

Biological control and precipitation effects on spotted knapweed (*Centaurea stoebe*): empirical and modeling results

ANASTASIA MAINES,[†] DAVID KNOCHEL,¹ AND TIMOTHY SEASTEDT

*Institute of Arctic and Alpine Research and Department of Ecology and Evolutionary Biology, University of Colorado,
Boulder, Colorado 80309 USA*

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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 experimental manipulations of spotted knapweed and long-term monitoring of seed production and insect abundance were used to parameterize a population matrix model for this species. Model predictions were compared against spotted knapweed densities observed in permanent transects, which were established after the release of biological controls. The model incorporated population-level compensation for adult mortality (increased growth from juveniles to adults). The model also incorporated newly reported results showing an interaction between precipitation and biological control impacts. We compared predictions from four alternate models against the observed population densities; models were: conspecific density-dependence, the effects of biological controls, precipitation, and biological control-precipitation interaction. The best model to explain population declines included the effects of biological control agents. Declines in population growth rates ($\lambda < 1$) were only predicted when reduced seed production and increased plant mortality due to biological controls were included. Results suggest that biological controls contributed to declines observed in field studies, and support the contention that biological control attenuates the ability of spotted knapweed to exploit favorable climatic conditions. The results also demonstrate that spotted knapweed control (i.e., conditions where $\lambda < 1$) depends upon a relatively large impact of biological control agents through high densities or large per capita impact; both of which are known to vary at our site and elsewhere. At our site spotted knapweed in certain habitats (e.g., disturbed riparian areas) is unlikely to be eradicated by insect herbivory, but spotted 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.

Key words: biological control; *Centaurea stoebe*; Colorado, USA; herbivory; invasive species; matrix modeling; population dynamics; precipitation; spotted knapweed.

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¹ Present address: Department of Integrative Biology, University of Colorado, Denver, Colorado 80217 USA.

[†] **E-mail:** Anastasia.maines@colorado.edu

INTRODUCTION

Understanding the mechanisms for a small portion of nonnative, introduced plant species to

become invasive, dominant species in introduced habitats has been a challenging subject for decades (e.g., Davis 2009, Moles et al. 2012, Heger et al. 2013). Interpretation of the domi-

nance traits of the introduced species requires an understanding of how niche characteristics and fitness traits of the invading species (MacDougall et al. 2009) interact with the physical and biological constraints imposed upon this species by the preexisting community (Seastedt and Pyšek 2011).

Modest advances have occurred in our predictive capabilities regarding the likelihood of a specific species becoming invasive (e.g., Blumenthal et al. 2009). However, once a species has become regionally abundant, finding successful ways of manipulating the community structure in order to reduce the densities of the problematic invasive species remains a difficult and time-consuming task. A common approach is to add host-specific natural enemies from the native habitats and attempt to reduce the fecundity and survivorship of the target species to levels where ecological and economic concerns regarding the presence of the species are diminished. Recent summaries of these attempts note that these approaches range from negligible to remarkably successful (Clewley et al. 2012, Stephens et al. 2013, Suckling 2013). A key point is that most biological control efforts must play ‘catch-up’ with established plant populations, some of which are perennial, have established seed banks, and have crossed over the ‘invasion cliff’ (Davis 2009). Hence, demonstration of density reductions and control may require an extended period of time.

A recent meta-analysis demonstrates that most biological control efforts on problematic species involve multiple natural enemies (Stephens et al. 2013). About three-quarters of the studies show negative, independent effects of the agents, while a subset show that antagonistic effects among the enemies result in effects being less than additive. Field studies where biological control agents are allowed to vary through time as a function of interannual factors that affect both biological and physical conditions are useful in evaluating potential interactions, and are essential to document actual cumulative impacts (Seastedt and Pyšek 2011).

Spotted knapweed (*Centaurea stoebe* L. subsp. *micranthos* [Gugler] Hayek [Asteraceae]) is an appropriate and important model system to evaluate the efficacy of biological control in eradication. This regionally abundant invasive

plant was dubbed the “Wicked weed of the West” due to the negative effects in western North America (Alper 2004). In the effort to control spotted knapweed, thirteen different species of biological control insects have been introduced (Shea and Kelly 1998). Several of the introduced biological control agents for spotted knapweed have been shown to have additive negative effects (Knoche et al. 2010b) and have been released in multiple locations across western North America for more than 10 years (Crowe and Bouchier 2006, Story et al. 2006, Seastedt et al. 2007). Thus an analysis of the population-level response of spotted knapweed to the released biological control agents is a valuable opportunity to evaluate long-term outcomes of biological control.

