻¹ one year after seed was sown (May 2012) and plants were assessed as either rosettes or bolting plants.
2.3.3 Experiment 2: Plant Competition x Precipitation

A factorial field experiment was established at a single site (site 4) to examine effects of plant competition and precipitation on spotted knapweed establishment. The location did not have a previous spotted knapweed population to eliminate possible confounding effects of a seed bank. Dominant cover at site four included Kentucky bluegrass (*Poa pratensis* L.), *Carex spp.*, and small-leaf pussytoes (*Antennaria paruifolia* Nutt.). While biological control agents were present within a 1 km of this site, no control insects were added to the site. The site was periodically monitored for presence of biological control insects.

I established 40 plots in late March 2012. A fully factorial design crossed plant competition with precipitation manipulations. Each treatment was replicated 10 times in an 8 by 8 m grid. Plot focal area was 0.1 m$^2$ with 16.6 cm buffer edges for a treated area of 0.25 m$^2$. As in experiment one, established vegetation remained intact in the plant competition treatment. Established vegetation was lightly raked in plant completion plots. I simulated a disturbance by removing all existing vegetation from the plot. After establishing plots, plot composition was not manipulated, thus non-knapweed seedlings could colonize the plot. Spotted knapweed seed was collected in the fall of 2011 for use in the 2012 study. Each plot was seeded with 200 seeds plot$^{-1}$ (2,000 m$^{-2}$) following a high seed rain density observed in the field (Maron & Marler, 2008; Knochel & Seastedt, 2010). To determine germination potential of collected seed, 100 seeds were kept moist in the lab under natural light for 14 days and the number of germinated seeds were recorded. Germination potential was calculated as the percent of seeds that germinated by the end of the trial. Due to low precipitation prior to establishment of plots, all plots were initially supplemented to 2.98 cm (average March precipitation for Boulder, CO; NOAA, 2012).
From April to August 2012, precipitation was monitored with a rain gauge at the field site. Ambient precipitation plots were supplemented biweekly as needed so that plots did not fall below one standard deviation of average precipitation. Supplemental precipitation plots were watered weekly to receive above-average precipitation. The number of seedlings in each plot was assessed in May and July. At each census, new individuals were marked with toothpicks and number of surviving seedlings was recorded. For this experiment, recruitment was assessed in August 2012.

On May 16, after some seedlings had germinated, 10 seedlings (or, if less than 10, as many seedlings as were present) in each of 20 randomly selected plots were marked with toothpicks to monitor the rate of herbivory. Seedlings were assessed weekly for survival and evidence of herbivory (bitten/removed leaves). Rate of herbivory was determined at each census as the total number of plants with herbivory in a plot divided by total number of plants in that plot.

In order to determine whether soil composition influenced differences between site establishment rates, I took composite soil samples of three cores per site from sites one to four (experiment one and two) in July 2012. Greenhouse studies have shown that seedling biomass increased with increased carbon (Knochel et al., 2010a). I analyzed soils for organic matter and soil composition. Soil composition and organic matter were not measured at site five (experiment 3 below) since seedling recruitment was not measured at this site. I used a linear regression to compare percent organic matter and recruitment. Recruitment was measured at the end of the growing season.
2.3.4 Experiment 3: Rosette survival

To determine the effect of herbivory on rosette survival and transition rate, 20 plots with an area of 0.1 m$^2$ were established in an existing spotted knapweed population in May 2011. This site was above site one on a south-facing slope, with moderate spotted knapweed cover (approximately 15 to 25%). Plots were evenly spaced in two parallel, 10 m long cross-slope transects separated by 3 m. Plots in the downhill transect received insecticide (Bayer Advanced Complete, containing Imadaicloprid systemic (0.72%) and β-Cyfluthrin non-systemic (0.36%), rate of 3 oz. product per 3.75 L water) while plots in the uphill transect were designated as a control and received an equivalent volume of water sprayed on the same days as the insecticide. Beginning May 10, 2011, plots were sprayed once every six weeks with insecticide or pure water using a handheld sprayer for a total of three insecticide or pure water applications over the summer. On May 17, 2011, 10 small rosettes per plot were randomly selected from all available rosettes within each plot for 100 focal rosettes per treatment. Small rosettes were defined as plants that had produced at least four true leaves and were smaller than 5 cm in diameter. Thus, all focal rosettes were from 1 to 5 cm in size. Small rosettes were chosen because they are likely to be younger plants that have never bolted.

Focal rosettes in each plot were mapped. At each census, I recorded evidence of herbivory, indicated by leaf tissue damage, and mortality, absence of rosette or a rosette with all aboveground tissue completely desiccated. Evidence of deer or elk grazing on vegetation within or outside of plots was occasionally observed, and these instances were noted as ungulate rather than insect herbivory. I scored plots for damage or mortality eight times (every three to four weeks and then one year after the start of the experiment). The rate of herbivory was determined at each census as the total number of plants with herbivory divided by total number of plants in
that plot. On the final census (May 2012), surviving plants were also identified as bolting adults or rosettes to determine transition and survival rates.

Plots contained spotted knapweed of varying stages from small rosettes to flowering adults that produced seed heads. To determine the effect of the insecticide treatment on adult plants, I conducted an assessment of seed production and number of insects present in seed heads. I collected three seed heads plot\(^{-1}\) in early September and assessed numbers of \textit{L. minutus}, \textit{Urophora} spp., and seeds present in seed heads to calculate average number of \textit{L. minutus}, \textit{Urophora} spp., and seeds present in each plot. I determined the effect of insecticide treatment on insect presence and seed production (\(n = 10\)).

