Factors Affecting Spotted Knapweed (*Centaurea stoebe*) Seedling Survival Rates

Anastasia P. Maines, David G. Knochel, and Timothy R. Seastedt*

Predicting site vulnerability to nonnative plant establishment remains a difficult goal. Seedling survival is an important component of population dynamics and can affect the success of control strategies. Field manipulations allow potential causal mechanisms of site vulnerability to be evaluated under realistic environmental conditions. We conducted field studies to determine the effects of plant competition and differing precipitation regimes on spotted knapweed seedling survival. We also examined the effect of herbivory on rosette survival and growth. Seeds were sown into plots with vegetation intact or removed at three sites. 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 accounted for much of the variation in emergence rate, while herbivory and plant competition affected seedling survival rates. We observed a wide range in emergence rates, with site averages ranging from 13.1 to 42.5%. Survival the following year ranged from 0.5 to 9.4% of sown seeds. Rosette survival was significantly higher when herbivores were excluded from plots. Below average precipitation reduced seedling survival; however, even with supplemental water, dry-down of exposed sites resulted in low seedling survival. Of the 8,000 seeds added to plots in one study, by autumn, only eight plants resulted, seven of which survived in watered plots with intact vegetation. Collectively, these results show that seedling survival is a critical phase in spotted knapweed population dynamics and can vary among habitats on the basis of plant competition and precipitation. Furthermore, herbivory affects all stages of the lifecycle from the seedling onward. The observed differences help explain the reported variability in seedling survival in the literature and inform efforts to control spotted knapweed using plant competition and biological controls.
Nomenclature: Spotted knapweed, *Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek.
Key words: Herbivory, invasive plants, plant competition, seedling survival, spotted knapweed.

Invasive plants are a management concern because of potential negative effects on ecosystems services. Possible ecological consequences include reduced native plant diversity (Hejda et al. 2009) and altered species interactions (Seastedt and Pyšek 2011). When invasive plants are unpalatable to livestock and wildlife, they can also become a financial concern for rangeland managers (DiTomaso 2000; Moore et al. 1989; Watson and Renney 1974). Because of 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 nonnative plants will establish and dominate the landscape remains challenging (Seastedt and Pyšek 2011).

A critical stage for understanding the invasion process is seed emergence and seedling survival. Before becoming dominant in the landscape, plants must gain a foothold in the environment through production of new individuals, and site characteristics are clearly important factors in this success (Evans et al. 2012; Ruprecht et al. 2013). The rate at which produced seed become new individuals can also affect the degree to which control methods, such as the use of biological controls, are effective. For example, emergence and survival rates can affect how much biological controls must reduce seed production to be an effective control measure (Knochel et al. 2010a). Plants with higher seedling survival will need to produce fewer seeds to maintain their population. Thus, biological controls would need to reduce seed production to a greater extent to reduce the population successfully.

Understanding variability of spotted knapweed emergence and seedling survival among sites could explain conflicting results over the effectiveness of biological controls for spotted knapweed (*Centaurea stoebe* L. ssp.

DOI: 10.1614/IPSM-D-13-00012.1

* Graduate student, Postdoctoral Researcher, and Professor, Department of Ecology and Evolutionary Biology and Institute of Arctic and Alpine Research, University of Colorado Boulder, Boulder, CO 80309. Current address of second author: Department of Integrative Biology, University of Colorado Denver, Denver, CO 80217. Corresponding author's E-mail: timothy.seastedt@colorado.edu

https://doi.org/10.1614/IPSM-D-13-00012.1 Published online by Cambridge University Press

Management Implications

Spotted knapweed is an invasive, perennial rangeland plant in North America. It is mostly unpalatable to livestock and has the potential to produce thousands of seeds yearly from dense monocultures that limit local plant diversity. Various biological control agents have been introduced to control this plant. They reduce plant reproduction both directly through seed predation and indirectly through reducing flower production and plant life span. This study was conducted in a spotted knapweed infestation on the Front Range of Colorado near Boulder, CO. We examined how established vegetation and herbivory affected the fate of the seeds that are produced. We added spotted knapweed seeds to field plots where vegetation and precipitation levels were manipulated. We also monitored plots in established spotted knapweed patches to examine the effect of herbivory on small rosettes. Spotted knapweed seedling survival was lower when plant competition was present but varied substantially among the sites. The effect of plant competition on seedling survival was subject to a moisture gradient; the presence of non-knapweed plants had a positive effect on seedling survival when precipitation levels were high. Herbivory negatively affected both seedling and rosette survival. The presence of established vegetation and insect herbivory decrease spotted knapweed survival, and biological control may be sufficient to control spotted knapweed at sites that have a high insect populations and low seedling emergence and survival.

micranthos (Gugler) Hayek). This regionally abundant invasive plant was introduced to North America from Eurasia (Sheley et al. 1998). In the absence of biological controls, it can produce more than 20,000 seeds plant^{-1} yr^{-1} for up to 5 yr, which results in dense monocultures in the introduced range (Watson and Renney 1974). Since its introduction in 1890, spotted knapweed has become a major invasive plant across the western United States and Canada (Sheley et al. 1998).

Some evidence suggests that spotted knapweed dominates opportunistically, taking advantage of disturbances and reduced herbivory in the introduced range. Spotted knapweed is less palatable for native insect herbivores, which results in fewer controls for spotted knapweed relative to native plants (Schaffner et al. 2011; Sheley et al. 1998). Field and greenhouse studies have demonstrated that plant competition can reduce spotted knapweed seedling survival, suggesting disturbances may contribute to initial establishment in some areas (Knochel et al. 2010a; Pokorny et al. 2005).