Reports of the efficacy of spotted knapweed control with specialist insects appear contradictory. In the 10–30 years following the release of biological controls, spotted knapweed populations have declined while biological control populations have 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 populations exhibited a compensatory response adult mortality: replacement of dead adults with increased numbers of rosettes becoming adults. The result was that there was no net change in adult density despite an increase in adult mortality due to biological controls (Ortega et al. 2012). This finding suggests that seed reduction and increased mortality of adult plants might not be sufficient to overcome growth from juvenile plants. A further complication when interpreting results is that drought has been suggested as a contributing factor to the observed declines in spotted knapweed populations (Corn et al. 2007, Story et al. 2008) or as an alternative explanation instead of reductions due to 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 are useful tools for comparing species population dynamics in different populations (Shea et al. 2005) and to examine the effect of herbivory on plant populations (Louda and

Potvin 1995, Eckberg et al. 2012). Stochastic elements such as environmental variability and factors such as density-dependence can also be incorporated into matrix models (Shea and Kelly 1998). Sensitivity analyses show the significance of individual parameters for population growth (Shea and Kelly 1998, Caswell 2001, Emery and Gross 2005). The contribution of each transition to the overall population growth can inform which life cycle stages should be targeted in management plans (Shea and Kelly 1998, Hansen 2007). Thus matrix models are valuable tools for determining the best control methods for specific circumstances and to evaluate seemingly contradictory results (Hansen 2007).

Recently Buhle et al. (2012) demonstrated the relationship between proactive ‘removal efforts’ (e.g., mechanical or chemical control) and the rate of invasive species decline during eradication management. Interestingly, biological control agents are legally prohibited from use in eradication efforts in certain regions of the U.S. because of their assumed inability to eradicate the species (Larimer County, CO). Here, we tested if biological control agents could facilitate proactive eradication activities. Such a finding could be very useful in revising management policies in areas where eradication efforts are ecologically and economically feasible.

We evaluated population-level effects by integrating responses to biological controls and precipitation in individual stages into a matrix model to evaluate predicted outcomes under the various proposed causal scenarios (Emery and Gross 2005, Smith et al. 2005). We used results from experimental manipulations of spotted knapweed and long-term monitoring of seed production in a stage-based matrix model to determine the population-level responses to precipitation, biological controls, and the combination of these factors. We tested the hypothesis that the cumulative impact of biological controls on individual stages has population-level effects. If reduced survival and seed production are insufficient to substantially decrease reproductive success, survival, and growth of juveniles to adult stages, then spotted knapweed densities will not differ whether biological controls are present or not. Populations in the introduced range without biological controls tend to exhibit near-monocultures, with densities controlled by

intraspecific competition.

METHODS

Study system and site description

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 and Renney 1974, Sheley et al. 1998, Ortega and Pearson 2005). It is a perennial plant that can live up to 9 years, with single plants producing up to 25,000 seeds per year (Watson and 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 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 and Seastedt 2010). One of the most effective combinations of biological control insects for this plant has been *C. achates* and *L. minutus*: collectively these insects increase spotted knapweed plant mortality and reduce seed production (Corn et al. 2006, Crowe and Bouchier 2006, Knochel et al. 2010b; A. P. Maines, D. G. Knochel, and T. R. Seastedt, *unpublished manuscript*).

Field studies were conducted in Lefthand Canyon (40.07 N, 105.19 W, elevation 1810–1989 m), located just north of Boulder, Colorado. Spotted knapweed biological controls *L. minutus*, *C. achates*, and *Urophora* spp. have been present at the site since 2001 (Seastedt et al. 2007). Biological controls *L. minutus* and *C. achates* were released from 2001–2005 (Seastedt et al. 2007). The site was grazed prior to 2006, but grazing by cattle has not occurred at the study area since 2006 (Knochel et al. 2010a). Further description of the study site is reported in sources (Table 1). The common garden study was conducted at the University of Colorado at the Boulder greenhouse (Knochel et al. 2010b).

Matrix model structure

We created a simulation model in R (R Development Core Team 2012) to test whether conspecific density-dependence, the effects of biological controls, precipitation, or an interaction best explained observed spotted knapweed

Table 1. Description of parameters used in spotted knapweed population model.

| Parameter description | Symbol | Estimate | | Source |
|---------------------------------------|----------------------|-------------|------------|--------|
| | | BC absent | BC present | |
| Seed survival in seed bank (SB) | S1 | 0.08 | nd | 1 |
| Survival of rosettes (R) | S2 | 0.40 | 0.20 | 2 |
| Survival of adults (A) | S3 | 0.89 | 0.7 | 3; 4 |
| Number of stems plant ⁻¹ | <i>a</i> | 5 | 3.33 | 4 |
| Number of flowers plant ⁻¹ | <i>n</i> | 80.2 | 45.8 | 4 |
| Number of seeds flower ⁻¹ | <i>s</i> † | 10.8 | 2.37 | 5 |
| Viability of fresh seed | <i>V</i> | 0.84 | nd | 2 |
| Seed production | <i>F1</i> | <i>nsV</i> | nd | |
| Precipitation (cm) (mean with SD) | <i>p_t</i> | 35.2 (5.49) | nd | 6 |
| Germination rate | <i>G1</i> ‡ | 0.25 | nd | 2 |
| Growth of seedling (S) to rosette | <i>G2</i> | 0.065 | nd | 1; 2 |
| Growth of seedling to adult | <i>G3</i> | 0.0039 | nd | 2 |
| Growth of rosette to adult | <i>G4</i> | 0.133 | nd | 2 |

