Population-level compensation by an invasive thistle thwarts biological control from seed predators

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Abstract. Pre-dispersal seed predators are often chosen as biocontrol agents because of their high impacts on plant fitness; however, they have a mixed record in realizing decreased plant population growth. Few studies have experimentally removed agents to explore their impact on weed population growth. Here, we used manipulative experiments with invasive yellow starthistle (YST), Centaurea solstitialis, and its pre-dispersal seed predator biological control agents, primarily Eustenopus villosus, the hairy weevil, and Chaetorellia succinea, the false peacock fly, to explore how these agents affect population growth of YST. We also use additional seed augmentation experiments to mimic effects of agents on seed inputs across a range of seed and adult plant densities.

We found that biocontrol agents reduced seed production by 70% and that seedling numbers were significantly related to seed inputs. However, several compensatory processes prevented effective population reduction of YST by seed predators. First, self-thinning reduced seedling numbers such that densities of plants in our agents-present and agents-absent treatments converged. Second, plots in which plants started at low density had particularly high population growth rates. In this case, plant plasticity and conservation of final yield, in which a small number of large plants produce as much seed as a large number of smaller plants occupying the same area, also provided avenues where plant populations can compensate for damage. Similarly, seed production on a per-plot basis was unchanged across a large range of YST densities. Our results suggest that at very low plant densities, biocontrol agents may reduce plant populations; however, other sources of mortality to YST (preferably imposed after self-thinning) will be needed to reduce populations to sizes where agents can become effective tools in weed control.

Key words: biological control; biological invasions; Centaurea solstitialis; compensation; constant final yield; fitness; lambda; pre-dispersal seed predation; seed limitation; self-thinning.

INTRODUCTION

Understanding the interactions between natural enemies and plant population dynamics is imperative for successful biological control of invasive weeds. Much of the theory underlying biological control of plants assumes herbivory or disease limits population growth. However, evidence suggests that reductions in individual fitness do not necessarily translate into reductions in population size or lambda (λ) (Harper 1977, Andersen 1989, Crawley 1989). Pre-dispersal seed predators are a common choice for biological control agents, but these seed feeders have generally had a low rate of success in controlling invasive plants (McFadyen 1998). Despite agent establishment and high levels of seed destruction, agents often have little impact on plant population size.

Several modeling efforts have addressed the demographic impact of biological control agents. These models are aimed at determining most effective means of control, either by identifying the most important life stage contributing to λ, or by examining the projected effects of a number of management strategies (Rees and Pyaynter 1997, Shea and Kelly 1998, 2004, McEvoy and Coombs 1999, Shea et al. 2005). Despite attention from modelers, however, relatively few empirical studies have taken an experimental demographic approach and manipulated the presence of agents to assess how impacts on individual plant fitness and population growth are linked (but see Lonsdale and Farrell 1998, McEvoy and Coombs 1999, Shea et al. 2005). Only such approaches can relate damage done by biological control agents to individual plants to population-level impacts.

While seed predators can have large impacts on seed production, compensation for seed loss through plant plasticity and density-dependent processes are potential explanations for the high percentage of failed biological control agents (Myers et al. 1988, Cullen 1996, Crawley 2000). Several empirical studies have shown that seed loss to invertebrate pre-dispersal seed predators failed to decrease plant population size because of microsite limitation (Louda 1983, Duggan 1985) or reproductive


Seed limitation is the most commonly studied link between seed number and population size. At the population level, seed loss will only limit populations if seed input is reduced below the adult carrying capacity because density-dependent seedling mortality can compensate for differences in seedling densities. Otherwise, seed predators are only eliminating surplus seeds that are not needed for maintaining population density (Harper 1977, Crawley 1990, 1997). In several nonexperimental studies, models built from population censuses predict that self-thinning offsets the effects of significant seed loss (Myers et al. 1988, Andersen 1989, Hoffman and Moran 1991, Kelly and McCallum 1995, Myers and Risley 2000); however, the role of self-thinning as a countering effect to seed predation has not been explored experimentally.

Finally, plant plasticity may provide compensation for the effects of seed predators on a per area basis through a phenomenon termed constant final yield. Such plasticity due to plant size hierarchies has the ability to equalize seed contributions across a wide range of plant densities. Thus, even if pre-dispersal seed predation reduces adult density, seed outputs between sparse and dense populations may be comparable. Plasticity, therefore, may contribute to unlinking individual fitness (number of seeds produced per individual) from population growth, lambda.

