

# Widespread seed limitation affects plant density but not population trajectory in the invasive plant *Centaurea solstitialis*

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**Abstract** In some plant populations, the availability of seeds strongly regulates recruitment. However, a scarcity of germination microsites, granivory or density-dependent mortality can reduce the number of plants that germinate or survive to flower. The relative strengths of these controls are unknown for most plant populations and for exotic invaders in particular. We conducted a seed addition experiment with a granivore exclusion treatment in a field setting to explore how these factors interact to regulate populations of the widespread invader *Centaurea solstitialis* (yellow starthistle) at three study sites across the plant's range in California. We coupled the experimental approach with observational studies within established *C. solstitialis* populations to estimate seed rain, recruitment and mortality at natural densities. Seed limitation occurred in both experimental and observational plots in all populations. Although vertebrate granivores were active at each site, they had no effect on *C. solstitialis* recruitment. Density increased mortality, but the effect was variable and weak relative to its effect on fecundity. The seed limitation that was evident at the seedling stage persisted to flowering. Seed-limited

populations such as these ought to be highly sensitive to losses to seed predators, and many biological control agents, including those established for *C. solstitialis*, are seed predators. However, flowering plant density was decoupled from seed production by a strong compensatory response in the surviving plants; seed production was nearly constant in plots across all seed addition levels. Thus, flowering plant density is reduced by the established biocontrol agents, but seed production compensates to replace the population every generation, and no long-term decline is predicted.

**Keywords** Biocontrol · Density dependent · Invasive · Seed predation · Yellow starthistle

## Introduction

A central goal of plant population biology is to understand the factors that regulate the recruitment of new individuals into a population (Harper 1977). In some populations, there is a strong relationship between the number of seeds produced and the number of seedlings that establish. In such populations, recruitment is limited by seed input, and any increase or decrease in seed production is expected to have a concomitant effect on recruitment (Louda 1982; Crawley 1989; Louda and Potvin 1995; Parker 2001; Herrera et al. 2002). However, the link between seed production and seedling abundance is rarely straightforward. Three processes have the potential to decouple recruitment from seed production: a scarcity of germination microsites, granivory, and density-dependent processes.

All seeds require a suitable microsite for germination. Although some species are more stringent in their microsite requirements than others, there is a finite number of such sites in any given patch of ground. Many plant populations

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produce seeds in excess of the available microsites, and in such cases an increase in seed input is not expected to increase recruitment (Louda 1983; Crawley 1989; Sheppard et al. 2002). However, seed and microsite limitation are expected to vary over spatial scales relevant to dispersal because seeds are rarely, if ever uniformly dispersed from the maternal plant. We often see microsite limitation near the maternal plant, and seed limitation becomes more pronounced at increasing distances from the seed source (Clark et al. 1998; Poulsen et al. 2007). For species with very limited dispersal, this may occur on a scale of meters or less. Microsite limitation also appears to vary temporally according to successional stage. Two recent reviews (Turnbull et al. 2000; Maron and Crone 2006) found that species are more likely to experience seed limitation in disturbed or early successional habitats and microsite limitation in late successional habitats.

Seed predators may strongly mediate recruitment, but only when they remove seeds in excess of the compensatory mechanisms at subsequent stages (e.g., microsite limitation, density-dependent competition, etc.) (Louda 1982; Louda and Potvin 1995; Maron and Simms 2001; Nuñez et al. 2008). When recruitment is microsite limited, granivory should not affect recruitment (Crawley 1989, 1992; Hunter 1992). In some populations, even very large reductions in seed production are not sufficient to reduce absolute seed numbers below what is necessary to fill the available microsites, and thus have no measurable effect on recruitment (Andersen 1989; Crawley and Gillman 1989). However, because seed and microsite limitation vary spatially, even granivory that has no effect on recruitment near a maternal plant may still be an important factor in limiting recruitment further away where seed limitation ought to prevail, e.g., near the edge of a population.

Finally, strong density-dependent mortality at any life stage prior to reproduction can reduce or even eliminate gains in recruitment due to higher seed input (Crawley 1997). Density can increase mortality by intensifying intraspecific competition for resources (Condit et al. 1994) or increasing the risk of pathogen or herbivore attack (Burdon and Chilvers 1982; Maron and Simms 2001; Gilbert 2002). Density-dependent processes are expected to be strongest where recruitment is microsite limited and weaker where it is seed limited (Clark et al. 2007; Poulsen et al. 2007). When density-dependent mortality is strong, even significant increases in seedling recruitment may not have a population-level effect if plants die before they reach flowering. Individual fecundity may also respond to changes in density, as plants will be smaller and less fecund at high densities. This ought to be especially important to species with an annual lifecycle because recruitment is entirely from seed and strong density effects

may eliminate differences among plots that began with high or low seed inputs.