Notes: Estimates are when biological controls are absent (BC absent) or present (BC present). The abbreviation “nd” indicates no difference for the value in the biological control model. Values for precipitation are mean with SD in parentheses. Sources for estimates are: 1, Knochel et al. (2010a); 2, A. P. Maines, *unpublished manuscript*; 3, Sheley et al. (1998); 4, Knochel et al. (2010b); 5, Knochel and Seastedt (2010); 6, NOAA (2012). *F1* is calculated from values in the table.

† Value varies when precipitation effects are included in the model (Eqs. 3 and 4).
 ‡ Value varies when precipitation effects are included in the model (Eq. 7).

population dynamics. The biological model used to simulate spotted knapweed population growth was a stage-based matrix model with a yearly time step (Caswell 2001; Fig. 1). Matrix **A** contains four spotted knapweed life cycle stages: seed bank, seedlings, rosettes, and flowering adults (Fig. 1). This model was parameterized

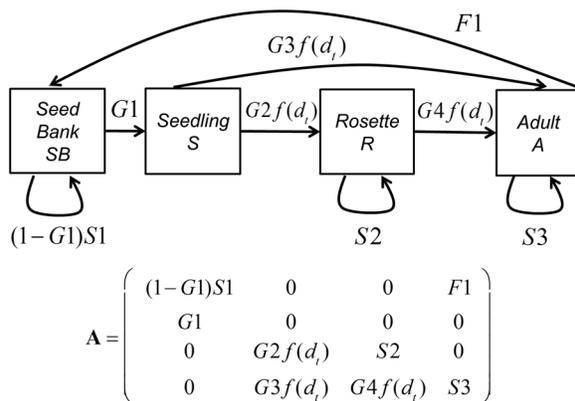


Fig. 1. *Centaurea stoebe* (spotted knapweed) life cycle diagram and projection matrix. Projection matrix columns correspond to life cycle stages and rows correspond to stages in year *t* + 1. Arrows represent transition probabilities from May of year *t* to May of the following year, *t* + 1. Parameters are probabilities of growth to next stage (*G1*–*G4*), survival in stage (*S1*–*S3*), and fecundity (*F1*). Growth is constrained by stem density, *d_t*.

for four different scenarios: (1) conspecific density-dependence only, (2) precipitation effects, (3) biological control effects, and (4) biological controls and precipitation effects. R code for the model is included in the Supplement.

All models included conspecific density-dependence, which reduced growth from early stage classes to the adult stage as conspecific stem densities, *d_t* densities reached a maximum. Stem density was the number of adults m⁻² in year *t*, *A_t*, multiplied by the number of stems adult⁻¹, *a*. Growth from seedlings to rosettes and from seedling and rosettes to adults was constrained by the stem density such that the growth probability decreased as stem density approached 120 stems m⁻²

$$f(d_t) = 1 - \frac{A_t a}{120}. \tag{1}$$

The model was constrained so that no juveniles (either seedlings or rosettes) became adults if the stem density exceeded the maximum.

Adult spotted knapweed plants often flower for multiple years. The number of adults in the next year, *A_{t+1}*, was calculated as the probability of surviving, *S3*, plus new adults growing from seedlings, *G4*, and rosettes, *G3*.

$$A_{t+1} = A_t S3 + R_t G4f(d_t) + S_t G3f(d_t) \tag{2}$$

The number of viable seeds produced by each adult, *F1*, was the product of the number of

flowers produced per adult, n , times the number of seeds flower⁻¹, s , and the germination potential of fresh seed, V (Table 1). When the effect of precipitation was included in the model, seeds flower⁻¹ was a function of October to June precipitation in cm, p_t , and the presence or absence of biological controls (Eqs. 3 and 4; D. G. Knochel et al., *unpublished manuscript*). Seed production in the absence of biological controls was:

$$s_p = 0.43p_t - 9.5 \quad (3)$$

and when biological controls were present:

$$s_{p,b} = 0.23p_t - 6. \quad (4)$$

Negative intercepts do not represent biologically reasonable values, thus when the effects of precipitation were included in the model, the number of seeds flower⁻¹ was constrained so that the minimum number of seeds was zero.