Here, we examine the efficacy of biocontrol agents by exploring these multiple linkages between individual fitness and population size using the experimental removal of biocontrol agents. Despite the large number of studies focusing on biocontrol by seed predators, several issues still need to be addressed: (1) In annual species, how does individual seed production in one generation translate into population densities in the next generation? (2) How does adult density affect reproductive output on an individual and per area basis? (3) Can population-level compensation and individual plasticity negate seed loss imposed by biocontrol agents? Answers to these questions are critical to assessing the value of seed predators as biocontrol agents. This study addresses these gaps by examining yellow starthistle (Centaurea solstitialis), an annual invasive weed, and its suite of introduced biocontrol pre-dispersal seed predators. We assess the effects of seed destruction by biocontrol agents on the viability of yellow starthistle populations through an herbivore exclusion experiment designed to detect compensatory responses, and a seed augmentation experiment that examines the extent of seed limitation, and simulates seed inputs prior to the establishment of biocontrol agents.

**STUDY SYSTEM**

*Centaurea solstitialis* (Asteraceae), yellow starthistle (YST), is an annual noxious weed that invaded California ca. 1860, probably from shipments of contaminated alfalfa seed (Gerlach 1997). YST is a winter annual that germinates after the first winter rains in California’s mediterranean climate. It grows slowly as a rosette until the early spring when it begins to bolt (Maddock 1981, Roche and Thill 2001). Flowering occurs during the dry hot summers where YST is one of the few annuals in the Central Valley that is still alive (Dyer and Rice 1999; J. M. Garren, personal observation). Capitula (seed heads) produce between 30 and 80 seeds each ( Benefield et al. 2001) with the number of seed heads per plant ranging from 1 to 3400 seed heads (Thomsen et al. 1996), with most plants producing <100 heads. Although 70–80% of seeds in the soil germinate, desiccate, or decay in the first year, it can take up to four years to deplete the seed bank (Joley et al. 1992, 2003, Benefield et al. 2001).

Biological control agents that attack the developing seed head were released to combat the spread of *C. solstitialis* in the 1970s through the 1990s. The two most abundant and effective agents are *Eustenopus villosus*, the hairy weevil, and *Chaetorellia succinea*, the false peacock fly; a third fly, *Urophora siranaseva*, is also established at our sites (Baliunas and Villegas 1999). Combined, these agents are reported to decrease seed production by 40–100% per head (Pitcairn and DiTomaso 2000). Despite the large amount of seeds destroyed, these insects may not significantly reduce yellow starthistle populations (Turner and Fornasari 1992, Sun and Ritland 1998, DiTomaso and Gerlach 2000).

To assess the degree of seed limitation and the efficacy of seed predators in reducing plant population size, we conducted experiments in two field sites over two years. An herbivore exclusion experiment in which seed
predators were removed took place at the Putah Creek Riparian Reserve on the University of California–Davis campus. A seed augmentation experiment took place in the Capay Valley on a privately owned fallow field located in Brooks, California. This agricultural field was last cultivated in 1998 and has been relatively undisturbed since fallow. On average 70–80% of seed heads per plant are infested with one or more of the insect agents (J. M. Garren, unpublished data).

Typical of highly variable Mediterranean ecosystems, the two seasons in which we conducted the experiments differed markedly. In 2003–2004, while there was below average rainfall overall, 87% of the precipitation occurred over a short time period in December and February and resulted in flooding of the field site. In 2004–2005, precipitation patterns were about average, but a severe heat wave caused temperatures between 37° and 39°C over an extended 10-day period.

**Materials and Methods**

**Experimental design**

*Herbivore exclusion.*—To determine the effects of seed predators on seed production, plant demography, and population size, we set up 50 plots, each a 3.5 m radius circle, in the spring of 2004. The plots consisted of a target population in (A) a 1 m diameter central circle, (B) a recruitment ring 1 m wide, and (C) a buffer zone 2 m wide (Fig. 1). Plots were paired by initial density of rosettes, and the treatments (sprayed with insecticide or water) were randomly assigned within each pair. Plant densities in these plots were not manipulated and ranged from 2 to 60 plants per plot. When treatment application began, the flowering plant densities did not differ between treatments (insecticide, 22.8 ± 3.3 plants/plot; control, 23.4 ± 3.0 plants/plot; t = −0.13588, df = 24, P < 0.89). Ortho Systemic Insect Killer (formerly Isotox; The Ortho Group, Marysville, Ohio, USA), an insecticide that does not affect pollinator behavior (Louda 1982a, b; see data below), was sprayed at a concentration of 2 fl oz/gallon (15.6 mL/L), as recommended by the manufacturer, on target populations in the inner circle (A) of plots when plants started flowering; control plots received a water spray as a spray control. Treatments were applied every two weeks while YST was flowering. Limiting our insecticide treatments to the flowering months had two advantages: (1) YST populations experienced the majority of growth and development under natural conditions with no spray, and (2) the indirect effects of insecticide spray (release of competitors from insects, for example) were expected to be minimal because other plant species had already completely senesced when spraying was initiated.