A host of biological control insects have been released to control this plant, from seed head predators to root feeders. Three biological controls have been found to be particularly effective. The seed head weevil *Larinus minutus* Gyl. and gall fly *Urophora* spp. larvae grow in the developing seed heads, and their presence causes a substantial reduction in spotted knapweed seed production (Seastedt et al. 2007; Story et al. 2008). Larvae of the root weevil *Cyphocleonus achates* Fahr. develop in spotted knapweed roots, and adults feed on spotted knapweed vegetation, resulting in decreased adult plant survival and flower production (Knochel et al. 2010b; Ortega et al. 2012).

In the decades following the introduction of biological controls that targeted spotted knapweed seed production and plant survival, some spotted knapweed populations have declined (Gayton and Miller 2012; Knochel et al. 2010b; Seastedt et al. 2007; Story et al. 2006, 2008). However, recent experimental results show that spotted knapweed populations compensate for increased adult mortality by increasing the number of rosettes growing to the adult stage in some conditions, which results in no net change in adult population size (Ortega et al. 2012). Thus, an alternative hypothesis has been proposed to explain the observed spotted knapweed declines; that is, the observed population dynamics are due to drought, rather than the released biocontrol insects (Corn et al. 2007; Ortega et al. 2012; Story et al. 2008). The drought and biological control hypotheses need not be mutually exclusive; both biological control and drought could reduce density (Maines et al., in press). Highly variable seedling survival rates complicate our understanding of how seed predators and other herbivores affect spotted knapweed populations, with survival from seed ranging from 0.06 to 36% (Knochel et al. 2010a; Pokorny et al. 2005; Sheley et al. 1998). While water addition increased rosette mortality (Corn et al. 2007), seedling survival may be particularly sensitive to drought because of an underdeveloped root system, which is less effective at obtaining water (Peters 2000). Furthermore, native plants may be less effective competitors against spotted knapweed under high precipitation regimes (Maron and Marler 2008). These conflicting findings about the effects of plant competition and precipitation suggest that certain habitats, climatic conditions, or some combination of these factors can affect the outcome of biological control efforts.

Field experiments can demonstrate the control factors that are significant under realistic environmental conditions. In our manipulative field study, we examined spotted knapweed seedling survival with and without plant competition across multiple sites and under controlled precipitation regimes. We also examined the effect of herbivory on rosettes before flowering. These experiments tested the hypothesis that spotted knapweed becomes an opportunistic invader when released from top-down controls in the introduced range. We predicted that seedlings would exploit resource opportunities from reduced plant competition, and we examined the following questions:

1. How does plant competition affect spotted knapweed seedling survival?
2. How does precipitation and the interaction between plant competition and precipitation influence spotted knapweed seedling survival?

https://doi.org/10.1614/IPSM-D-13-00012.1 Published online by Cambridge University Press

3. How does herbivory influence spotted knapweed rosette survival and growth?

Materials and Methods

Field Site Description. The study area was north of Boulder, CO, in Lefthand Canyon (40.07°N, 105.19°W, elevation 1,810 to 1,989 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*, *Cyphocleonus achates*, *Sphenoptera jugoslavica* Obenb., and gall flies *Urophora* spp., have been previously released and established at the site (Seastedt et al. 2007). The studies were conducted from March 2011 to September 2012. The experimental manipulations were conducted at five sites differing in slope, aspect, and vegetative composition. Sites were within 2 km of each other. We determined the seed germination potential for these studies by placing 100 seeds in moist paper towels in the lab under natural light for 14 d. Germination potential was calculated as the percentage of seeds that germinated by the end of the trial.

Experiment 1: Plant Competition Effects on Seedling Survival. In March 2011, 20 experimental plots were established in each of three sites. Site 1 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 2 was flat in aspect and slope with a primary cover of orchardgrass (*Dactylis glomerata* L.) and smooth brome (*Bromus inermis* Leyss.) with a moderate spotted knapweed population (15 to 20% relative cover). Site 3 was north-facing. Dominant cover at site 3 was bluegrass (*Poa* spp.), snowberry (*Symphoricurpos* Duh.), 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 any existing seed bank was not manipulated. Spotted knapweed seed bank viability after the first year is estimated between 5 and 8% (Jacobs and Sheley 1998; Knochel et al. 2010a). Seed production at these sites has been low because of insect seed consumption (Knochel and Seastedt 2009). Therefore, the resident seed contribution to the experiment was assumed to be small relative to the number of seeds added here.

At each site, 20 plots of 0.1 m^2 separated by 0.25 m were established along two parallel transects. Plots were randomly assigned to plant competition present or absent treatments with 10 plots per treatment per site. Established vegetation remained intact in plant competition treatments (trt=present), or plots were weeded to remove vegetation from the plot area (trt=absent). After initial 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}) in late March on the basis of a high seed rain density observed in the field (Knochel and Seastedt 2010; Maron and Marler 2008).

Spotted knapweed seed emergence and mortality of emerged seedlings were assessed bimonthly from May to August 2011. At each census, we marked new seedlings with toothpicks. Seedling mortality was determined from the number of toothpicks that no longer marked a plant. We recorded the number of seedlings that were alive in the plot and the number of seedlings that died since the previous count. After determining seedling mortality, we removed toothpicks for seedling that had died. Emergence was the sum of all new spotted knapweed seedlings we observed over the entire monitoring period. Survival was the total number of plants present in the plot at the final census (May 2012: 1 yr after the seed was sown). We also recorded the number of seedlings with damaged leaves at each census to obtain estimates of the herbivory rate for seedlings. We calculated the herbivory rate from 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: insects were present at all sites.