2.3.5 \textit{Statistical analysis}

Total number of seedlings (germination), total number of plants alive at the end of the census (established plants), and proportion of seedlings with herbivory were square root transformed to fulfill assumptions of normality and back-transformed for readability of figures. Seedling herbivory rate per plot was averaged for all counts to determine the average percent herbivory over the monitoring period. For experiment 1, the number of seedlings with herbivory and seedling mortality were ln(x+1) transformed to meet normality assumptions. A regression between herbivory at time \(t\) and mortality at \(t + 1\) was conducted to determine if herbivory predicted mortality in the following census. An ANOVA was used to determine the effect of site, plant competition, and their interaction on spotted knapweed seedling germination, herbivory, and recruitment from experiment one. Tukey post-hoc tests were performed to determine significant differences. Due to the high number of zeros, seedling establishment values from experiment two did not meet assumptions of normality so a Kruskal-Wallis test was used to compare recruitment probabilities for precipitation by plant competition treatments.
For experiment 3, a t-test was used to compare rosette herbivory rates when herbivores were excluded (insecticide treatment) or present (no insecticide) to ensure that insecticide application was successful in excluding herbivores. Proportion of seedlings with herbivory was averaged for each plot for all counts to determine average herbivory over the monitoring period. I used paired t-tests to determine the effect of insecticide on rosette survival and transition to adult. Where data did not meet normality assumptions I used Kurskal-Wallis tests. I also compared seed production, Larinus spp. presence, and presence of Urophora spp. for seed heads between control and insecticide treatment using paired t-tests and Kurskal-Wallis tests, as appropriate. All statistical analyses were conducted using R programming language and figures were created using the plotrix package (R Core Team, 2012, R Foundation for Statistical Computing, Vienna, Austria; Lemon, 2006).

2.4 Results

2.4.1 Experiment 1: Plant Competition

Precipitation during March to July 2011 was 304 mm, which was 110% of average Boulder precipitation (Figure 2.1; Janet Prevéy, unpublished results; NOAA, 2012). Germination was significantly different among sites, but plant competition and the interaction of site and plant competition were not significant predictors of germination (Table 2.1). There was a wide range in germination rates, with site averages ranging from 13.1% to 42.5% of seeds germinating in the first year (Figure 2.2). In the Tukey post-hoc test, germination at site one and three was significantly lower than germination at site two.
Table 2.1. ANOVA results for the effect of site, plant completion, and their interaction on percent germination, herbivory rate, and number of recruited plants. Results from ANOVA for effect of precipitation, plant completion, and their interaction on average herbivory rate.

<table>
<thead>
<tr>
<th></th>
<th>Competition</th>
<th>Site</th>
<th>Competition x Site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Germination</td>
<td>1</td>
<td>0.076</td>
<td>0.78</td>
</tr>
<tr>
<td>Recruitment</td>
<td>1</td>
<td>6.49</td>
<td><strong>0.014</strong></td>
</tr>
<tr>
<td>Herbivory</td>
<td>1</td>
<td>0.52</td>
<td>0.48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Competition</th>
<th>Precipitation</th>
<th>Competition x Precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Herbivory</td>
<td>1</td>
<td>12.6</td>
<td><strong>0.003</strong></td>
</tr>
</tbody>
</table>

P values significant at alpha = 0.05 are bold.

Figure 2.1. Cumulative March to July precipitation (mm) for study area against 93-year average for Boulder, CO (NOAA, 2012). Precipitation measurements for ambient measurements were collected approximately weekly from rain gauges at the field site (Ambient 2011, Janet Prevéy, unpublished results).
Figure 2.2. Mean percent germination of added seeds by site and plant competition present (dark bars) or removed (light bars) (n = 60, 10 per bar). Only significant predictor for germination is site. There is no effect of plant competition or an interaction between site and plant competition for germination. Bars are means ± 1 standard error.

Percent recruitment (plants seeds⁻¹) measured in October ranged from 2.0 ± 0.33%, 5.4 ± 0.80%, or 12.8 ± 1.3% of seeds across three sites. There was a trend of lower recruitment in plots with plant competition, but it was not significant (data not shown). By the final census, one year after seeds were sown, both site and plant competition were significant predictors of seedling establishment (Table 2.1). Establishment rates varied from 0 to 11.2% of seeds becoming plants the following May (Figure 2.3A). The majority of first year recruits were rosettes with 0 to 0.5 bolting plants m⁻² (Table 2.2). In a Tukey post-hoc test, recruitment differed significantly among sites.

The herbivory at time t was a significant predictor of seedling mortality in the next census (t+1) (R² = .15, p < 0.0001) (data not shown). The herbivory rate varied by site. In an ANOVA, a significantly greater percent of seedlings experienced herbivory at site 1 than at the other two sites (Table 2.1, Figure 2.4A).
Table 2.2. Mean (standard error) number of recruited rosettes and adults from seed by site and plant competition treatment from 200 seeds sown per plot (n per cell =10).

<table>
<thead>
<tr>
<th></th>
<th>Rosettes</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vegetation Absent</td>
<td>Vegetation Present</td>
</tr>
<tr>
<td>Site 1</td>
<td>1.6 (.85)</td>
<td>0</td>
</tr>
<tr>
<td>Site 2</td>
<td>9.0 (2.2)</td>
<td>6.2 (0.9)</td>
</tr>
<tr>
<td>Site 3</td>
<td>22.4 (3.2)</td>
<td>15.1 (3.7)</td>
</tr>
</tbody>
</table>
Figure 2.3. Mean percent recruitment (plants/seeds sown) A) one year after seeding by site and plant competition present (dark bars) or removed (light bars) (n=60, 10 per bar) and B) the fall after added by precipitation treatment and plant competition present (dark bars) or removed (light bars) (n=40, 10 per bar). In B, less than 1% of the seeds added were plants by fall. Bars are means ± 1 standard error.
Figure 2.4. Boxes represent the range of values for percent of seedlings with herbivory for A) 2011 study by plant competition and site (n=60) and B) 2012 study by plant competition and precipitation level (n=40). Boxes connect upper and lower quartile. Box color indicates plant competition present (dark boxes) or removed (light boxes). The bars are medians, pluses indicate mean values, whiskers indicate range of values, and open circles are outliers. Note different y-axes.
2.4.2 Experiment 2: Plant Competition x Precipitation

Precipitation for March to July 2012 in ambient plots was supplemented to 239 mm, which is 85% of average Boulder precipitation (Figure 2.1; NOAA, 2012). In supplemented precipitation plots, precipitation was 532 mm, which is 190% of average Boulder precipitation (Figure 2.1; NOAA, 2012). The germination rate of spotted knapweed seed collected in the fall of 2011 was 84%. The recruitment probability was significantly different among the treatments (F = 9.69, df = 3; p = 0.021) (Figure 2.3B). Out of 8000 seeds added, only 8 plants survived. The only recruitment rate significantly different than zero was from plots with plant competition and supplemented precipitation (0.35% of seeds). The rate of herbivory was significantly lower in disturbed plots than in plots with plant competition, but there was no effect of the precipitation treatment on rate of herbivory (Table 2.1, Figure 2.4B).