To determine any direct effect of the insecticide on growth or reproduction, a separate experiment was conducted in controlled environmental chambers. Forty YST plants were grown from seed in 30.5-cm tree pots for seven months under temperature conditions following those taken from a Davis weather station and adjusted monthly; insecticide and control treatments as before were applied biweekly for the last 10 weeks during flowering. Because YST is a pollinator-dependent obligate outcrosser (Maddox et al. 1996), each plant was hand-pollinated inside the chamber. Plants were paired based on height before treatment application, and a paired t test was used to compare height and seed production of sprayed and unsprayed plants. We found no evidence of direct effects of insecticide on either height or head production (insecticide, 56 ± 2.6 seed heads/plant, mean ± SE; control, 58 ± 4.8 seed heads/plant, t = 0.3995, P = 0.6940; height, insecticide, 76 ± 2.1 cm, control, 75 ± 2.5 cm, t = 0.1051, df = 19, P = 0.9174, α = 0.05, β = 0.8).

To examine whether there were effects of insecticide on pollinators, we conducted pollinator observations in the field in all of the sprayed and unsprayed plots after each insecticide application. We randomly chose 10 plots per treatment after each insecticide application (only five per treatment for the last spray because plots with flowers were scarce) and observed pollinator visitation. We recorded the behavior of single pollinators as they foraged in plots, counting numbers of
flowers visited and duration of visit per flower. When the first pollinator left, we followed the next one to enter the plot. In order to test the effects of the insecticide on pollinator behavior, our nested ANCOVA model included available flowers per plot as the covariate plus date, treatment, and plot (treatment) as fixed factors. There were no effects of spray on either the number of pollinators visiting plants or on the duration of visits to flowers (N = 70 visits, all values expressed as least square mean ± SE, insecticide, 4.2 ± 0.86 pollinators/observation; control, 3.9 ± 0.81 pollinators/observation; total visitation time, insecticide, 254 ± 33 s, control, 264 ± 35 s; Appendix C). This result is consistent with those of Louda (1982a, b), who also showed no effect of this insecticide on pollinators.

To determine the effect of biocontrol agents on population dynamics of YST, we compared recruitment dynamics in our different treatments. Recruitment and buffer rings outside each inner circle of plants (Fig. 1B, C) were created for sampling in the next generation (2005). In these areas, we cut back all YST, using a weed whacker, in early summer after flowering began, but before seed set (Appendix B). After the initial clearing, we hand-sheared new recruits and new shoots to maintain an environment free of yellow starthistle reproduction in the 3 m wide zone (Fig. 1B, C, 2004). Again, because YST begins flowering much later than almost all other species, the majority of other plants had completed flowering and reproduction by that time. We chose to remove only flowering stems and not whole plants to minimize disturbance or alteration of recruitment conditions. By eliminating all YST reproduction in these areas, we hoped to create relatively isolated experimental populations whose dynamics were independent. A 3-m buffer zone was expected to minimize outside seed input because 95% of YST seeds fall within 1 m of the parent plant (Roche 1992). Results suggest that we were successful in preventing extensive exchange of seed propagules across the experiment (see Results).

In 2005, the first recruitment ring filled with reproducing plants from year 1, and our target population increased in size from 1 to 3 m in diameter; we extended the buffer ring 3 m outside of the initial recruitment ring (Appendix B).

To determine the effect of biocontrol agents on seed production, we conducted head dissections. Because we did not want to influence plot reproductive output extensively, we used limited destructive sampling within plots. We limited head removal to 30 randomly selected senesced seed heads from each treatment once each month during reproduction; four plots with fewer than 35 heads were only sampled once (one head). Senesced seed heads were selected within numbered partitions of each plot using randomly generated numbers. All heads were dissected to assess infestation rates and seed production.

Because we did limited destructive sampling of our experimental plots, we created additional experimental plots that we could sample destructively. We ran two transects in the same area of Putah Creek where our plots were; along each transect, plants were randomly chosen every other meter and treated with insecticide or water in the same manner as the experimental plots. There were a total of 30 insecticide and 30 control plants. These transect plants were visited twice a week for 15 weeks during the summer; senescing heads were processed by recording the week in which each head senesced, measuring the capitula width, and bagging the head to capture seeds. Attack from agents occurs before or during flowering, so bagging after flowering did not affect infestation rates. Once all the heads were bagged and plants had senesced, plants were brought to the laboratory for head dissection. All heads were dissected in plants with 20 heads or less; otherwise, 20 heads/plant were randomly chosen for dissection. Upon dissection, agent presence or absence was recorded along with viable seed number (N = 426 insecticide heads, N = 383 control heads).

The transect plants provided detailed information on infestation rates and seed production on a weekly basis at the site. Data from dissections from these plants were used to calculate mean viable (filled) seeds per head per week for infested and intact heads throughout the season. Contrary to other studies (Pitcairn et al. 1998, Enloe and Spencer 2004), the capitula width was not a good predictor of viable seed production in this study; thus, we used alternative methods to estimate seed production.