Experiment 2. Effect of Competition and Precipitation on Survival. A factorial field experiment was established at a single site (site 4) to examine the effects of plant competition and precipitation on spotted knapweed seedling survival. The location did not have a previous spotted knapweed population, to eliminate possible confounding effects of a seed bank. Dominant cover at site 4 included Kentucky bluegrass (*Poa pratensis* L.), *Carex* spp., and small-leaf pussytoes (*Antennaria parvifolia* Nutt.). Although biological control agents were present within 1 km of this site, no control insects were added to this area.

We 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. Like experiment 1, established vegetation remained intact in the plant competition plots. Established vegetation was lightly raked in plant competition plots. For control plots, existing vegetation was weeded to completely remove vegetation from the plot area. Each plot was seeded with 200 seeds $plot^{-1}$. After establishing plots, the plot composition was not manipulated, thus non-knapweed seedlings could colonize plots. Spotted knapweed seed was collected in the fall of 2011 for use in the 2012 study. Because of low

https://doi.org/10.1614/IPSM-D-13-00012.1 Published online by Cambridge University Press

precipitation before establishment of plots, all plots were initially supplemented to meet the 93-yr average for March precipitation in 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 one standard deviation 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 the number of surviving seedlings was recorded. We also examined plots for the presence of biological control insects at each census. For this experiment, survival was assessed in August 2012.

On May 16, after emerged seedlings were observed, we marked 10 seedlings (or, if < 10, as many seedlings as were present in the plot) with toothpicks in each of 20 randomly selected plots. Once marked, the same individuals were followed for the duration of the experiment. We assessed focal seedlings weekly for evidence of herbivory (bitten/removed leaves). Since the number of focal seedlings varied throughout the study period because of seedling mortality, the rate of herbivory for each plot at each census was the number of focal seedlings with herbivory divided by total number of focal seedlings currently alive in the plot.

To evaluate the relationship between soil composition and seedling survival, we took composite soil samples 10 cm deep from sites 1 to 4 on July 2012. Samples were analyzed for soil texture. Soil measurements were not made at site 5 (experiment 3, described below) because seedling survival was not measured at this site.

Experiment 3: Effects of Herbivory on Rosette Survival. We tested the effect of herbivory on new rosettes in the presence of adult plants by establishing 20 plots with an area of 0.1 m^2 in an existing spotted knapweed population in May 2011. This site was above site 1 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. The downhill transect received insecticide (Bayer Advanced Complete, containing imadicloprid systemic [0.72%] and β-cyfluthrin nonsystemic [0.36%], rate of 3-oz. [88-ml] product per 3.75 L water), and the uphill transect was 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 6 wk with insecticide or pure water using a handheld sprayer for a total of three insecticide or pure water applications over the summer. Ten small rosettes per plot were randomly selected in mid May 2011 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 diam. Thus, all focal rosettes were between 1 and 5 cm diam. Small rosettes were chosen because they are likely to be younger plants that have never bolted. Focal rosettes were mapped and monitored throughout the summer.

At each census, we recorded evidence of herbivory, which was indicated by leaf tissue damage and rosette mortality, defined as the absence of rosette or a rosette with all aboveground tissue completely desiccated. Evidence of damage from deer or elk moving through plots was occasionally observed, and these instances were noted as ungulate damage rather than insect herbivory. We scored plots for damage and mortality eight times (every 3 to 4 wk and then 1 yr after the start of the experiment). Because rosette mortality occurred throughout the summer, the rate of herbivory for each plot at each census was calculated from the total number of focal rosettes with herbivory divided by total number of focal rosettes in that plot. On the final census (May 2012), focal plants were 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. To determine the effect of the insecticide treatment on adult plants in the plots, we conducted an assessment of seed production and number of insects present in seed heads. We collected three seed heads per plot in early September and counted the number of *L. minutus, Urophora* spp., and seeds present to calculate average number of *L. minutus, Urophora* spp., and seeds per flower present in each plot. This allowed us to measure the effect of insecticide treatment on insect presence and adult seed production ($n = 10$).

Statistical Analysis. The total number of seedlings observed in the plots over the course of the experiment (emergence) was natural log–transformed to fulfill assumptions of normality and then back-transformed for readability of figures. To account for repeated counts of seedling herbivory, the herbivory rate for each plot was averaged for all censuses to determine the average herbivory rate over the monitoring period. For experiment 1, both the number of seedlings with herbivory and seedling mortality were $\ln(x + 1)$ transformed to meet normality assumptions for the regression. A regression examined the relationship between herbivory at time t and seedling mortality at $t + 1$ to determine whether herbivory rate predicted seedling mortality observed in the following census. In experiment 1, a mixed model using the nlme package in R was used to determine the effect of plant competition on spotted knapweed seedling emergence (R Core Team, R Foundation for Statistical Computing, Vienna, Austria; Pinheiro et al. 2012). We used a mixed model in the lme4 package in R using the Poisson family to

https://doi.org/10.1614/IPSM-D-13-00012.1 Published online by Cambridge University Press

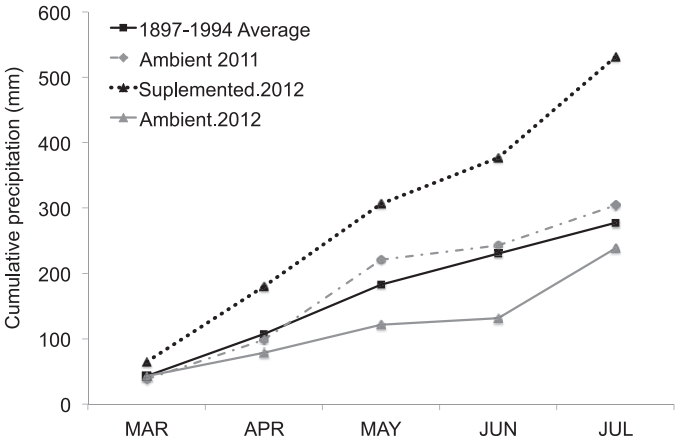


Figure 1. Cumulative March to July precipitation (mm) for study area with Boulder, CO, 93-yr average rainfall (NOAA 2012). Ambient precipitation measurements were collected approximately weekly from rain gauges at the field site (Ambient 2011; Janet Prevéy, unpublished data).

meet assumptions because seedling survival was count data (Bates et al. 2012). The model included the fixed effects of plant competition and herbivory rate and the random effect of site. Variables were untransformed for this analysis. Because of the high number of zeros, seedling survival values from experiment 2 did not meet assumptions of normality, so a Kruskal–Wallis test was used to compare survival probabilities among precipitation by plant competition treatments.