Results from the analyses of the composite samples for organic matter and soil composition did not explain site differences. Site 1 and site 3 were composed of sandy loam and sites 2 and 4 were loamy sand. The concentration of soil organic matter was 11% (site 1), 6.7% (site 2), 16.0% (site 3), and 6.9% (site 4) and was not significantly correlated with number of seedlings (p = 0.26, n = 4) (data not shown).

2.4.3 Experiment 3: Rosette survival

The insecticide treatment successfully reduced the rate of herbivory experienced by rosettes, but it did not affect the presence of seed predators in seed heads (Figure 2.5, 2.7; Table 2.3). Reduced herbivory did not increase rosette survival to the end of the summer (Figure 2.6, Table 2.3). However, by the following May (2012) there was a significantly higher percent of seedlings surviving as rosettes in plots that received the insecticide treatment. There was no
difference in the percent of rosettes transitioning to adults (bolting) (Figure 2.6, Table 2.3).

There was no difference in seed production between treatments (Figure 2.7, Table 2.3).

Table 2.3. Paired t-test (t) and Kruskal-Wallis (W) results for (a) percent of rosettes with herbivory, (b) rosette survival, and (c) seed head measures.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>df</th>
<th>Statistic</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Percent of rosettes with herbivory</td>
<td>1</td>
<td>W = 28.4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>(b) Percent of identified Rosettes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer survival (%)</td>
<td>9</td>
<td>t = -1.55</td>
<td>0.15</td>
</tr>
<tr>
<td>Survival as Rosette (t+1) (%)</td>
<td>9</td>
<td>t = -3.02</td>
<td>0.014</td>
</tr>
<tr>
<td>Transition to Adult (t+1) (%)</td>
<td>1</td>
<td>W = 0</td>
<td>1</td>
</tr>
<tr>
<td>(c) Seed head measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed production</td>
<td>1</td>
<td>W = 0.53</td>
<td>0.47</td>
</tr>
<tr>
<td>Larinus presence</td>
<td>1</td>
<td>W = 2.10</td>
<td>0.15</td>
</tr>
<tr>
<td>Urophora presence</td>
<td>9</td>
<td>t = 0.75</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Note: n = 100 rosettes and 30 seed heads.

Figure 2.5. Range of percent of focal rosettes experiencing herbivory in plots without insecticide (light boxes) and with insecticide applied (dark boxes) (n = 20). Boxes connect upper and lower quartile. The bars are medians, pluses indicate mean values, whiskers indicate range of values, and open circles are outliers.
Figure 2.6. Percent of focal rosettes that survived the summer, survived to the following year, and grew to the adult stage (Bolting\(_{t+1}\)) when no insecticide was applied (None) or with insecticide (Applied) (n=20). Boxes connect upper and lower quartile. The bars are medians, pluses indicate mean values, whiskers indicate range of values, and open circles are outliers.

Figure 2.7. Mean numbers of seeds, *Larinus minutus*, and *Urophora spp.* present in seed heads from plots without insecticide (light bars) or with insecticide applied (dark bars) (n= 20). Bars are means \pm 1 standard error.
2.5 Discussion

Biological control insects have been demonstrated to reduce spotted knapweed seed production and adult survival in Colorado (Knochel et al., 2010b; Wooley et al., 2011). Yet how this reduction translates to population-level dynamics depends partially on recruitment. A significant question for management of spotted knapweed is whether seed reduction can contribute to reduced population size and if so, how much must seed production must be reduced for this control to be effective (Knochel et al., 2010a). Estimates of the reduction needed to control spotted knapweed populations vary based on observed establishment values. Based on the recruitment values observed here, an adult would have to produce between 9 to 105 seeds to produce one new plant, depending on site characteristics. This ten-fold difference in establishment success in part explains discrepancies among published studies in evaluating the efficacy of biological control. At some sites with high recruitment, additional measures beyond biological agents would be required to control the population. Seed reduction as a control mechanism will be most effective in suppressing plant densities in areas with low recruitment.

Plant competition reduced spotted knapweed establishment at all sites, except under increased resource availability. Site characteristics play an important role in vulnerability to invasion and may help explain the wide variation in spotted knapweed seedling establishment rates in the literature. Factors, such as precipitation, explain some of differences in the effect of plant competition on spotted knapweed. Resource availability, including precipitation, has been shown to mediate how plant competition limits spotted knapweed establishment (Maron & Marler, 2008; Knochel et al., 2010a; Blumenthal et al., 2008). However the effects on seedlings are not consistent. Ortega et al. (2012) found no difference in seedling density between drought and average soil moisture conditions. I found that spotted knapweed was unable to colonize
disturbed plots with supplemented precipitation (Figure 2.3), although I did observe non-knapweed seedlings colonizing the plots (A. P. Maines, personal observation). This variability contributes to the difficulty in predicting where introduced plants will thrive (Davis et al., 2000). It is possible that other plants could also benefit from increased resources. In some cases, native plants may be better able to take advantage of increased resources than non-native plants (Ruprecht et al., 2013). These results suggest that the effect of decreased precipitation on seedling recruitment may not be a major factor in spotted knapweed declines across the west. However, a landscape that is fairly resistant to spotted knapweed establishment (such as site four) could be invaded in a year with above average precipitation.