Ultimately, we used these estimates of seed production on a weekly basis in transect plants to calculate total seed outputs of our experimental plots. Our assumptions are that the plants in the transects receiving a treatment behaved similarly to plants in plots with the same treatment. Because these plants were located in the same site, we are comfortable with these assumptions.

To calculate our per-plot seed output required several steps. First, we calculated the average proportion of seed heads senesced per week (of the total seed head production). We also calculated the weekly average proportion of infested and intact heads produced by plants in each treatment. We then applied these proportions from the transect plants to the total number of heads produced per plot to calculate the number of infested and intact heads per plot on a per week basis. Next, we calculated the number of seeds produced per week by multiplying the average number of seeds per infested or intact head by the number of heads of that type produced each week per plot. Weekly calculations were summed to estimate total seed production per plot. Because both seed head production and insect infestation rates change through the season, we feel that this approach gives a good estimate of total seed production.

In 2005, seed production per plot was calculated in a similar, but simpler, way: estimates were based on monthly averages from dissections of heads from plots, rather than weekly measurements. We used head
dissections from plots because individual plants were not sprayed for destructive sampling in this year.

To examine seed quality, we weighed a subsample of seeds from the different treatments and also performed germination trials to examine the effects of treatments on seed quality. Average mass was calculated using five seeds (if available) from each head for three randomly chosen heads from each plant in one transect (29 plants total, one destroyed during the season). Percentage germination was determined when these same 15 seeds per plant were planted separately in plug trays in the greenhouse. ANOVA models were used to detect differences between treatments for both average mass and percentage germination using treatment and plant nested within treatment.

Seed rain throughout the plot and recruitment areas was monitored with seed traps. Seed traps were modeled after traps used by Roche (1992), except we used 10.2-cm PVC pipe, instead of coffee cans, for added stability. Traps sampled an area of 81 cm². A screen funnel was attached to the top to reduce seed predation, and clear vinyl was attached to the bottom for visibility using duct tape. Five traps were placed on the ground in each plot. One was placed in the middle of the target population, two rays extending north and east from the center (to capture seeds dispersed by the prevailing winds from the south and west) had two seed traps each; one was placed on the target population’s edge (0.5 m from center) and the other was placed 1.5 m from center at the beginning of the buffer ring. Seeds found in the traps were counted and returned to the system.

Individual seed head production was monitored in the experimental plots during both years. Seed heads were counted on five plants in each plot in the summer of 2004 and six plants were monitored in the summer of 2005. These individual head counts gave us insight into average reproduction per plant and proportion of reproductive plants at the end of the season.

Permanent subplots were set up in each plot just after germination to monitor YST recruitment into the experimental plots. Subplot densities were censused monthly between November 2004 and July 2005 in six 20 × 20 cm subplots: one in the center target ring, three in the recruitment ring (north, east, southwest), and two randomly assigned in the buffer ring. The buffer subplots were included to assess buffer effectiveness in reducing seed inputs from surrounding YST, our target areas, as well as to assess inputs from the seed bank. Seed heads were counted in two randomly placed quadrants spanning the width of the recruitment ring (1 × 0.5 m).

We estimated lambda for each plot and compared population growth rates in the experimentally imposed presence and absence of the biocontrol agents.

Seed augmentation experiment.—To test whether seed limitation occurs for YST, and to examine the potential for biocontrol agent efficacy through seed destruction, we added locally collected seed to plots at varying densities at our Capay Valley field site. YST seeds were collected from the field site in the summer of 2004. Thirty 0.5 × 0.5 m plots were randomly placed in the fallow field and seed heads per plot were counted. These preliminary counts gave us an estimate of local reproductive output and site quality. We used these data to create three pretreatment (initial) head-density ranges: low, 0–10; mid, 30–60; high, 80–110 head/m² plot. We also used the initial head density as a covariate in our analyses. Head density and infestation rates from the previous year (data not included) were used to estimate seed rain per area. We used these calculations as a guide in choosing our treatments. A total of 110 0.5 × 0.5 m plots were located as follows: 40 plots in the low range, 40 plots in the mid-range and 30 plots in the high range.

Seed addition treatments were applied after the first rains in 2004 across the three density ranges. For the treatments we distributed either 1000 seeds, 3000 seeds, or no additional seeds (control) to each plot. Seeds were locally collected outside of the experimental area. In the end, we had a total of 60 control plots (20 plots in each head-density range), 20 plots with 1000 seeds added (10 plots in the low and mid-ranges), 30 plots with 3000 seeds added (10 plots in each fitness range).

Seedling density was monitored using two permanent randomly placed 10 × 10 cm subplots once a month from December 2004–April 2005. Adult density and reproductive output per plot were recorded in August of 2005.

Statistical analysis

Infestation rate and buffer effectiveness.—Infestation rate was calculated as the percentage of heads dissected harboring one or more of the biological control agents within plots.