For experiment 3, a *t* test was used to determine whether the insecticide treatment reduced the rate of herbivory on rosettes. We used *t* tests to examine the effect of treatment on rosette survival and growth to the adult stage. To account for repeated plot-level measurement of herbivory rate, the herbivory rate used in *t* tests was the average rate for each plot over the monitoring period. We used paired *t* tests to determine the effect of insecticide on rosette survival and transition to adult. Where data did not meet normality assumptions, we used Kruskal–Wallis tests. We 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 Kruskal–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).

Results and Discussion

Experiment 1. Because of a germination rate of 84% in the lab germination experiment, we expected high seedling emergence in the field. Precipitation during March to July 2011 was 304 mm, which was 110% of average Boulder precipitation (Figure 1; Janet Prevéy, unpublished results; NOAA 2012). The soil texture at sites 1 and 3 was sandy loam, and at site 2 loamy sand.

The presence of plant competition did not significantly affect the proportion of seeds that emerged ($F_{1,56} = 0.086$, $P = 0.77$). Differences between sites account for 54% of the variance in emergence rate. Range of emergence was wide, with site averages ranging from 13.1 to 42.5% of added seed observed over the course of the summer.

Percent survival measured in October was 2.0 ± 0.33%, 5.4 ± 0.80%, and 12.8 ± 1.3% of added seed across three sites. Plots with plant competition had a nonsignificant trend of lower survival ($F_{1,56} = 0.087$, $P = 0.77$). The best model to predict the number of seedlings surviving to the following year included an interaction effect between plant competition and herbivory rate ($z = 4.89$, $P < 0.0001$). Herbivory rate had a much greater effect in plots with plant competition than in plots without plant competition (Figure 2B). The mean herbivory rate significantly affected final seedling survival ($z = -5.72$, $P < 0.0001$). Plant competition significantly decreased the number of seedlings surviving to the next year ($z = -0.887$, $P = 0.37$; Figure 2B). Site explained 50% of the variation in seedling survival. Plants surviving to the following year varied from 0.47, 4.0, and 9.4% of added seeds, demonstrating additional seedling mortality over the winter. The herbivory rate was a significant predictor of seedling mortality in the next census ($R^2 = 0.15$, $P < 0.0001$) (data not shown).

Experiment 2. Soil texture at site 4 was loamy sand. Precipitation for March to July 2012 in ambient plots was amended to 239 mm, which is 85% of average Boulder precipitation (Figure 1; NOAA 2012). In supplemented precipitation plots, precipitation was 532 mm, which is 190% of average Boulder precipitation (Figure 1; NOAA 2012). The seedling survival rate was significantly different among the four plant competition by precipitation treatments ($W = 9.69$, $df = 3$, $P = 0.021$; Figure 3A). The only seedling survival rate significantly different than zero was in plots with plant competition and supplemented precipitation (0.35% of seeds). Overall, out of 8,000 seeds added, only eight plants survived. The rate of herbivory was significantly lower when plant competition was absent than when it was present. There was no effect of the precipitation treatment on the herbivory rate (Table 1; Figure 3B).

Experiment 3: Rosettes. The insecticide treatment successfully reduced the rate of herbivory experienced by rosettes (Figure 4A), but the number of seed predators (*Urophora* spp. and *L. minutus*) and the number of seeds in seed heads were not significantly different between the treatments (Table 2). The insecticide treatment had no effect on rosette survival by the end of the first summer or the percentage of rosettes that became adults the following year (Figure 4B; Table 2). However, rosette survival to the following year was significantly higher in plots that received the insecticide treatment.

https://doi.org/10.1614/IPSM-D-13-00012.1 Published online by Cambridge University Press