One factor explaining the differences in establishment rates between sites could be variation in the level of herbivory. Lower rates of herbivory correlated with higher first year establishment and rosette survival. Although site one and two had similar germination rates, they had very different establishment. The high establishment rate at site three could be due to germination of the higher seed bank present on North-facing slopes. Despite similar cover, other studies have shown a larger surviving seed bank on north-facing slopes than in other locations (David Knochel, unpublished data). The insects affecting the seedling stage are unknown. The rosette mortality rates I observed were higher than previously reported (Corn et al., 2007). Rosettes have been shown to have a higher rate of damage from generalist herbivores than adult plants (Collins and Müller-Schärer, 2012). It is possible that spotted knapweed seedlings are subject to generalist herbivores since herbivory rates were high at site four where biological controls were not present prior to the experiment. Herbivory could also be due to biological controls eating the seedlings, though only one seed head weevil was found on site four during the course of the experiment.
Plant competition and herbivory constrain recruitment under realistic conditions. The range of recruitment rates found in this study was consistent with the variation in recruitment reported in previous studies (Figure 2.3; Pokorny et al., 2005; Knochel et al., 2010a; Ortega et al., 2012). Under high levels of seed production, such as was added here, spotted knapweed can be a successful, dominant competitor at certain sites (Ortega et al., 2012). Yet these results and others also demonstrate that spotted knapweed is not successful at all sites. Seed reduction could have significant effects in limiting the success and spread of spotted knapweed at some sites.
CHAPTER 3 MODELING EFFECTS OF BIOLOGICAL CONTROL INSECTS AND PRECIPITATION ON POPULATION DYNAMICS
A. P. Maines

3.1 Abstract

The literature is inconsistent regarding the ability of herbivory to control or reduce densities of a major invasive plant species of North America, spotted knapweed (*Centaurea stoebe*). Here, findings from long-term monitoring and experimental manipulations of spotted knapweed densities, seed production, and survivorship were used to parameterize a population matrix model for this species. The model incorporated compensatory responses (increased transition from rosettes to adults) that occur when densities of adult knapweed plants are reduced. The model also incorporated newly reported results showing precipitation effects on biological control impacts. I compared how well biological controls and precipitation explained the observed spotted knapweed declines. The best model to explain population declines included the effects of biological control agents. Declines in population growth rates ($\lambda \leq 1$) were only predicted when reduced seed production and increased plant mortality due to biological controls was included. Results suggest that biological controls contributed to declines observed in field studies and support the contention that biological control attenuates the ability of knapweed to exploit favorable climatic conditions. The results also show that the ability to achieve control of knapweed (i.e., conditions where $\lambda \leq 1$) depend upon a relatively large impact of biological control agents, with high densities or large per capita impact, both of which are known to vary at the site and elsewhere. Knapweed in certain habitats (e.g., disturbed riparian areas) is unlikely to be controlled by insect herbivory, but knapweed densities found in most other habitats can be reduced by biological control agents. Regardless, the management implications from these findings are that biological controls can intensify the efficacy of other control methods.
Keywords: matrix modeling, biological control, spotted knapweed, population dynamics, herbivory

3.2 Introduction

Spotted knapweed (Centaurea stoebe) is an invasive forb in the US and Canada that is an undesirable forage species for cattle and wildlife and a concern to native biological diversity (Watson & Renney, 1974; Sheley et al., 1998; Ortega and Pearson 2005). It is a perennial plant with a maximum lifespan of 9 years, with single plants producing up to 25,000 seeds per year (Watson & Renney, 1974; Sheley et al., 1998). Biological control insects that have been released to control spotted knapweed include insects that target plant tissues such as the root weevil Cyphocleonus achates and a beetle Sphenoptera jugoslavica, and those which target seed production such as the weevil Larinus minutus and several flies (Urophora spp.) (Sheley et al., 1998; Story et al., 2008; Knochel & Seastedt, 2010). Reports of the efficacy of spotted knapweed control with specialist insects appear contradictory.

One of the most effective combinations of biological control insects for this plant has been C. achates and L. minutus: collectively they increase spotted knapweed plant mortality and reduce seed production (Corn et al., 2006; Crowe & Bourchier, 2006; Knochel et al., 2010b; A.P. Maines, unpublished data). In the 10-30 years following the release of biological controls, long-term monitoring has shown spotted knapweed populations to decline as biological control populations increased or persisted (Story et al., 2006, 2008; Seastedt et al., 2007; Gayton and Miller, 2012). However, an experiment by Ortega et al. (2012) demonstrated that spotted knapweed exhibited a population-level compensatory response to increased adult mortality caused by C. achates, through increased rosette transition to the adult stage. Thus there was no
net change in adult density between spotted knapweed populations with and without seedlings (Ortega et al., 2012). This finding suggests that seed reduction and increased mortality of adult plants might not be sufficient to overcome increased survival and growth from juvenile plants (rosettes). A further complication when interpreting results is the possible effects of drought as a contributing factor for observed declines in spotted knapweed populations (Corn et al., 2007; Story et al., 2008) or as an alternative explanation to reductions by biological controls (Ortega et al., 2012). Experimental manipulations have been conducted on many stages of the life cycle; however, integration of these findings to compare how individual effects impact long-term population dynamics has not been undertaken.

Matrix models allow integration of responses of life-history stages to treatments to predict long-term, population-level effects of treatments (Emery & Gross, 2005; Smith et al., 2005). Stochastic elements such as environmental variability and factors such as density-dependence can also be incorporated into matrix models (Shea & Kelly, 1998). Sensitivity analyses provide information about the contribution of each transition to the overall population growth, informing which spotted knapweed stages should be targeted in management plans (Shea & Kelly, 1998; Hansen, 2007). The significance of individual effects at the population-level can also be determined (Shea & Kelly, 1998; Caswell, 2001; Emery & Gross, 2005). Matrix models are useful tools for comparing population dynamics in different populations (Shea et al., 2005) and to examine the effect of herbivory on plant populations (Eckberg et al., 2012; Louda and Potvin, 1995). Thus matrix models are valuable tools for determining the best control methods for specific circumstances and to evaluate seemingly contradictory results (Hansen, 2007).
I combined results from studies on precipitation and biological control impacts on spotted knapweed with a stage-based matrix model to compare the population-level responses to precipitation and biological controls. I tested the hypothesis that the cumulative impact of biological controls would reduce population growth. If reduced survival and seed production are insufficient to substantially decrease reproductive success and the increased growth of rosettes to adults resulting from reduced adult densities, then densities of knapweed with biological controls will not differ from populations without such controls. Such populations tend to exhibit near-monocultures, with densities controlled by intraspecific competition.