To examine whether our buffer was effective in isolating seed deposition across plots, total seed trap capture and November 2004 seedling densities (center, recruitment ring, and buffer ring) were compared between treatments using two-way ANOVA with treatment and distance from center as fixed factors.

Impacts of agents on seed production and later life stages.—Head production, viable seed production per plot, seedling density, and adult density were compared between treatments using MANCOVA. Treatment was the predictor variable, and pretreatment plant density in each plot was used as a covariate to control for variance due to possible differences in site suitability. For seedling (November census) and adult plant density (July census) comparisons, the average density from the recruitment ring subplots was used as the response variable. In 2004, two outliers from the insecticide treatment had extremely high head and seed production. These plots were removed from the analysis to meet the assumption of normality. Results do not qualitatively change when the plots are included in the same analysis or in nonparametric tests. Because the MANCOVA was significant, univariate ANCOVAs were also done to
ment head-density range not only tells us about seed inputs, but may also serve as an indicator of site suitability and potential carrying capacity. Reproductive output per plot between treatments was compared using the nonparametric Kruskal-Wallis test, owing to strong non-normality of the data.

**RESULTS**

**Herbivore exclusion experiment**

Infestation rate and buffer effectiveness.—As expected, infestation rates of seed heads decreased in insecticide plots; in 2004, 71% of dissected control heads were infested with one or more agent but only 7.6% of insecticide-treated heads were infested ($N = 283$). In 2005, infestation in insecticide heads increased to 28% ($N = 165$); however, this was not due to an overall increase in insect abundance, as control infestation levels remained at 71%. This increase in infestation level of sprayed plants appeared to be due to an increase in densities of *E. villosus*, the weevil, which may be less vulnerable to the insecticide. This weevil was responsible for the majority of infestations in insecticide heads in both years. Only 8% of infestations in insecticide heads were by *C. succinea*, the false peacock fly, while 37% of control heads contained evidence of the same biocontrol agent. Overall, the presence of biological control agents decreased seed production by 79% per head in 2004 and 71% in 2005 compared to insecticide-treated heads.

The buffer treatment of removing all flowering stems of YST was successful in isolating our target populations. Control treatments and distance from center both significantly decreased the number of seeds captured in traps (treatment, SS = 15.3, $F = 19.40$, df = 3, 146, $P < 0.0001$; distance, SS = 135.5, $F = 85.7$, df = 3, 146, $P < 0.0001$). Seed capture in insecticide plots was 117% more than in control plots (Fig. 2). Seed capture decreased as distance from the center increased with an average of <1 seed/trap (81 cm$^2$) in the buffer zone. There was no detectable difference between treatments in the buffer areas (Fig. 2), seed densities were very low in buffer areas, and strong differences in seed numbers were present between treatments in the inner rings. Therefore, seed dynamics between plots can be viewed as independent.

Agent impacts on seed production and later life stages.—The MANCOVA examining the effects of biocontrol agent exclusion on the density of heads, seeds, seedlings, and adults was significant (Wilks' lambda = 0.18, $df = 84$, $P < 0.0001$); these results were explored further using univariate tests. Agent exclusion significantly increased seed production per plot by 98% in 2004 and 88% in 2005 (Table 1). Analysis with 2004 seed production per plot is reported with two outliers removed; however, the results were qualitatively similar in nonparametric tests with outliers included.

An increase in seed number could be compromised by a decrease in seed quality, but we observed no differences between treatments in average seed mass.
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Table 1. ANCOVA of effects of excluding seed predators with insecticide in experimental plots on the production of seed heads per plot and number of viable seeds/m², and on subsequent seedling density (20 cm²), adult density (20 cm²), seed head density, and seed output/m² in the next generation of Cenntera solstitialis.

<table>
<thead>
<tr>
<th>Response</th>
<th>Treatment</th>
<th>Initial adult density 2004</th>
<th>Insecticide</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SS</td>
<td>F</td>
<td>P</td>
<td>SS</td>
</tr>
<tr>
<td>2004</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed head density†</td>
<td>7024</td>
<td>0.262</td>
<td>0.61</td>
<td>453,933</td>
</tr>
<tr>
<td>Viable seeds†</td>
<td>230,347,799</td>
<td>25.33</td>
<td>&lt;0.0001</td>
<td>107,844,805</td>
</tr>
<tr>
<td>Seedling density†</td>
<td>1379</td>
<td>10.41</td>
<td>0.002</td>
<td>1277</td>
</tr>
<tr>
<td>2005</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult density‡</td>
<td>0.006</td>
<td>0.054</td>
<td>0.82</td>
<td>0.064</td>
</tr>
<tr>
<td>Seed head density</td>
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<td>0.169</td>
<td>0.68</td>
<td>29,160</td>
</tr>
<tr>
<td>Viable seeds‡</td>
<td>30,101,075</td>
<td>6.31</td>
<td>0.016</td>
<td>7,757,631</td>
</tr>
</tbody>
</table>

Notes: Initial adult plant density was included as a covariate. Degrees of freedom are as follows: treatment, 1; initial adult density 2004, 4; error, 47. There was no difference in initial adult densities in 2004 when insecticide application began.
† Reported ANCOVA results and means are with outliers removed; df = 2, 45. Results were qualitatively the same when outliers were included in Kruskal-Wallis tests.
‡ Adult density was log-transformed to meet assumptions. Raw means are reported.