Seeds present in the seed bank, SB_{t+1} , were yearly produced seeds plus seed surviving in the seed bank, $S1$, minus germinated seeds, $G1$:

$$SB_{t+1} = SB_t S1(1 - G1) + A_t F1. \quad (5)$$

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 that germinated, $G1$:

$$S_{t+1} = SB_t G1. \quad (6)$$

For models including precipitation effects, germination probability varied with yearly precipitation. The probability of germination, $G1$, was multiplied by precipitation received in October to June in a given year, p_t divided by the average precipitation (35.2 cm) (germination was proportional to the proportion of average precipitation received):

$$G1_p = \frac{p_t}{35.2} G1. \quad (7)$$

Rosettes are non-reproductive plants that germinated in a previous season and survive in the stage or grow to flowering adults. The proportion of seedlings growing to rosettes was dependent on stem density. Rosettes in year $t + 1$ consisted of surviving rosettes, $S2$, plus growth from seedlings, $G2$:

$$R_{t+1} = R_t S2 + S_t G2 f(d_t). \quad (8)$$

Model descriptions

All models were parameterized with survival and transition estimates obtained from experimental studies in Colorado. Survival probabilities were estimated from the number of surviving individuals in a stage divided by the initial number of individuals. Transition probabilities were calculated as the proportion of individuals in a stage in year t that grew to the next stage in the following year. In the case of multiple mean values for a specific transition value (such as when plant competition was a variable within the experiment), the average of the reported values was used in the model. While some parameter estimates are averages from knapweed plants grown with and without plant competition, an explicit interspecific plant competition effect was not included in the models. Model parameter estimates and sources are summarized in Table 1.

The conspecific density-dependence (density) model was parameterized with transition and survival estimates from spotted knapweed populations in the introduced range (Table 1). The only control on population growth was conspecific density. Seed bank survival was determined from a germination test conducted on seed isolated from soil cores taken from five locations at the study site (Knochel et al. 2010a). This value was used under all scenarios, since biological control should not affect seed bank survival. The estimate for seeds flower⁻¹ was obtained from yearly surveys of seed heads at the field site (Knochel and Seastedt 2010). For the density model, the number of seeds flower⁻¹ was calculated from the average number of seeds when *L. minutus* and *Urophora* spp. were absent from the flower for all reported years (Knochel and Seastedt 2010). Other parameter estimates came from a common garden experiment where plants in enclosures had biological controls excluded or added at densities observed in the field (Knochel et al. 2010b) and from a field experiment where known densities of spotted knapweed seeds were sown and the resulting number of seedlings and rosettes were assessed (Knochel et al. 2010a; A. P. Maines, D. G. Knochel, and T. R. Seastedt, *unpublished manuscript*). The yearly seed bank germination probability was the average of estimates reported by Knochel et al. (2010a) and A. P. Maines, D. G. Knochel, and T. R. Seastedt (*unpublished manu-*

script). The estimate for the adult survival probability, S_3 , was based on a 9-year lifespan (Sheley et al. 1998).

Models with precipitation effects had seed production, F_1 , and germination, G_1 that varied with precipitation. Precipitation effects were obtained from analysis of 10 years of measurements of seeds flower⁻¹ against precipitation (D. G. Knochel et al., *unpublished manuscript*). The probability of seedling emergence increased with above average precipitation and decreased with below average precipitation. The data are inconsistent about the effects of precipitation on other survival and growth stages. Other results suggest that drought relief increased rosette mortality or has minimal effects on juvenile densities (Corn et al. 2007, Ortega et al. 2012), so no precipitation effects were included for growth or rosette survival. There is an interaction between precipitation and the effect of plant competition on spotted knapweed (Maron and Marler 2008); however, data do not indicate a correlation between precipitation and stem density or number of flowers (Ortega et al. 2012; T. R. Seastedt, *unpublished data*). Thus we did not include a precipitation effect for adults.

When the effects of biological controls were included, parameters were taken from spotted knapweed populations with biological controls present. Studies have shown that biological controls insects, *C. achates*, *L. minutus*, and *Urophora* spp. reduce rosette survival, S_2 , adult survival, S_3 , and fecundity, F_1 . Herbivory has been shown to reduce rosette survival, but has no effect on the rate of rosette growth to the adult stage (A. P. Maines, D. G. Knochel, and T. R. Seastedt, *unpublished manuscript*). The difference between adult mortality rates when biological controls were present or absent was subtracted from the 9-year lifespan (Knochel et al. 2010b; Table 1). We obtained the estimate for seeds flower⁻¹ when biological controls are present from the average the seed production for the two sites where biological controls were released (Knochel and Seastedt 2010). This value reflected overall attack rate as it included affected and unaffected heads (Knochel and Seastedt 2010). Biological controls also reduce the production of flowers plant⁻¹ (Knochel et al. 2010b, Knochel and Seastedt 2010; Table 1). The number of stems plant⁻¹ was smaller when biological controls

were present than when they were absent (Knochel et al. 2010b; Table 1). There is no difference for transition probabilities of growth of seedlings and rosettes to adults when biological controls were present or not. Herbivory may reduce seedling growth to rosettes (G_2), but this study did not include a control where herbivores were excluded, thus no effect was included in the model simulations (A. P. Maines, D. G. Knochel, and T. R. Seastedt, *unpublished manuscript*). Models that included biological controls assumed an insect population with consistent effects through time.