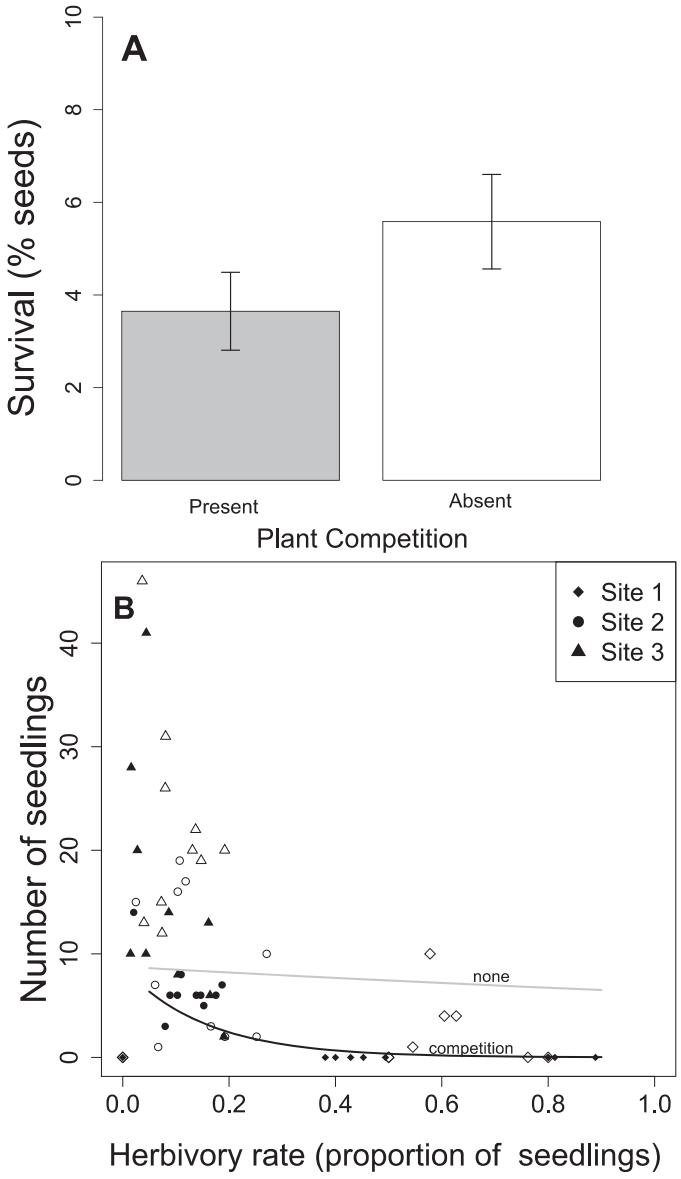


Figure 2. Effects of plant competition and herbivory rate on seedling survival. (A) Mean percent seedling survival (plants per seeds sown) 1 yr after seeding when plant competition was present (dark bars) or removed (light bars) (n = 60). Plots with plant competition had a nonsignificant trend of lower survival ($F_{1,56}$ = 0.087, P = 0.77). (B) The number of seedlings surviving to the second year by observed herbivory rate. Filled symbols are plots with plant competition, and open symbols are plots without plant competition. Best fit model for herbivory rate effect and plant competition by herbivory rate interaction. Equation (using the Poisson link): $\ln(y) = -6.42x_1 + 6.09(x_2 - x_{1)} + 2.17$, where x_1 is herbivory rate and x_2 is plant competition present (0) or absent (1).

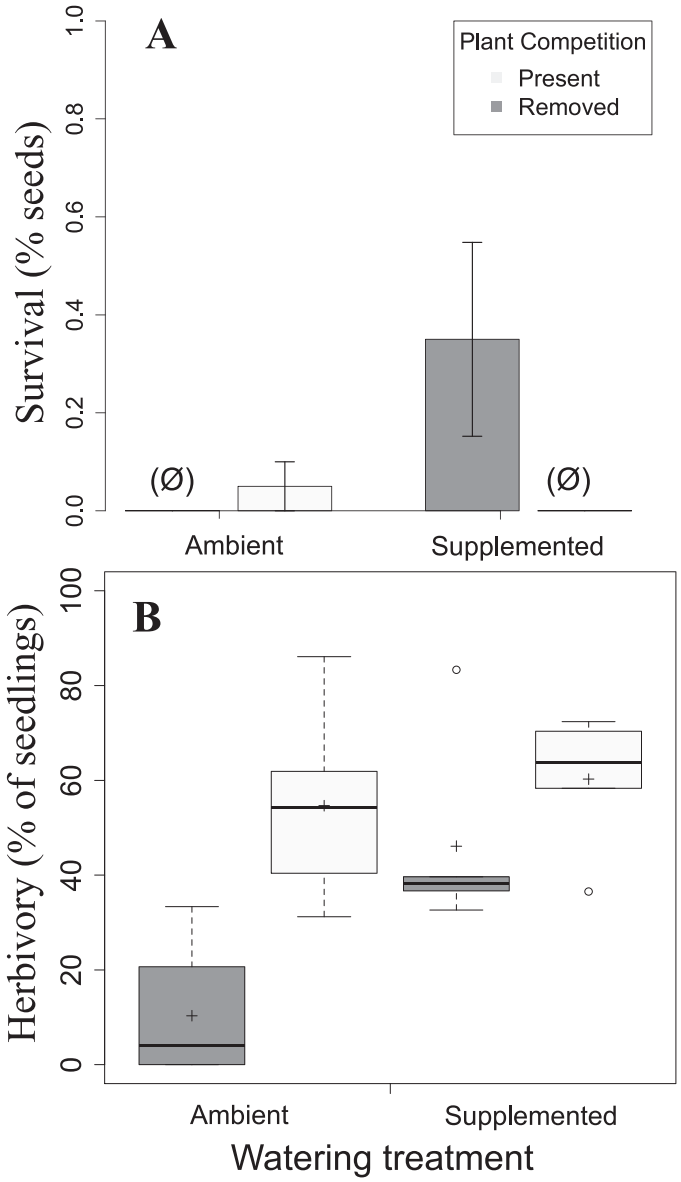


Figure 3. Boxes represent the range of values for 2012 study (A) for plants surviving to August as the percentage of sown seeds by precipitation treatment and plant competition present (dark bars) or removed (light bars) (n = 40, 10 per bar). Less than 1% of the seeds added were plants by fall. Bars are means ± 1 standard error. (B) Herbivory rate by plant competition and precipitation treatments (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.

A significant question for spotted knapweed management is whether seed reduction by a combination of plant competition and herbivory 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 in seed production needed to control spotted knapweed populations have varied based on observed seedling survival probabilities. The seedling survival values observed in our 2011

https://doi.org/10.1614/IPSM-D-13-00012.1 Published online by Cambridge University Press

Table 1. ANOVA analysis[a] of the effect of precipitation, plant competition, and their interaction on average herbivory rate from experiment 2.