3.3 Methods

3.3.1 Matrix Model Structure

I created a simulation model in R (R Core Team, 2012) to test whether the effects of biological controls, precipitation, the interaction of biological controls and precipitation or density-dependence only best explained observed spotted knapweed population dynamics. The biological model used to simulate knapweed population growth was stage-based with a yearly time step (Caswell, 2001; Figure 3.1). Matrix A contains four spotted knapweed life cycle stages: seed bank, seedlings, rosettes, and flowering adults (Figure 3.1).

The model was parameterized to simulate four alternate scenarios for observed spotted knapweed population dynamics. Models included the effects of A) conspecific density-dependence, B) variability in precipitation, C) biological control, and D) biological controls and variability in precipitation. The conspecific density-dependent (density) model did not include effects of precipitation. In the density model, the only control on population growth was conspecific density-dependence. In the precipitation effects models, seed production, $F1$, and germination, $G1$, varied with yearly precipitation. Models for biological control included the
reduce rosette survival, $S_2$, adult survival, $S_3$, and fecundity, $F1$. The interaction model included both decreased rosette and adult survival due to insect presence and reduced seed production that fluctuated as a function of precipitation. Models that included the effects of biological controls assumed an insect population with consistent effects through time. The effects of interspecific plant competition were not included in any model.

All models were parameterized with probability estimates obtained from experimental studies or long-term monitoring in Colorado. The study area for field studies was in Lefthand Canyon (40.07 N, 105.19 W, elevation 1810-1989 m), located just north of Boulder, Colorado. Grazing by cattle has not occurred at the study area since 2006 (Knochel et al., 2010a).

Knapweed biological controls $L.\ minutus$, $C.\ achates$, $Urophora$ sp., and $Sphenoptera$ $jugoslavica$ have been present at the site since 2001 (Seastedt et al., 2007). The effects of $S.\ jugoslavica$ were not incorporated into the model; they are present at very low densities at the research site. Further description of study site is reported in sources (Table 3.1). Measures for
Table 3.1. Description of parameters used in spotted knapweed population model. Parameter estimates for models without the effects of biological controls (absent) and with the effects (present).

<table>
<thead>
<tr>
<th>Parameter Description</th>
<th>Symbol</th>
<th>Absent</th>
<th>Present</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed survival in seed bank (SB)</td>
<td>S1</td>
<td>0.08</td>
<td></td>
<td>Knochel et al., 2010a</td>
</tr>
<tr>
<td>Survival of rosettes (R)</td>
<td>S2</td>
<td>0.40</td>
<td>0.20</td>
<td>A.P. Maines, Chap. 2</td>
</tr>
<tr>
<td>Survival of adults (A)</td>
<td>S3</td>
<td>0.89</td>
<td>0.7</td>
<td>Knochel et al., 2010b</td>
</tr>
<tr>
<td>Number of stems per plant</td>
<td>a</td>
<td>5</td>
<td>3.33</td>
<td>Knochel et al., 2010b</td>
</tr>
<tr>
<td>Number of flowers per plant</td>
<td>n</td>
<td>80.2</td>
<td>45.8</td>
<td>Knochel et al., 2010b</td>
</tr>
<tr>
<td>Number of seeds per flower</td>
<td>s*</td>
<td>10.8</td>
<td>2.37</td>
<td>Knochel &amp; Seastedt, 2010</td>
</tr>
<tr>
<td>Viability of fresh seed</td>
<td>V</td>
<td>0.84</td>
<td></td>
<td>A.P. Maines, Chap. 2</td>
</tr>
<tr>
<td>Seed Production</td>
<td>F1</td>
<td>n×s×V</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Mean (std) for precipitation (cm)</td>
<td>p_r</td>
<td>35.2 (5.49)</td>
<td></td>
<td>NOAA, 2012</td>
</tr>
<tr>
<td>Germination rate</td>
<td>G1*</td>
<td>0.25</td>
<td></td>
<td>A.P. Maines, Chap. 2</td>
</tr>
<tr>
<td>Growth of seedling (S) to rosette</td>
<td>G2</td>
<td>0.065</td>
<td></td>
<td>Knochel et al., 2010a;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A.P. Maines, Chap. 2</td>
</tr>
<tr>
<td>Growth of seedling to adult</td>
<td>G3</td>
<td>0.0039</td>
<td></td>
<td>A.P. Maines, Chap. 2</td>
</tr>
<tr>
<td>Growth of rosette to adult</td>
<td>G4</td>
<td>0.133</td>
<td></td>
<td>A.P. Maines, Chap. 2</td>
</tr>
</tbody>
</table>

Note: Centered values were used in models regardless of biological control presence.
* Values vary when effects of precipitation are included in the model (see eq. 3a and b, 6)

Fecundity and adult survival were obtained from a common garden experiment conducted at the University of Colorado at the Boulder greenhouse (Knochel et al., 2010b). In the case of multiple estimates for a specific transition value the average of reported estimates was used in the model. Model parameter estimates and sources are summarized in Table 3.1.

### 3.3.2 Model Description

Population growth in all simulations was constrained by conspecific stem density, d_t. Stem density was the number of adults m⁻² in year t, A_t, multiplied by the average number of stems adult⁻¹, a. The number of stems plant⁻¹ was smaller when biological controls were present than when they were absent (Knochel et al., 2010b; Table 3.1). Growth from seedlings to rosettes and from seedling and rosettes to adults was constrained by the stem density, d_t, such that the probability of growth decreased as stem density approached 120 stems m⁻².

\[
f(d_t) = 1 - \frac{A_t a}{120}
\]  

(1)
If the stem density exceeded the maximum, then no additional seedlings or rosettes became adults.