(insecticide, 1.69 ± 0.06 mg, mean ± SE; control, 1.74 ± 0.05 mg; F = 1.09, df = 28, 58, P = 0.38). Germination rate was also consistent between treatments. Insecticide seeds had an average germination rate of 74% while control seeds had an average rate of 75% (F = 0.69, df = 28, 58, P = 0.85). Overall these data suggest that only seed number, and not seed quality, was affected by seed predators.

Seedling densities at emergence in November 2004 were 45% greater in the insecticide plots than in those experiencing attack from biocontrol agents. Therefore, seed limitation does affect seedling populations, and agents significantly reduce populations of seedlings (Table 1). Seedling densities also decreased dramatically as distance from the plot center increased; indicating that seed deposition was mostly contained within our plots (treatment, F = 12.13, df = 1, 146, P = 0.0007; distance, F = 37.4, df = 1, 146, P < 0.0001; Fig. 2). Overall, there was a strong correlation between seed input in the fall and seedling numbers the following winter across all areas (insecticide > control, center > recruitment ring > buffer; Fig. 2). Therefore, seedlings arising from the seed bank are few, and do not overwhelm the seed density to seedling density relationship predicted from seed inputs.

Despite large differences in seedling densities in our treatments, densities of adult plants were not significantly different in plots with and without agents (control, 3.35 ± 0.56 adults/subplot, mean ± SE; insecticide, 4.07 ± 0.79 adults/subplot; P = 0.18, α = 0.05, β = 0.94, analysis using log-transformation; Fig. 3, Table 1). Plant densities slowly converged each month and were no longer different between treatments in February (F = 3.2, df = 1, 48, P = 0.08; Fig. 3). Therefore, the presence of biocontrol agents decreased seedling densities but did not affect adult densities. Head production per plot was not significantly different between insecticide and control treatments in either year (Table 1); thus, no significant reproductive compensation to herbivory through increased head production occurred.

We find that biocontrol agents do not significantly change the direction or speed of population growth, despite having huge effects on seed numbers over most of the range of densities in YST populations. Lambda was not significantly different between treatments (Kruskal-Wallis, χ² = 0.17, df = 1, P = 0.68, median λ for insecticide = 2.6, control = 2.4).

Seed-to-seedling transition: seed limitation.—Regression analysis of 2004 seed production per square meter data and 2004 mean seedling density per plot revealed positive linear relationships for each treatment (Fig. 4B). Regression analysis with values of both treatments combined revealed a significant quadratic relationship (Fig. 5B). Seedling recruitment becomes saturated at 6500 seeds/m², at which point recruitment becomes limited by factors other than seed availability (Appendix A). Eleven of our control plots have seed production of this magnitude or greater, suggesting that there is a large range of YST density over which seed destruction by biocontrol agents has little impact. These results are also

![Figure 3](image-url)

FIG. 3. Effect of excluding pre-dispersal seed predators on plant density: mean plant density in 20 × 20 cm subplots (±SE) from November 2004 (seedlings) to July 2005 (adults).

* P < 0.05.
supported by the results of our seed augmentation experiment.

Seedling-to-adult transition: self-thinning.—There was no significant correlation between seedling and adult densities when analysis was conducted on treatments separately (Fig. 4C) or when combined (Fig. 5C); these results are the same whether or not adult data are log-transformed. Hence, compensatory processes like self-thinning remove the relationship between seed and adult population size, obviating the numerical effects of biocontrol agents on seeds (Appendix A and Fig. 5C).

Adult production to seed production transition: constant final yield.—In 2004 and 2005 we found an overall positive linear relationship between adult density and seed head production (Fig. 5A, D) when treatments were combined. However, when treatments were regressed separately, results differed between treatments and years (Fig. 4A, D).