The interaction model included both effects of biological controls and precipitation. There was a significant interaction between the presence of biological controls and the effect of precipitation, thus seed production decreased when both precipitation effects and biological controls were included in the model (Eq. 4; D. G. Knochel et al., *unpublished manuscript*). In our model the abundance of biological control populations did not vary with precipitation, as we have not seen a precipitation effect on insect attack rates. The abundance of *L. minutus* and *Urophora* spp. in seed heads and the number of flowers stem⁻¹ has been monitored at the site since 2007 (D. G. Knochel et al., *unpublished manuscript*). During this period, the abundance of *L. minutus* was not correlated with yearly precipitation or growing season precipitation ($p = 0.61$ and 0.054 , respectively) nor was the number of flowers stem⁻¹ correlated with either measure ($p = 0.30$ and 0.12 , respectively; T. R. Seastedt, *unpublished data*). In general, as long as the plants can flower and initiate seed set, the seed head insects can persist. The abundance of the root weevil is not affected by the plant's ability to produce flowers. Due to these data, our model did not incorporate any variation in biological population due to precipitation.

Density measures

Model predictions were compared to spotted knapweed densities monitored in permanent transects established at our study site north of Boulder (40.07 N, 105.19 W, elevation 1810–1989 m). Stem and rosette densities have been monitored since 2007 (Seastedt et al. 2007; D. G. Knochel et al., *unpublished manuscript*). The number of stems m⁻² and rosettes m⁻² were

recorded yearly within sequential plots along a permanent transect. Sampling procedures are described in detail in Seastedt et al. (2007). Plant densities m^{-2} from the permanent transect were used as a starting point for model simulations, but were not used to calculate parameter values.

Model analysis

We calculated the dominant eigenvalues (λ) to estimate population growth under the four model scenarios. For models that included the effects of precipitation, we evaluated the intrinsic population growth (λ) at three levels: when precipitation was one standard deviation above average, equal to average, and one standard deviation below average. We did this by calculating lambda with matrix entries for *F1* and *G1* evaluated at the three levels of precipitation. We 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 from which the parameter estimate came. Density-independent lambda (λ) and 95% confidence intervals were calculated using the *popbio* package in R version 2.15.1 (Stubben and Milligan 2007, R Development Core Team 2012).

We also calculated transition elasticities 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 allow comparison of different scenarios (de Kroon et al. 1986), both of which were true for these models. We calculated density-independent elasticities using the *popbio* package in R (Stubben and Milligan 2007, R Development Core Team 2012). For models that included precipitation effects, we evaluated parameters at precipitation one standard deviation below average to predict how changes in parameters would impact population growth under reduced precipitation.

We conducted model simulations to compare population persistence under varying levels of flowering plant (adult) removal in each scenario. A suggested management practice is to remove adults prior to seed production (Sheley et al. 1998). We modeled this management practice under all four scenarios to determine the years of management at each level needed to eradicate

the invasive population (Buhle et al. 2012). We used 100 years to examine long-term outcomes. We conducted 1000 simulations of 100 years of control for management levels from 0 to 100% adult removal. Since this model simulates densities the values would never reach zero, so we chose a threshold under which we could consider the population to be eradicated. For the purposes of this model, the population was considered to persist if more than 1 adult plant m^{-2} was present. If all simulations for a management level did not predict control, then the mean time to control was not reported.

Finally, we compared model predictions in the four scenarios against population densities in the permanent transect at the field site. Transect data were not used to calculate transition or survival probabilities, but were used to calculate the error for negative log likelihood values (Hilborn and Mangel 1997). The actual precipitation in Boulder, Colorado during the data collection period was used to calculate predicted densities in models that included precipitation effects (NOAA 2012). We assumed normally distributed errors for both rosette and stem density measurements. We selected the best model based on Akaike Information Criterion adjusted for small sample sizes (AIC_c) and model weights (Anderson et al. 2000, Bolker 2008). AIC_c model weights show the relative value of each model in explaining the data compared to the other examined models (Anderson et al. 2000).

Start values for all simulations were the 2007 densities from the permanent transect: 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} (estimated from the 2006 seed production), 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 biological control scenario has a larger estimate for the number of adults due to the smaller estimate of stems $adult^{-1}$ when biological controls are present; the actual number of adults was not determined in the survey (Seastedt et al. 2007; Table 1). The model estimated many, small adults in the biological control scenario and a few, large adults when biological controls were not present. For future forecasts, yearly precipitation (cm) was randomly chosen from a normal

distribution with the mean and standard deviation of the 93-year October–June precipitation (cm) in Boulder, Colorado (NOAA 2012; Table 1).

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 and Seastedt 2010, Knochel et al. 2010a; b; A.P. Maines, *unpublished manuscript*). Confidence intervals overlapping or below zero growth ($\lambda \leq 1$) were projected only when biological controls were present (Fig. 2). While declines in growth rates ($\lambda < 1$) were only present under a low-precipitation scenario, the population was ‘controlled’ ($\lambda \leq 1$) when biological controls were present under all precipitation levels except above-average rainfall.