At the scale of a species' geographic range, the degree to which any one population is seed or microsite limited will likely vary along gradients of environmental conditions that are relevant to germination. Recruitment may be microsite limited where environmental conditions are optimal and plant density is high. Recruitment may be microsite limited even where density is low if environmental conditions are suboptimal. For example, at the edge of a species range where the plant may be near its physiological limit, equilibrium density ought to be low and microsites scarce. Expanding populations will always be seed limited at the edge where current population density goes from low to zero (Parker 2001).

Geographic variation in the degree of seed limitation means that granivory may have very different effects on seedling recruitment in different populations. For example, the cinnabar moth, *Tyria jacobaea*, feeds on the inflorescences of its host plant, *Senecio jacobaea*, which substantially reduced fecundity and seedling recruitment in arid coastal dunes (van der Meijden 1979) but not in mesic grasslands (Crawley and Gillman 1989) because recruitment was seed limited in the former location and microsite limited in the latter. The abiotic factors that can ameliorate or exacerbate density-dependent mortality are also likely to vary across a species' geographical range. Likewise, plant fecundity at low density will vary across the range as determined by site characteristics. Such geographic variation may be especially true for species that occupy large ranges or ranges that span steep environmental gradients, such as many invasive species in their introduced ranges.

The undesirable impacts caused by invasive plants are often the result of the high densities reached by the invader (Parker et al. 1999). It is therefore critical to understand how recruitment of new individuals into an exotic plant population is regulated. One characteristic often associated with success as an invader is high reproductive output (Baker 1965; Reichard and Hamilton 1997). Thus, in established invasions, seed production is usually high and so is plant density, and such populations are more likely to be microsite than seed limited. In contrast, seed limitation may be widespread in expanding populations, populations under intensive control treatments, or in populations with natural heterogeneity in density caused by environmental or demographic stochasticity.

Classical biological control is the practice of introducing enemies from the invader's native range with the expectation that the enemies will reduce plant density or the persistence of the population. For those exotic species that have come to occupy large areas, biological control is the principal management tool and many biocontrol agents are seed predators (Coombs et al. 2004). Biocontrol seed

predators are likely to have quite different effects on populations in which recruitment is seed limited than on populations in which it is microsite limited. Surprisingly, the frequency of seed limitation has not been widely studied in invasive plants (but see Parker 2001; Nuñez et al. 2008; Garren and Strauss 2009).

We used a combination of experimental and observational approaches to explore the role of seed availability, density-dependent processes and seed predation in three *Centaurea solstitialis* invasions across California. *C. solstitialis* L. (Asteraceae, yellow starthistle) is native to Eurasia. It was first introduced to California nearly 150 years ago and is still expanding its range in the state (Pitcairn et al. 2006). It is considered one of the state's most problematic invasive species (Pitcairn et al. 2006). *C. solstitialis* has an annual lifecycle so recruitment each year is entirely by seed. Thus, elucidating how the factors described above interact is essential to understanding and managing *C. solstitialis* invasions in California.

Our goal was to quantify the shape of the recruitment response to experimental seed addition in uninvaded areas and in unmanipulated established invasions. We conducted a seed addition experiment in uninvaded areas and caged half the plots to exclude vertebrate granivores. By following the plants for their entire life cycle, we were able to determine if density significantly reduced the survival of seedlings or fecundity. In addition, we measured natural seed production, seedling recruitment, and survivorship in established populations. We conducted this work in three populations across a broad geographic distribution to elucidate conditions under which seed limitation or microsite limitation was most important.

We asked the following questions. (1) How is seedling recruitment influenced by seed input and post-dispersal granivory? (2) Does density influence mortality and therefore the number of plants that reach flowering? (3) Does density influence fecundity and therefore the number of seeds produced per plot? (4) Do the results vary across a broad geographic range? We discuss the potential for biocontrol seed predators to control *C. solstitialis* invasions.