Adult spotted knapweed plants may bolt multiple years. The number of adults in the next year, $A_{t+1}$, was surviving adults, $S_3$, plus new adults growing from seedlings, $G_4$, and rosettes, $G_3$. Adult survival, $S_3$, was based on a 9-year lifespan (Sheley et al., 1998). When the effects of biological controls were included, adult mortality due to insects was subtracted from 9-year lifespan (Knochel et al., 2010b; Table 3.1).

$$A_{t+1} = A_t S_3 + R_t G_4 f(t) + S_t G_3 f(t)$$ (2)

Number of viable seeds produced by each adult, $F_1$, was the product of the number of flowers produced per adult, $n$, times the number of seeds produced per flower, $s$, and the germination potential of fresh seed, $V$ (Table 3.1). Both seed production and flowers plant$^{-1}$ were reduced in the presence of biological controls (Knochel et al., 2010b; Seastedt & Knochel, 2010). When the effects of biological controls are included, flower production was reduced (Table 3.1). When effects of biological controls and precipitation were not included in the model, the number of seeds seed head$^{-1}$ was the average number of seeds when $L. minutus$ and $Urophora$ spp. were absent from the seed head, averaged over all reported years (Knochel & Seastedt, 2010). When only the effects of biological controls were included in the model, the number of seeds seed head$^{-1}$ was averaged of values reported for sites (Knochel & Seastedt, 2010). For models that included the effects of precipitation, seed production was a function of October to June precipitation in cm, $p_t$, and the presence of biological controls (D. G. Knochel, Univ. Colo., unpublished data). For future forecasts, yearly precipitation (cm) was randomly chosen from a normal distribution with the mean and standard deviation equal to the 93-year average and standard deviation for October-June precipitation (cm) in Colorado (NOAA, 2012; Table 3.1).
When comparing observed and predicted densities, actual precipitation was used (NOAA, 2012). When effects of precipitation were included in the model, yearly seed production as a function of precipitation, $s_p$, was specified as:

$$s_p = 0.43p_t - 9.5$$

(3a)

and when the interaction of effects of biological controls and precipitation were included, yearly seed production as a function of insect presence and yearly precipitation $s_{p,b}$ was:

$$s_{p,b} = 0.23p_t - 6$$

(3b)

Both relationships were specified using 10 years of measurements from D. G. Knochel (Univ. Colo., unpublished data). When the effects of precipitation were included in the model, the number of seeds head$^{-1}$ was controlled so that the minimum number of seeds was zero.

Seeds present in the seed bank, $SB_{t+1}$, were calculated as new seeds produced plus seed surviving in the seed bank, $SI$, minus germinated seeds, $G1$:

$$SB_{t+1} = SB_t S1 (1 - G1) + A_t F1$$

(4)

For this model, seedlings are newly germinated plants and remain in this stage only one year. Number of seedlings in a year, $S_{t+1}$, was the proportion of the seed bank germinating, $G1$. Proportion of seed bank germinating was the average of estimates reported by Knochel et al. (2010a) and unpublished data (A. P. Maines, Univ. Colo.).

$$S_{t+1} = SB_t G1$$

(5)

For models including precipitation effects, germination probability varied with yearly precipitation. The probability of germination, $G1$, varied with the proportion of average precipitation, $p_{ave}$, received in a given year, $p_t$.

$$G1_p = \frac{p_t}{p_{ave}} G1$$

(6)
Rosettes are non-reproductive plants that germinated in a previous season and survive in the stage or grow to flowering adults. Recruitment from seedlings to rosettes was dependent on stem density. Rosette survival was reduced when the effects of biological controls were included in the model since herbivory has been shown to reduce rosette survival (A. P. Maines, Univ. Colo., unpublished data). The effect of precipitation on rosettes has not been examined in Colorado, however other results suggest that precipitation has minimal effects on rosette survival, so no effects were included in the model (Corn et al., 2007; Ortega et al., 2012).

Rosettes in year \( t+1 \) consist of surviving rosettes, \( S_2 \), plus growth from seedlings, \( G_2 \):

\[
R_{t+1} = R_t S_2 + S_t G_2 f(d_t)
\]  

(7)

### 3.3.3 Density Measures

Model predictions were compared to plant densities monitored in permanent spotted knapweed transects established at the study site north of Boulder (40.07 N, 105.19 W, elevation 1810 to 1989 m). Spotted knapweed was accidently introduced to the study area in the 1980s and biological controls were released from 2001-2005 (Seastedt et al., 2007). Biological controls present at the site include gall flies of the \textit{Urophora} spp. observed at the site in 2001 and introduced weevils \textit{L. minutus} and \textit{C. achates} (Knochel and Seastedt, 2010). Stem and rosette densities have been monitored since 2007 (David Knochel, unpublished data; Seastedt et al., 2007). Sampling procedures are described in detail in Seastedt et al. (2007). The number of stems and rosettes m\(^{-2}\) were recorded yearly within sequential plots along a permanent transect.

### 3.3.4 Model Analysis

The dominant eigenvalues (\( \lambda \)) were used to estimate population growth for each model scenarios. For models that included the effects of precipitation, I evaluated the intrinsic
population growth \((\lambda)\) at three levels. For lambda analyses, transition rates that depended on precipitation \((F1\) and \(G1\)) were evaluated at precipitation one standard deviation above average, equal to average, and one standard deviation below average. I calculated 95% confidence intervals for lambda estimates using 2000 parametric bootstrapped matrices (Caswell, 2001). Sample sizes used in bootstrapping were based on the sample size used to estimate the parameter. Density-independent lambda \((\lambda)\) and 95% confidence intervals were calculated using the popbio package in R version 2.15.1 (Stubben and Milligan, 2007; R Core Team, 2012).

I also calculated transition elasticity to determine how changes in individual parameters would impact population growth rates (de Kroon et al., 1986; Caswell, 2001). Elasticities compare the relative contribution of parameters when parameters are on different scales and when comparing responses in different cases (de Kroon et al., 1986), both of which were true for these models. I calculated density-independent elasticities using the popbio package in R (Stubben and Milligan, 2007; R Core Team, 2012). I evaluated parameters at precipitation one standard deviation below average for models that included the effects of precipitation to predict how changes in parameters would impact population growth under low precipitation.