Elasticity analyses showed that biological control increased the importance of adult survival, S_3 , for population growth while decreasing the importance of seed production, F_1 (Fig. 3). However, none of the models predicted eradication in 100 years. The predicted number of stems in precipitation models had a mean and standard deviation because precipitation was randomly chosen from a normal distribution with a mean and standard deviation equal to that of Boulder, Colorado’s 93-year mean (NOAA 2012). When only conspecific density-dependence was included in the model, the spotted knapweed population reached 107 stems m^{-2} in 100 yrs, (which is essentially the density-dependent maximum created in the model). The mean predicted number of stems under the precipitation effects model was 98 (SD = 4.5) stems m^{-2} in 100 years. The predicted stem density in 100 yrs was substantially lower when biological controls were included: 5 (SD = 3.3) and 12 stems m^{-2} predicted when precipitation effects were included or not, respectively.

Due to the shift in importance to adult survival when biological controls are present, the efficacy of other management efforts could be increased. In the presence of biological controls, twenty years of actively removing 20% of adult plants annually will control the population. In their absence, it would take removal of 75% of adult plants annually to obtain the same result, assuming no interaction effect between the

management action (e.g., mechanical, chemical) and efficacy of insects (Fig. 4).

When we compared 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 (Fig. 5, Table 2). Models including the precipitation effects did not add to the predictive power of the model. The biological controls effects model (Biocontrol) had the smallest AIC_c values and a model weight of 93% (Table 2). The model including the effects of precipitation and biological controls was distinguishable from Biocontrol model since the difference between AIC_c 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 2).

DISCUSSION

This study is the first to compare the measured effects of biological controls and precipitation on spotted knapweed stages against observed population dynamics in permanent transects. These results support the contention that biological controls have contributed to the observed decline in spotted knapweed populations in Colorado, and support similar findings from 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.

Our 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, Rose et al. 2005, Eckberg et al. 2012, Stricker and Stiling 2012). Biological control contributes to spotted knapweed population-level effects directly through reducing spotted knapweed seed production and plant survival and indirectly by decreasing spotted knapweed’s ability to exploit favorable climatic conditions. The model predicts that biological controls *C. achates*, *L. minutus*, and *Urophora* spp. can contribute to long-term spotted knapweed population reduction.

Our results are also consistent with the finding

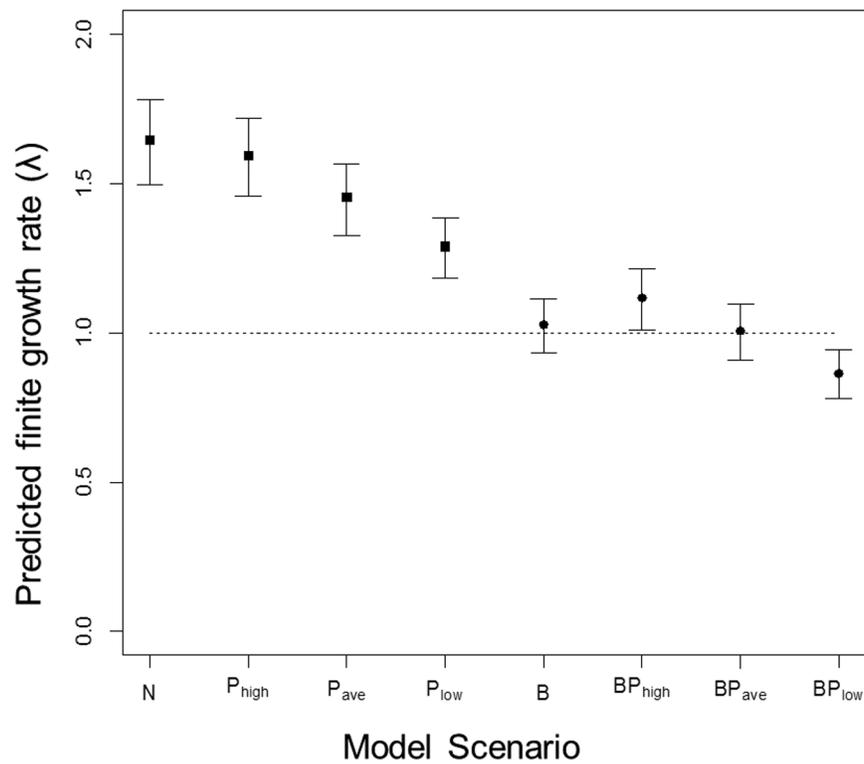


Fig. 2. Population growth rate estimates (λ values) with bootstrapped 95% confidence intervals. Estimates were calculated when the effects of biological controls are present (B) or absent (N) and when precipitation was included in the model (P). When precipitation was included, subscripts indicate level of precipitation: a standard deviation above average (high), average precipitation (ave), and a standard deviation below average (low). Dotted line indicates zero population growth ($\lambda = 1$).