	Competition			Precipitation			Competition × Precipitation		
	df	*F*	P	df	*F*	P	df	*F*	P
Herbivory	1	12.6	**0.003**	1	4.05	0.61	1	3.42	0.083

[a] P values significant at alpha = 0.05 are bold.

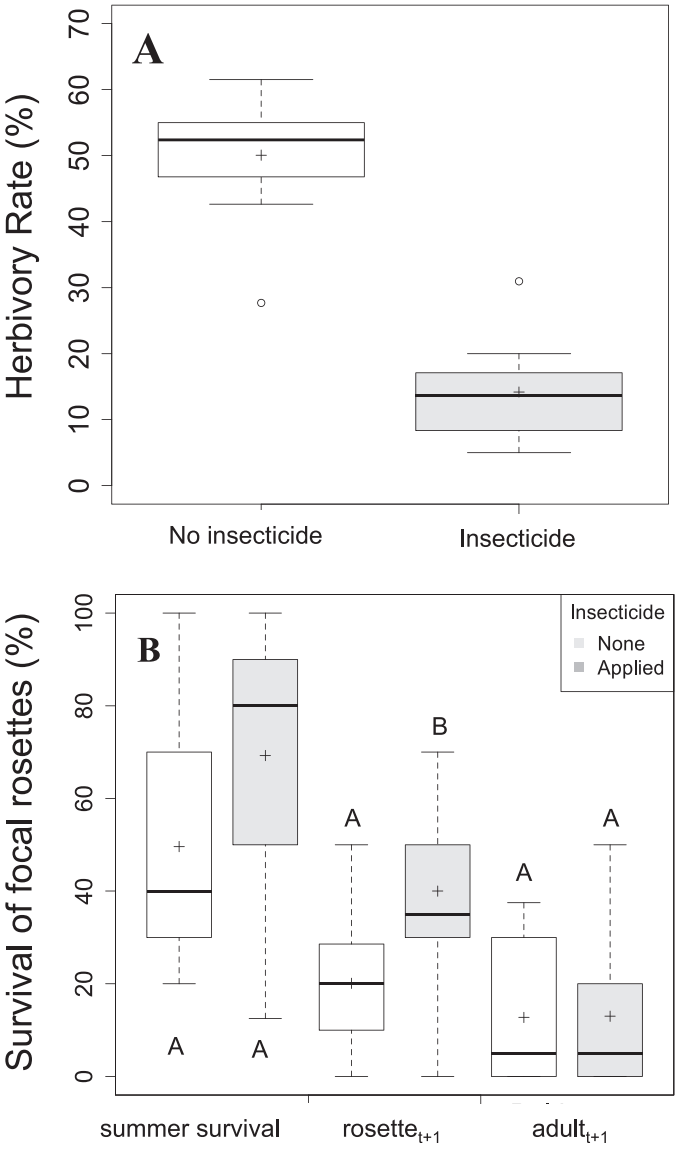


Figure 4. (A) Percentage of focal rosettes experiencing herbivory in plots without insecticide (light boxes) and with insecticide applied (dark boxes; $n = 20$). (B) Percentage of focal rosettes that survived the summer, survived as rosettes the following year, and grew to the adult stage the following year when no insecticide was applied (light bars) or with insecticide applied (dark bars, $n = 20$). Boxes connect upper and lower quartile. The bars are medians, pluses indicate mean values, whiskers indicate range of values, and open circles are outliers. In panel B, capital letters indicate significant differences between bars in the same category.

experiment (percentage of seeds that reached the rosette stage) translate to an adult plant needing to produce between 11 and 212 seeds to produce one new plant, depending on site characteristics. A plant from sites with survival rates of our 2012 experiment would have to produce 1,000 seeds to generate one new plant that survived until the second year. Because spotted knapweed is a short-lived perennial, the persistence of a given density of this weed could be maintained by even lower seed production. Regardless, this 100-fold difference in seedling survival probabilities can explain discrepancies among published studies in evaluating the efficacy of biological control. At some sites with high seedling survival, additional measures beyond biological agents would be required to control the population. Seed reduction as a control mechanism will be effective in suppressing plant densities in areas with low to very low seedling survival, such as the site of our second experiment.

Biological control insects are known to reduce spotted knapweed seed production and adult survival (Knochel et al. 2010b; Wooley et al. 2011), and our results identify some possible constraints on seedling and rosette survival that are important under realistic field conditions. The range of seedling survival rates found in this study was consistent with the variation in seedling survival reported in previous studies (Figure 3; Knochel et al. 2010a; Ortega et al. 2012; Pokorny et al. 2005). The high survival rate at site 3 (experiment 1) could be due to emergence of a seed bank. Other studies have shown a larger surviving seed bank on north-facing slopes than in other locations despite similar spotted knapweed cover (D. G. Knochel, unpublished data). Under high levels of seed production, as was added here, spotted knapweed can be a dominant competitor at certain sites (Ortega et al. 2012), but not at all sites, as demonstrated here.

Site characteristics play an important role in vulnerability to invasion and may help explain the wide variation in spotted knapweed seedling survival rates in the literature. Seastedt and Pyšek (2011) list the abiotic and biotic constraints that benefit or constrain successful seedling invasion and persistence within a specific site. That list includes a number of variables not measured here, such as potential positive and negative effects of endophytes (Newcombe et al. 2009) or soil mycorrhizae (Harner et al. 2010); therefore, our findings cannot provide a comprehensive analysis of the critical controls on weed densities.

https://doi.org/10.1614/IPSM-D-13-00012.1 Published online by Cambridge University Press

Table 2. Paired t test (t) and Kruskal–Wallis (W) results for measures of focal rosettes (a and b) and survey of adult seed heads (c).[a]

Measurement	df	Statistic	P
(a) Percentage of rosettes with herbivory	1	$W = 28.4$	< 0.0001
(b) Focal rosette fate			
Summer survival	9	$t = -1.55$	0.15
Rosette$_{t+1}$	9	$t = -3.02$	0.014
Adult$_{t+1}$	1	$W = 0$	1
(c) Seed head abundance			
No. seeds	1	$W = 0.53$	0.47
No. *Larinus*	1	$W = 2.10$	0.15
No. *Urophora*	9	$t = 0.75$	0.47

[a] $n = 100$ rosettes and 30 seed heads.