All model simulations were run with conspecific density-dependent controls on the number of adults and transitions to rosette and adult stages. Start values for all simulations were based on 2007 densities from permanent transects: 48.1 stems m\(^{-2}\), 4.3 rosettes m\(^{-2}\), and 0 seed m\(^{-2}\) produced in 2006 (Seastedt et al., 2007). These values translate to 4.3 rosettes m\(^{-2}\), 0 seedlings m\(^{-2}\), and 0 seeds m\(^{-2}\). The initial number of adults varied between models when biological controls were present (14.4 adults m\(^{-2}\)) or absent (9.62 adults m\(^{-2}\)). The greater number of adults estimated in the biological control scenario was due to the smaller number of stems adult\(^{-1}\) when biological controls are present (Table 3.1). Thus the model estimated more adults with a few
stems in the biological control scenario and fewer, larger adults when biological controls were not present.

I also compared population persistence under varying levels of flowering plant (adult) removal for each model. A suggested management practice is to remove adults prior to seed production (Sheley et al., 1998). I modeled this management practice under all four scenarios. I conducted 100 simulations of 100 years of control to predict population density under each model under varying percent removal of adult plants. The population was considered to persist if more than 1 adult plant per m$^{-2}$ was present.

Finally, I determined which model best fit observed stem and rosette densities measured in the permanent transect from 2007-2012. I calculated negative log likelihood values for all models (Hilborn and Mangel, 1997). I assumed normally distributed errors for both rosette and stem density measurements. I selected the best model based on Akaike Information Criterion adjusted for small sample sizes ($\text{AIC}_c$) and model weights (Anderson et al., 2000; Bolker, 2008). AIC model weights show the relative value of each model in explaining the data compared to the other examined models (Anderson et al., 2000).

### 3.4 Results

The sample sizes used to calculate bootstrapped 95% confidence intervals for lambda were 386 (seed bank), 3,000 (seedlings), 100 (rosettes), and 72 (adults) (Knochel & Seastedt, 2010; Knochel et al., 2010a, 2010b; A.P. Maines, unpublished data). Confidence intervals overlapping or below zero growth ($\lambda \leq 1$) were projected only when biological controls were present (Figure 3.2). While only one scenario produced significant declines in growth rates ($\lambda < 1$), the population was ‘controlled’ ($\lambda = 1$) when biological controls were present under all precipitation regimes except above-average rainfall.
Figure 3.2. Population growth rate estimates ($\lambda$ values) with bootstrapped 95% confidence intervals. Estimates were calculated when the effects of biological controls are present (B) or absent (N) and when precipitation effects were include in the model (P). Subscripts indicate level of precipitation: a standard deviation above average (high), average precipitation (ave), and a standard deviation below average (low). The dotted line indicates zero population growth.

Elasticity analyses showed that biological control increased the importance of adult survival, $S3$, for population growth while decreasing the importance of seed production, $F1$ (Figure 3.3). However, none of the models predicted eradication in 100 years. When only conspecific density-dependence was included in the model, the spotted knapweed population reached 107 stems $m^{-2}$ in 100 years, (which is essentially the density-dependent maximum created in the model). Since models with precipitation effects had precipitation values randomly chosen from a normal distribution with a mean and standard deviation equal to that of Boulder, Colorado’s 93-year mean (NOAA, 2012), the predicted number of stems had a mean and standard deviation. The mean predicted number of stems under the precipitation effects model
Figure 3.3. Life cycle diagrams with elasticity analysis results for a) conspecific density-dependence only, b) variable precipitation (evaluated at low precipitation), c) biological control, and d) biological controls and variable precipitation (evaluated at low precipitation). Values above arrows indicate percent contribution of transition to overall growth rate. Elasticities < 1% are omitted. Arrows are bolded for elasticities > 10%.

was 98 (sd = 4.52) stems m$^{-2}$ in 100 years. The predicted number of stems m$^{-2}$ in 100 years was substantially lower when biological controls were included, with 5 (sd = 3.33) and 12 stems m$^{-2}$ predicted when precipitation effects were included or not, respectively.

Biological control could increase the efficacy of other management efforts by reducing the threshold required for eradication. Due to the shift in importance to adult survival when biological controls are present, the efficacy of other management efforts could be increased. The threshold required for eradication in 20 years was reduced from about 70% removal of adults a year to a little more than 20%, if there is no interaction between the management action (e.g. mechanical, chemical) and efficacy of insects (Figure 3.4). The time to eradication converged when greater than 75% of adult plants were removed.
Figure 3.4. Mean persistence time of spotted knapweed population under varying levels of adult removal. Percent adult removal is the percent of adult plants removed prior to producing seed each year. Models include the effects of conspecific density-dependence only (Density), variable precipitation (Precipitation), biological control, and biological controls and variable precipitation (Bio x Precip).

When comparing model predictions to the observed population dynamics, models that included biological controls were the best predictors of observed stem and rosette densities in the permanent transect (Figure 3.5, Table 3.2). The biological controls effects model (Biocontrol) had the smallest AICₖ values and the greatest model weight (93%; Table 3.2). Models including the precipitation effects did not add to the predictive power of the model. The model including the effects of precipitation and biological controls was distinguishable from Biocontrol since the difference between AICₖ values was > 5 (Bolker, 2008). Thus the candidate model with the
greatest likelihood given these data was the model with the effects of biological controls only (Table 3.2).

Table 3.2. Estimates for negative log likelihoods (NegLogLik) and AIC values for analysis of model fit for permanent transect stem and rosette densities.