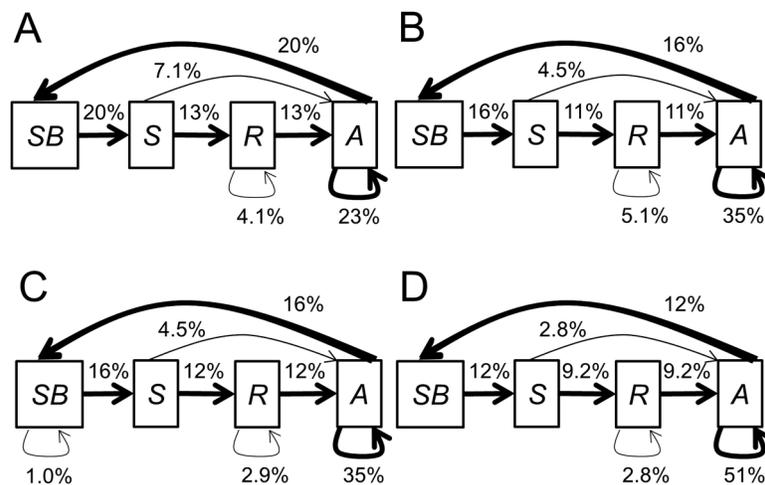


Fig. 3. Life cycle diagrams with elasticity analysis results for (A) conspecific density-dependence only, (B) precipitation (evaluated at low precipitation), (C) biological control, and (D) biological controls and precipitation (evaluated at low precipitation). Values above arrows indicate percent contribution of transition to overall growth rate. Elasticities $< 1\%$ are omitted. Arrows are bolded for elasticities $> 10\%$.

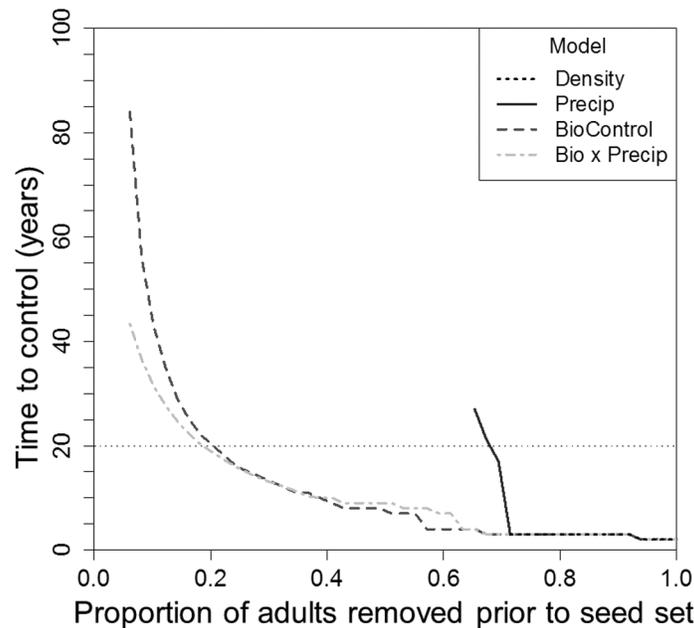


Fig. 4. Mean persistence time of spotted knapweed population under varying levels of adult removal. Percent adult removal is the percent of adult plants removed prior to producing seed each year. Models include the effects of conspecific density-dependence only (Density), precipitation (Precip), biological control (BioControl), and biological controls and precipitation (Bio \times Precip). All models converge at greater than 71% removal of adults. Average time to control was not included when all 1000 simulations did not predict control.

that spotted knapweed exhibits a compensatory response to low levels of adult mortality. In any scenario, low levels of adult removal can be compensated for at the population level. 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 more or larger individuals. However, negative impacts from herbivore addition can overcome initial advantages such as strong seedling establishment (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 spotted knapweed densities (Fig. 5). Thus, two observations appear warranted. First, the role of non-knapweed plant competition at relatively low spotted knapweed densities likely is more important than the values used in our model portray (cf. Pokorny et al. 2005). The second observation is

that the population growth rate estimated (λ 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. Plant competition is included implicitly in average values; however a valuable next step would be to include it explicitly in the model. In general, spotted knapweed cover is negatively correlated with background forb and grass cover (D. G. Knochel et al., *unpublished manuscript*). While the general trend is clear, the exact effects can be highly variable (Pokorny et al. 2005, Maron and Marler 2008, Knochel et al. 2010a). Our seeding experiments have shown a large range in seedling survivorship across sites (0–11.2%; A. P. Maines, D. G. Knochel, and T. R. Seastedt, *unpublished manuscript*). Pokorny et al. (2005) and Knochel et al. (2010a) demonstrated that the success of spotted knapweed seedlings decreases in the presence of plant competition; however, the strength of the effect varies with the type of plant competition sown or present. Furthermore,