Our findings do show that the presence of established plant competition reduced spotted knapweed survival at all sites, except under increased water availability. Climate factors such as precipitation explain some differences in the effect of plant competition on spotted knapweed. Precipitation and interannual variation in precipitation has been shown to mediate how plant competition limits spotted knapweed seedling survival (Blumenthal et al. 2008; Knochel et al. 2010a; Maron and Marler 2008). However, these effects on seedlings are not consistent as might be expected under a variable climate or resource regime. Furthermore, it is possible that background vegetation could also benefit from increased resources. Spotted knapweed was unable to colonize disturbed plots in our second experiment, even with supplemented precipitation (Figure 3A), yet we did observe non-knapweed seedlings colonizing the plots (A. P. Maines, personal observation). Some, though not all, native plants may be better able to take advantage of increased resources than nonnative plants (Ruprecht et al. 2013). This variability contributes to the difficulty in predicting where introduced plants will succeed (Davis et al. 2000).

Herbivory does appear to contribute to differences in seedling survival rates among sites. Areas with lower rates of herbivory were correlated with higher seedling and rosette survival. In our first experiment, two of the sites had similar emergence rates, but they had very different seedling survival. The herbivores affecting the seedling stage were not determined. It could be due either to biological controls or to generalist herbivores. Rosettes were shown to have a higher rate of damage from generalist herbivores than adult plants (Collins and Müller-Schärer 2012). Because herbivory rates were high in our second experiment (site 4), where the introduced biological controls were not present, it is possible that spotted knapweed seedlings may be subject to generalist herbivores in some habitats. During the course of our study, we only observed one seed head weevil at site 4. Although these insects do attack the foliage of rosettes and adult plants, we have never observed seedlings being attacked by the released biological control agents.

Results presented here showed that the combined biotic and abiotic characteristics of specific habitats affected seedling and rosette survivorship of spotted knapweed, but these effects varied across the landscape. Plant competition and herbivory were important, and these controls may be amplified or attenuated by other site factors, such as soil symbionts and pathogens, not measured here. Consistent with monitoring studies (cf. Gayton and Miller 2012), sustainable controls generated by plant competition and biological control agents can negatively affect spotted knapweed densities and appear appropriate and sufficient management tools in some areas. However, our results also indicate that other habitats exist in which these controls are unlikely to meet management goals, as indicated by modeling outcomes presented by Maines et al. (in press). At sites with high seedling survivorship, plant competition and herbivory can only expedite the more proactive measures used to reduce current spotted knapweed densities.

Acknowledgments

Field research reported here was funded by USDA grants NRI 2009 55320-05072 and NRI 2006-35320-17269 to the University of Colorado. We thank Sara Torres, Bradley Vander Vliet, Nohal Amir, Adriana Manrique, Robin Reibold, Marissa Reyes, Meredith Chedsey, Martha Mendoza, and Keith Maines for help in the field. We thank Linda and Sergio Sanabria for use of their land for field studies. We also thank Akasha Faist for helpful comments on a draft and Janet Prevéy for plant species identification.

Literature Cited

Bates, D., M. Maechler, and B. Bolker. 2012. Lme4: Linear Mixed Effects Models Using S4 Classes. R package version 0.999999-0. Vienna, Austria: The Comprehensive R Archive Network, http://CRAN.R-project.org/package=lme4.

Blumenthal, D., R. A. Chimner, J. M. Welker, and J. A. Morgan. 2008. Increased snow facilitates plant invasion in mixedgrass prairie. New Phytol. 179:440–448.

Collins, A. R. and H. Müller-Schärer. 2012. Influence of plant phenostage and ploidy level on oviposition and feeding of two specialist herbivores of spotted knapweed, *Centaurea stoebe*. Biol. Control 60:148–153.

Corn, J. G., J. M. Story, and L. J. White. 2007. Effect of summer drought relief on the impact of the root weevil *Cyphocleonus achates* on spotted knapweed. Environ. Entomol. 36:858–863.

Davis, M. A., J. P. Grime, and K. Thompson. 2000. Fluctuating resources in plant communities: a general theory of invisibility. J. Ecol. 88:528–534.

https://doi.org/10.1614/IPSM-D-13-00012.1 Published online by Cambridge University Press