In 2004 there was no significant relationship between adult density and seed production in insecticide plots. There was, however, a positive linear relationship for control plots. Thus, conservation of final yield results in equal amounts of seed production per area across a range of adult densities in the absence of seed predators, but that seed destruction by agents recouples adult plant density and seed production. In 2005, these relationships were somewhat different: densities of plants in 2005 were much higher, probably reflecting and abiotic differences in the growing seasons. In 2005, there was a positive linear relationship between adult density and seed production per plot in insecticide plots, and a significant quadratic relationship for control plots. Seed production became saturated at 25 plants/m² and was thus independent of adult plant density in >80% of the control plots. Notice that in 2005, the density of adult plants was over fourfold greater than densities in 2004, and individual plants within plots were smaller (the average seed head production per plant was 45.3 seed heads/plant in 2004 and only 6.9 seed heads/plant in 2005).
Seed augmentation experiment

Seedling recruitment was significantly related to our seed augmentation treatments, but not to the covariate of pretreatment seed input range (as measured by pretreatment adult head densities; Table 2). Seed addition into plots increased seedling recruitment when 1000 seeds/0.25 m$^2$ were added, but not when 3000 seeds were added. This result suggests that the population experiences a combination of seed and microsite limitation, and perhaps also suggests seed or seedling interference with one another at very high densities. As in our other experiment, adult density per plot was not affected by seed number (Fig. 6). In fact, though seedling numbers reflected our seed augmentation treatments and not the natural pretreatment seed input range, adult density corresponded to pretreatment seed input range, a result that supports the idea that preexisting differences in density among plots reflect differences in plot suitability or $K$ and not treatment (Table 2; Appendix D). These data imply that seed input affects numbers of seedlings recruiting, but not adult

Table 2. Effects of seed augmentation on seedling recruitment, adult establishment, and head production, tested with pretreatment seed input range as the fixed blocking factor.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Treatment</th>
<th></th>
<th>df</th>
<th>Pretreatment seed input</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>$F$</td>
<td>$\chi^2$</td>
<td>$P$</td>
<td></td>
</tr>
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<td>Seedling density</td>
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<td>0.0007</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Adults</td>
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<td>0.18</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Heads (Kruskal-Wallis)</td>
<td>2</td>
<td>1.2</td>
<td>0.55</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>
population size. Head production per plot was independent of both treatment and pretreatment seed input range (Table 2).

**DISCUSSION**

Using experimental removals of biocontrol agents and seed addition experiments at two different sites, we show that, despite large losses of individual plant fitness to seed predators (>70% seeds destroyed), biocontrol agents failed to reduce the population size of an invasive thistle, or to alter k. There was also no evidence of reproductive compensation to herbivory in seed head production: head density per plot was not significantly different between treatments. This result is contrary to another study showing that yellow starthistle has a high capacity for reproductive compensation under extreme simulated herbivory (Enloe and Spencer 2004). Our data show, however, that under normal herbivory levels in field conditions, this ability to compensate in seed head number is not expressed, or at least was not in this year.

Agents reduced seed production and resulted in significantly fewer seedling recruits, but despite this effect on seedling densities, adult densities of YST remained unchanged in plots with and without agents, or with and without seed addition. The thwarting of these seemingly effective agents occurred primarily through density-dependent seedling mortality through self-thinning, plant plasticity, and size hierarchies, resulting in constant final yield in seed production. A combination of seed and microsite limitation affected recruitment. Seedling recruitment is seed-limited at low densities and microsite-limited at moderate to high densities (microsite limitation occurred in 44% of our control plots; see also Eriksson and Ehrlen 1992, Munzbergova and Herben 2005).

Our seed addition experiments show that the best predictor of adult density was the previous year’s adult density, not the amount of seed input. Because we found that adult densities were unrelated to seed inputs and to seedling numbers, our results suggest that, if sufficient seed inputs occur, plant density is determined by habitat quality and carrying capacity at small scales.

Although it has been suggested that self-thinning may compensate for 40–98% seed loss (Myers et al. 1988, Hoffman and Moran 1991, Kelly and McCallum 1995, Myers and Risley 2000, Myers and Bazely 2003), this is the first herbivore exclusion study to demonstrate experimentally that density-dependent seedling mortality can override the effects of extensive seed loss from biocontrol agents. In fact, self-thinning has been negligible in other herbivore exclusion studies that have investigated impacts of seed predation on recruitment in perennial species (Louda 1982a, b, 1983, Louda and Potvin 1995, Maron and Gardner 2000, Kelly and Dyer 2002, Maron et al. 2002), and Maron and Gardner (2000) predict that pre-dispersal seed predation will affect populations, regardless of moderately strong density-dependent seedling mortality.

Although adult densities were not reduced by pre-dispersal seed predation, biocontrol might still be effective if seed bank depletion plays an important role in population dynamics. In our case, YST appears to have a limited seed bank. Our study showed extremely low recruitment of seedlings in areas where YST was prevented from flowering in the previous summer (prevention of a single year’s reproduction). Similarly, Joley et al. (2003) found almost complete depletion of the seed bank after four years, and Benefield et al. (2001) found that only 7% of seeds produced remain in the seed bank after the first season. While 7% of millions of seeds is a large number, YST appears to have substantial mortality of seeds in the soil (Benefield et al. 2001). From our study, biocontrol agents do deplete seed banks substantively, but the main population dynamics of YST appear to be driven by the previous year’s plant success, and self-thinning reduces large numbers of seedlings to much lower adult densities, negating ecological contributions by seed banks except at low plant densities.