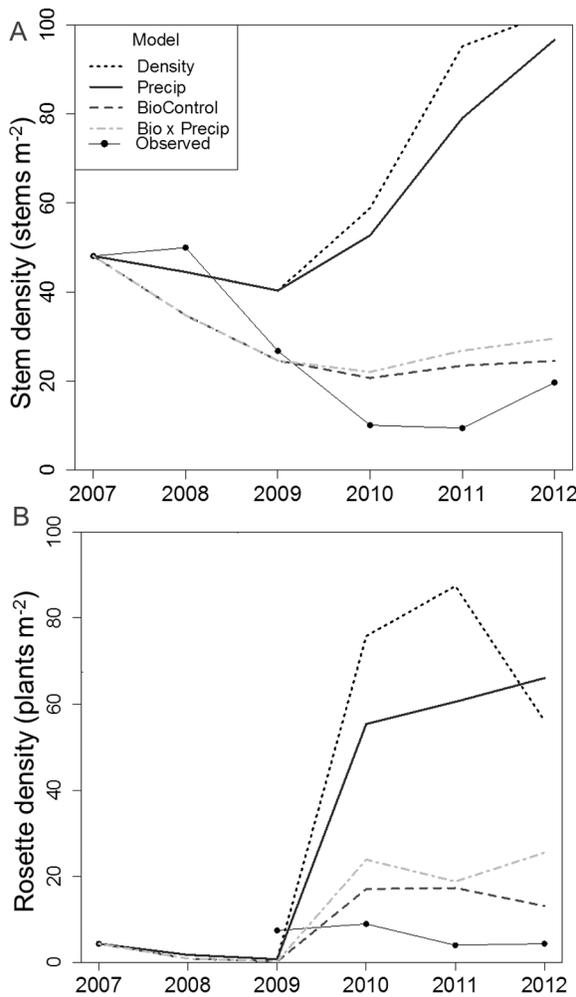


Fig. 5. Model predictions against observed densities for (A) stems m^{-2} and (B) rosettes m^{-2} . Observed densities are from permanent transects (D. G. Knochel et al., *unpublished manuscript*). Models include the effects of conspecific density-dependence only (Density), variable precipitation (Precip), biological control (BioControl), and biological controls and precipitation (Bio \times Precip).

there are interactions between resource variability, such as precipitation, and the competitive effects of non-knapweed plants (Knochel and Seastedt 2010; A. P. Maines, D. G. Knochel, and T. R. Seastedt, *unpublished manuscript*). When precipitation increases, the competitive impact of plant competition can decrease (Maron and Marler 2008, Ortega et al. 2012). Thus a next step to increase the accuracy of this model would be to include non-knapweed plant cover in the model.

Variability in precipitation does not explain the observed reduction in spotted knapweed densities from 2007–2012. Our results demonstrate that the recent climatic conditions would have slightly depressed the population in comparison to a conspecific density-dependence. In Montana, spotted 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 spotted 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 amplify 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. 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. 2010b, Eckberg et al. 2012). Insect dynamics could result in varying intensity of insect attack rates from year to year. 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.

Table 2. Analysis of model predictions against stem and rosette densities observed in permanent transect.

| Model | n | K | NegLogLik | AIC _c | Δ AIC | w |
|-----------------------------------|-----|-----|-----------|------------------|--------------|---------|
| Density | 9 | 10 | 49.4 | 38.9 | 31.5 | <0.0001 |
| Precipitation | 9 | 10 | 47.9 | 35.8 | 28.4 | <0.0001 |
| BioControl | 9 | 10 | 33.7 | 7.42 | 0.00 | 0.93 |
| BioControl \times Precipitation | 9 | 10 | 36.3 | 12.7 | 5.3 | 0.066 |

Notes: Estimates for negative log likelihoods (NegLogLik) and Akaike Information Criterion (AIC). Table includes the sample size (n) and the number of parameters for AIC_c calculations (K). For each model we include the difference between the model and the model with the smallest AIC_c value (Δ AIC) and the model weight (w).

Knoche et al., *unpublished manuscript*). Biological impacts could also 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 the limitations of the model, model predictions mimicked the general trend in the monitoring data. Densities under a biological control scenario were substantially lower than 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. Furthermore, 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 (Fig. 3). 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.

Weed managers are under substantial pressure from stakeholders and vested interests to rapidly reduce the ecological and economic impacts of regionally abundant invasive species. Findings such as ours demonstrate that a regionally abundant species can be controlled by biological control agents, but perhaps only in a subset of habitats and over an extended time frame. Our study joins those that support the contention that biological controls have contributed to the observed declines in spotted knapweed populations. Our findings also show that biological control agents have the ability to speed up the

more costly, proactive management activities necessary to eradicate large populations of invasive plants in those areas where such management is warranted.

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SUPPLEMENTAL MATERIAL

SUPPLEMENT

R script file to run spotted knapweed density model simulations for each scenario ([Ecological Archives C004-009-S1](#)).