DiTomaso, J. M. 2000. Invasive weeds in rangelands: species, impacts, and management. Weed Sci. 48:255–265.
Evans, J. A., A. S. Davis, S. Raghu, A. Ragavendran, D. A. Landis, and D. W. Schemske. 2012. The importance of space, time, and stochasticity to the demography and management of *Alliaria petiolata*. Ecol. Appl. 22:1497–1511.
Gayton, D. and V. Miller. 2012. Impact of biological control on two knapweed species in British Columbia. J. Ecosyst. Manag. 13:1–14.
Harner, M. J., D. L. Mummey, J. A. Stanford, and M. C. Rillig. 2010. Arbuscular mycorrhizal fungi enhance spotted knapweed growth across a riparian chronosequence. Biol. Invasions 12:1481–1490.
Hejda, M., P. Pyšek, and V. Jarosik. 2009. Impact of invasive plants on the species richness, diversity and composition of invaded communities. J. Ecol. 97:393–403.
Jacobs, J. and R. Sheley. 1998. Observation: life history of spotted knapweed. J. Range Manag. 51:665–673.
Knochel, D. G., C. Flagg, and T. R. Seastedt. 2010a. Effects of plant competition, seed predation, and nutrient limitation on seedling survivorship of spotted knapweed (*Centaurea stoebe*). Biol. Invasions 12:3771–3784.
Knochel, D. G., N. D. Monson, and T. R. Seastedt. 2010b. Additive effects of aboveground and belowground herbivores on the dominance of spotted knapweed (*Centaurea stoebe*). Oecologia 164: 701–712.
Knochel, D. G. and T. R. Seastedt. 2009. Sustainable control of spotted knapweed, (*Centaurea stoebe*). Pages 211–225 *in* Inderjit, ed. Management of Invasive Weeds. Dordrecht, The Netherlands: Springer Science + Business Media B.V.
Knochel, D. G. and T. R. Seastedt. 2010. Reconciling contradictory findings of herbivore impacts on spotted knapweed (*Centaurea stoebe*) growth and reproduction. Ecol. Appl. 20:1903–1912.
Maines, A. P., D. G. Knochel, and T. R. Seastedt. 2013. Biological control and precipitation effects on spotted knapweed (*Centaurea stoebe*): empirical and modeling results. . Ecosphere 4:art80. http://dx.doi.org/10.1890/ES13-00094.1
Maron, J. L. and M. Marler. 2008. Field-based competitive impacts between invaders and natives at varying resource supply. J. Ecol. 96: 1187–1197.
Moore, W., C. Doyle, and A. Rahman. 1989. Economics of controlling *Carduus nutans* on grazed pasture in New Zealand. Crop Prot. 8:16–24.
Newcombe, G., A. Shipunov, S. D. Eigenbrode, A.K.H. Raghavendra, H. Ding, C. L. Anderson, R. Menijivar, M. Crawford, and M. Schwarzländer. 2009. Endophytes influence protection and growth of an invasive plant. Commun. Integr. Biol. 2:29–31.
[NOAA] National Oceanic and Atmospheric Administration. 2012. Boulder Monthly Climate Data: Precipitation. Washington, DC: National Oceanic and Atmospheric Administration, Earth System Research Laboratory (Physical Sciences Division), http://www.esrl.noaa.gov/psd/boulder/Boulder.mm.precip.html. Accessed April 11, 2012.
Ortega, Y. K., D. E. Pearson, L. Waller, N. J. Sturdevant, and J. L. Maron. 2012. Population-level compensation impedes biological control of an invasive forb and indirect release of a native grass. Ecology 93:783–792.
Peters, D. 2000. Climatic variation and simulated patterns in seedling establishment of two dominant grasses at a semi-arid-arid grassland ecotone. J. Veg. Sci. 11:493–504.
Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, the R Development Core Team. 2012. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-104. Vienna, Austria: The Comprehensive R Archive Network, http://cran.r-project.org/web/packages/nlme/index.html. Accessed September 12, 2012.
Pokorny, M., R. Sheley, C. Zabinski, R. Engel, T. Svejcar, and J. Borkowski. 2005. Plant functional group diversity as a mechanism for invasion resistance. Restor. Ecol. 13:448–459.
R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
Ruprecht, E., A. Fenesi, and I. Nijs. 2013. Sudden changes in environmental conditions do not increase invasion risk in grassland. Acta Oecol. 47:8–15.
Schaffner, U., W. M. Ridenour, V. C. Wolf, T. Bassett, C. Mueller, H. Mũller-Schärer, S. Sutherland, C. J. Lortie, and R. M. Callaway. 2011. Plant invasions, generalist herbivores, and novel defense weapons RID A-2783-2012. Ecology 92:829–835.
Seastedt, T. R., D. G. Knochel, M. Garmoe, and S. A. Shosky. 2007. Interactions and effects of multiple biological control insects on diffuse and spotted knapweed in the Front Range of Colorado. Biol. Control 42:345–354.
Seastedt, T. R. and P. Pyšek. 2011. Mechanisms of plant invasions of North American and European grasslands. Annu. Rev. Ecol. Evol. Syst. 42:133–153.
Sheley, R., J. Jacobs, and M. Carpinelli. 1998. Distribution, biology, and management of diffuse knapweed (*Centaurea diffusa*) and spotted knapweed (*Centaurea maculosa*). Weed Technol. 12:353–362.
Story, J. M., W. Callan, J. G. Corn, and L. J. White. 2006. Decline of spotted knapweed density at two sites in western Montana with large populations of the introduced root weevil, *Cyphocleonus achates* (Fahraeus). Biol. Control 38:227–232.
Story, J. M., L. Smith, J. G. Corn, and L. J. White. 2008. Influence of seed head-attacking biological control agents on spotted knapweed reproductive potential in western Montana over a 30-year period. Environ. Entomol. 37:510–519.
Watson, A. and A. Renney. 1974. Biology of Canadian weeds. 6. Centaurea diffusa and Centaurea maculosa. Can. J. Plant Sci. 54: 687–701.
Wooley, S. C., B. Smith, C. King, T. R. Seastedt, and D. G. Knochel. 2011. The lesser of two weevils: physiological responses of spotted knapweed (*Centaurea stoebe*) to above- and belowground herbivory by *Larinus minutus* and *Cyphocleonus achates*. Biocontrol Sci. Technol. 21:153–170.

Received February 12, 2013, and approved July 26, 2013.

https://doi.org/10.1614/IPSM-D-13-00012.1 Published online by Cambridge University Press