## Methods

### *Centaurea solstitialis* life cycle

*Centaurea solstitialis* is an annual thistle. Seeds germinate in response to winter precipitation, which in California typically begins in November and continues through the spring. Seeds can germinate at any time during the winter if they have sufficient moisture and do not require freezing, scarification or dormancy (Benefield et al. 2001; Swope,

unpublished data). In early summer, plants bolt and begin producing flowers, which they will do continuously until they complete their lifecycle in mid to late summer. The majority of the seeds disperse in a relatively compressed period of time towards the end of the flowering period, typically between late July and late August.

*C. solstitialis*' seedbank is short-lived in California. The majority of its seeds germinate or die in the first year (Benefield et al. 2001; Joley et al. 1992; Garren and Strauss 2009). Benefield et al. (2001) found that within a year of dispersing from the maternal plant, only 7% of the seeds remain alive in the soil. Garren and Strauss (2009) also found that the seedbank is nearly depleted after 1 year, and that recruitment from the seedbank was extremely low and overwhelmed by recruitment from current seed rain.

### Study sites

*Centaurea solstitialis* was first recorded in California in the San Francisco Bay Area in 1869 and has persisted in coastal grasslands since then, although it is typically found in smaller, lower density populations in coastal areas as compared to populations in the interior parts of the state (Pitcairn et al. 1998). *C. solstitialis* began to invade the interior part of the state in the 1930s and 1940s (Benefield et al. 2001), and has since formed high-density, persistent populations in this region. For the past decade, *C. solstitialis* has been expanding its range eastward into the mid- and high- elevations of the Sierra Nevada Mountains (Pitcairn et al. 2006). The eastern edge of its range in California is now as far east and as high in elevation as Donner Summit (2,260 m elev., El Dorado Co.).

For this study, we selected sites that span this range from coastal areas to the Sierra Nevada. Our "Coast" site is located in the Loma Alta Open Space Preserve (Marin County; 38°00'N, 122°36'W) approximately 25 km from the first vouchered *C. solstitialis* specimen in California. Our "Interior" site is located in Mount Diablo State Park (Contra Costa County: 37°51'N, 121°55'W), where *C. solstitialis* is the most widespread weed in the park (Marla Hastings, Mt. Diablo State Park, personal communication). Our "Sierra" site is located in the El Dorado National Forest (El Dorado County: 38°46'N, 120°25'W). The Coast and Interior sites are 65 km apart, and the Sierra site is 165 km from the Interior site and 208 km from the Coast site. See Table S1 in the "[Electronic Supplementary Material](#)" for more information about the study sites.

### Insect biocontrol agents

Six insect seed predators have been introduced as biocontrol agents for *C. solstitialis*. Two agents are present at our study sites: the weevil *Eustenopus villosus* (Coleoptera:

Curculionidae; released in 1990) and the fly *Chaetorellia succinea* (Diptera: Tephritidae; released in 1988). Both agents lay eggs in the inflorescences and the larvae consume seeds as they develop. Each leaves species specific evidence of attack visible on the outside of the inflorescence, making it possible to nondestructively identify those inflorescences that have been attacked and by which species. We quantified attack rates for each of the seed predators at all of the sites by categorizing every inflorescence in our plots as unattacked, attacked by *Eustenopus*, or attacked by *Chaetorellia*. Each inflorescence rarely contained more than one larva (just over 1% of 3,337 inflorescences destructively sampled in the lab contained more than one insect, Swope, unpublished data).

In other experiments, we have attempted to eliminate or reduce attack by *Eustenopus* and *Chaetorellia* without success. Even high concentrations of Ortho Systemic Insect Killer (The Ortho Group, Marysville, OH, USA) applied every four days was insufficient to significantly reduce insect attack rates (Swope, unpublished data), but it did appear to have phytotoxic effects. We therefore chose not to try to reduce or exclude insect seed predators in this study. We consider the importance of these insects in the “Discussion.”

#### Experimental seed addition

Experimental seed addition plots were located ~20 m upslope from an established invasion where slope angle, aspect, soil type and land use were identical to invaded areas found downslope. We therefore assumed that these areas were suitable habitat for *C. solstitialis* but had not been invaded yet because of dispersal limitation upslope. Seed addition plots measured 0.5 m × 0.5 m and were randomly located 2–5 m apart. We used a wire frame secured to the ground with garden staples to permanently mark each plot's boundaries.