Another factor that diminishes the efficacy of biocontrol agents, and that has received much less attention in the literature, is the combination of plant plasticity and plant size hierarchies that result in constant levels of seed production across a large range of densities of YST. In these cases, even if biological
control agents were to reduce the number of individuals dramatically, fewer, larger plants could still produce as much seed as many smaller plants on a per area basis; insecticide plots of 2004 produced a constant final yield resulting in no correlation between adult density and seed production. As we mentioned earlier, there was flooding in February of 2004. Surviving seeds germinated after the floods receded. Late germination resulted in a flush of late season competitors. Adults had a difficult time reproducing with consistent rainfall resulting in a flush of late season competitors and herbivores, from the insecticide treatment, low densities of competitors. This release from competition with dense competition from *Lotus purshianus* and *Melilotus indica*. This stressful environment reduced reproduction per area and per plant; the double whammy of competition and herbivory stress created a low constant final yield in 80% of the control plots and linearized the adult and seed production relationship for insecticide plots. These kinds of effects are not easily detectable from studies in which individual plants are measured because they operate on the larger scale of plant neighborhood and the reproductive rate must be measured on a per contiguous area basis. To our knowledge, few other studies of biological control have considered this effect.

Although we got consistent results in sites that were 121 km apart, a caveat of our study is that it was only conducted over one year. The processes that we observed, however, were important across a large range of the density of naturally occurring YST populations, and hence could also be expected to be important across years in which YST fluctuates in abundance.

Despite the huge impact that biological control agents have on seed production, they do not appear to be causing a detectable population decline in YST in our heavily infested field sites. Two compensatory mechanisms may be negating the effects of seed loss. First, seedling recruitment was seed limited, but self-thinning removed surplus plants in high density plots. Second, constant final yield obscured the relationship between adult density and reproductive output on a per area basis. There was also microsite limitation at higher seed outputs disrupting the seed-to-seedling relationship. These data suggest that YST habitat is saturated, even in the presence of the biocontrol agents, and that available resources are regulating the population. Even when biocontrol agents can reduce plants to low densities, these few plants can grow large and produce immense quantities of seed. Thus, it remains to be seen whether seed limitation can persist for long periods in the absence of other stressors (such as competition, flooding, drought).

**Implications for management and biocontrol**

Multiple strategies targeting several life stages may be required to overcome the compensatory mechanisms that reduce the efficacy of pre-dispersal seed predators. These strategies should be employed after density-dependent mortality has done some of the work; ideally new agents or management strategies will impact juvenile and adult plants. For example, sowing effective plant competitors or introducing a new biological control agent that reduces survival of juveniles and adults could lower plant population densities, thereby linearizing the relationship between adult density and seed production (Roche et al. 1994, Pitcairn et al. 1997, Shelly and Larson 1997, Duques 2001, Gelbard and Harrison 2005) and increasing the amount of seed limitation. When densities of YST become low enough, our data suggest that the effects of the current biocontrol agents will strengthen, as the relationships between seed input and adult densities are correlated at lower plant densities.

Studies investigating the interactions between population dynamics and biological control agents should be conducted to assess biological control efficacy not only on individuals, but on populations as a whole. Such exercises expose agent strengths and weakness and illuminate vulnerable life stages that can be exploited in management strategies. Understanding impacts of agents on population dynamics of invasive species will improve our ability to choose the most effective management plan.

**Acknowledgments**

This research could not have been completed without the support of Ray Carruthers and funding from the USDA-ARS-EFW, the California Agricultural Experiment Station, and NSF DGE-0114432 IGERT on Biological Invasions (S. Y. Strauss, principal investigator). We are grateful for critical comments from John Maron, Joe Ditomaso, Kevin Rice, and conversation with K. Shea. An anonymous reviewer greatly improved this manuscript. We would like to thank April Cole, Rick Lankau, Jen Lau, Adrianna Muir, Justin Weber-Stover, and Ali Weber-Stover for their invaluable field assistance, as well as Larry Dew and UCD Putah Creek Preserve for allowing us to conduct experiments on their property.

**Literature Cited**


APPENDIX A
A flowchart showing how compensatory processes can counteract seed loss to disrupt biocontrol efficacy of seed predators (Ecological Archives A019-029-A1).

APPENDIX B
Photos of representative experimental plots from the summers of 2004 and 2005 (Ecological Archives A019-029-A2).

APPENDIX C
ANOVA of effects of insecticide application on total visitation time of pollinators and total number of visitors (Ecological Archives A019-029-A3).

APPENDIX D
Mean density of life history stages for seed augmentation treatments separated by pretreatment seed input range (Ecological Archives A019-029-A4).