<table>
<thead>
<tr>
<th>Model</th>
<th>n</th>
<th>K</th>
<th>NegLogLik</th>
<th>AICc</th>
<th>ΔAIC</th>
<th>w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>9</td>
<td>10</td>
<td>49.4</td>
<td>38.9</td>
<td>31.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Precipitation</td>
<td>9</td>
<td>10</td>
<td>47.9</td>
<td>35.8</td>
<td>28.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Biocontrol</td>
<td>9</td>
<td>10</td>
<td>33.7</td>
<td>7.42</td>
<td>0.00</td>
<td>0.93</td>
</tr>
<tr>
<td>Precipitation x Biocontrol</td>
<td>9</td>
<td>10</td>
<td>36.3</td>
<td>12.7</td>
<td>5.3</td>
<td>0.066</td>
</tr>
</tbody>
</table>

Note: Table includes the sample size (n) and the number of parameters for AICc calculations (K). For each model I include the difference between the model and the model with the smallest AICc value (ΔAIC) and the model weight (w).
Figure 3.5. Model predictions against observed densities for A) stems m\(^{-2}\) and B) rosettes m\(^{-2}\). Observed densities are from permanent transects (D. G. Knochel, unpublished manuscript). Models include the effects of conspecific density-dependence only (Density), variable precipitation (Precipitation), biological control, and biological controls and variable precipitation (Bio x Precip).
3.5. Discussion

This study is the first to compare observed the effects of biological controls and precipitation on spotted knapweed against observed population dynamics from permanent transects. These results support the contention that biological controls have contributed to the observed decline in spotted knapweed populations in Colorado, Montana, and British Columbia. Although biological control agents alone may not be able to reduce densities below desired threshold levels in all habitats, these agents contribute to a population that is more responsive to other types of management.

Biological control contributes to spotted knapweed population-level effects directly through reducing spotted knapweed seed production and plant survival and indirectly by decreasing knapweed’s ability to exploit favorable climatic conditions. These results join others in suggesting that the net contributions of seed reductions and increased plant mortality resulting from biological control can have population-level effects (Louda and Potvin, 1995; Eckberg et al., 2012; Stricker and Stiling, 2012; Rose et al., 2005). The model predicts that biological controls \textit{C. achates}, \textit{L. minutus}, and \textit{Urophora} spp. can contribute to long-term spotted knapweed population reduction.

These results are also consistent with the finding that low levels of adult mortality can be compensated for by additional recruitment from the juvenile stage. In any scenario, low levels of adult removal were not sufficient to eradicate spotted knapweed populations. Plant density can indirectly reduce seed production by negatively impacting stem size thus reducing the number of flowers per stem (Underwood and Halpern, 2012). Low levels of mortality can result in greater resource availability, resulting in new or larger individuals. However, even with initial advantages such as strong seedling establishment, the addition of herbivores can reduce the
success of the invasive plant (Suwa and Louda, 2012). Biological control can reduce the threshold of the level of control needed for populations to be reduced or eradicated.

The model parameterized with the best data available overestimates observed knapweed densities (Figure 3.3). Thus, two observations appear warranted. First, the role of non-knapweed plant competition at relatively low knapweed densities likely is more important than my model portrays (c.f. Pokorný et al. 2005). Second, the population growth rate estimation ($\lambda$ estimate) at low precipitation could be an overestimate of plant performance under dry conditions. Non-knapweed plant competition is perhaps the ‘missing’ piece to the model at low spotted knapweed densities. Pokorný et al. (2005) and Knochel et al. (2010a) demonstrated that plant competition decreased the success of knapweed seedlings when seeds were sown together. Knapweed seedlings also have lower establishment rates in areas with established plant competition (A. P. Maines, Univ. Colo., unpublished manuscript). Further, there could be an interaction between resource variability, such as precipitation, and the competitive effects of plant competition (Knochel & Seastedt, 2010). When precipitation increases, the impact of plant competition on population growth is lower (Maron & Marler, 2008). Thus including plant cover to account for effects of competition on seedling establishment could improve the accuracy of this model both when precipitation effects are included or not.

Variability in precipitation does not explain reduction in spotted knapweed densities from 2007-2012. October to June precipitation has varied greatly in Colorado over the past 10 years, which could explain why precipitation might not be a significant explanatory factor for spotted knapweed declines in Colorado (D. G. Knochel, unpublished manuscript). In Montana, knapweed populations declined when biological controls were present even during above average precipitation years when comparison sites remained at high densities (Story et al., 2006).
Similarly, Gayton and Miller (2012) found that knapweed populations in British Columbia, Canada declined in the presence of biological controls regardless of climatic trends. However, model results suggest that below average precipitation could compound the effects of biological controls and contribute to the rate of decline in a population.

Another model assumption was that the effect of biological controls remained constant over time. My seeding experiments have shown a large range in seedling survivorship (0-11.2%, A. P. Maines, unpublished manuscript). Although herbivory can negatively impact seedling survival, the effect can vary by site (Eckberg et al., 2012; A. P. Maines, unpublished manuscript). Thus the average value used in the model may not reflect actual recruitment values from the single monitoring site used here. Studies of effects of biological control on plant mortality have shown that there is a relationship between the intensity of weevil attack and plant vigor and mortality (Corn et al., 2006; Knochel et al., 2010; Eckberg et al., 2012). We have observed year-to-year variation in insect densities, but have not observed a directional change at our field site (Seastedt et al, 2007; D. G. Knochel, unpublished manuscript). Insect dynamics could result in varying intensity of insect attack rates from year to year. Furthermore, biological impacts might accumulate with the age of the individual plant (cumulative stress across years) (Underwood and Halpern, 2012), so that change in plant age structure could be an unmeasured biological control factor. Stephens and Myers (2012) found that the insect load per plant could vary with the plant density. Incorporating feedbacks in the system between insect and plant density could increase the generalizability of the model to new biological control introductions.

Despite its limitations, the model predicted the general trend observed in field populations. Biological controls decreased densities substantially from the densities projected in non-biological control scenarios. A reduced equilibrium density with herbivores has also been
predicted with the invasive weevil *Rhinocyllus conicus* on the native Platte thistle (Rose et al., 2005). The strong recruitment rate of the invasive thistle *Cirsium vulgare* was not sufficient to maintain the population when herbivores were added (Suwa and Louda, 2012). The reduced equilibrium density can increase the impacts of variation in other environmental factors, such as plant competition and precipitation. Biological control shifts the contribution of population growth from a variety of stages towards adult survival, which is easiest to target with other forms of control (Figure 3.2). In any scenario, low levels of control could be compensated for by increased growth from immature to adult classes. However when biological controls were present, the threshold for effective control of an established population was much lower than when they were absent. These results support the contention that biological controls have contributed to the observed decline in spotted knapweed populations in Colorado, Montana, and British Columbia. Biological controls could be a great part of a management program, provided that the other management strategies don’t interfere with the dynamics of the biological controls.
LITERATURE CITED