We collected inflorescences from >300 plants in established invasions at each site over a three-week period at the peak of seed dispersal (August 2007). We extracted seeds from the inflorescences in the lab, saving only viable seeds and discarding damaged or unfilled achenes, and pooled seeds by site. Seeds for the experimental additions were drawn from these mixes and seeds from different sites were not mixed together so there was no site-to-site transfer of seeds.

There were six replicates of four seed addition levels at each site: no seeds added (0), 50, 500, and 1,000 seeds added. Plots to which we added no seeds served to determine if a seedbank was present in these uninvaded areas or if there was any immigration from the adjacent invasion. Seeds were added in the last week of August 2007 to coincide with the peak of natural seed dispersal in that

year. Seeds were systematically dispersed into each plot to achieve a uniform distribution. To mimic natural seed dispersal, seeds were not buried or scratched into the soil.

#### Seed predation: vertebrate granivores

At the time of seed addition, we caged half of the replicates in each treatment level at each site to exclude vertebrate granivores; the other half of the replicates were left open to granivores. Cages were constructed from hardware fabric (0.5 cm × 0.5 cm mesh openings), measured 1 m × 1 m (this created a 25 cm buffer between the edge of the plot and the cage), were 40 cm high, and were secured to the ground with garden staples. To determine if granivores were present at each site, we placed six Petri dishes, each with 20 millet seeds and 20 barley seeds, randomly between the plots (but not in them, so as to avoid drawing granivores to the plots). We checked each dish after 48 h to see if seeds were missing and assumed that missing seeds had been taken by granivores. To determine if the cages successfully excluded granivores, we placed one Petri dish with 20 millet seeds and 20 barley seeds inside of each caged plot in the buffer zone between the plot and the cage wall so as not to interfere with any seeds that might germinate in the plot and checked for seed removal after 48 h. We removed the dishes (with any remaining seeds) after the 48-h period and replaced the dishes with seeds at each census (every three weeks; see “Census”). Cages were removed from all plots simultaneously at each site when plants began to bolt and no additional seedlings had emerged for ≥6 weeks (Coast and Interior: late April; Sierra: early May).

#### Census

At three-week intervals following plot establishment and seed addition, we visited each plot and marked every seedling that had emerged with a 15 cm wooden stake. We also recorded how many seedlings had died since the last visit. It is possible to distinguish *C. solstitialis* seedlings from other forbs as soon as the first true leaves emerge (typically one week after germination). Plots at the Sierra site were periodically covered in snow and so we were occasionally unable to census these plots on the scheduled dates. We censused these plots as soon as possible after snowmelt, but this site still received two fewer total visits between January and March (2008) than the other sites. We continued to census the plots until all of the plants had completed their lifecycle (late July 2008).

#### Observational plots

In addition to the experimental work, we also collected observational data in established *C. solstitialis* invasions at

the three sites. At the Coast and Interior sites, we established 15–20 randomly located observational plots in the invasions downslope of the experimental plots, and another 15–20 randomly located plots in another *C. solstitialis* invasion at the same site but 1–3 km away. At the Sierra site, we established 20 observational plots in the downslope invasion, but did not establish a second set of plots in another invasion due to logistical constraints. Plot size, census schedule and census methods were identical to those used for the experimental seed addition plots, but we had no vertebrate granivore exclusion treatment for the observational plots.

#### Estimating seed rain and fecundity

To estimate natural seed rain into the observational plots, we counted the total number of inflorescences in each plot and multiplied that by the mean number of viable seeds produced by inflorescences at that site in that year ( $N = 757$  inflorescences from the Coast, 1,041 inflorescences from the Interior, and 1,590 inflorescences from the Sierra). We assumed that emigration of seeds out of each plot was equivalent to seed immigration into that plot. We felt that this was a reasonable assumption given the scale of the spatial heterogeneity of plant density in these populations.

We also estimated the number of seeds produced by each plant and in each plot at the end of the experiment. To do this, we counted the number of inflorescences per plant for all the plants in each plot and multiplied by the mean number of seeds per inflorescence.

#### Competitive environment

To determine if there were differences among the sites in terms of the plants with which *C. solstitialis* seedlings would compete, we identified all of the plants in each plot to species and estimated canopy cover for each. To maximize both accuracy and consistency, we estimated canopy cover using a frame equal to the size of the plots (0.5 m × 0.5 m) that was divided into 5 cm × 5 cm sub-frames. For each plot, we recorded the number of sub-frames in which each species occurred and calculated the percent cover for the whole 0.5 m × 0.5 m plot. These censuses were conducted when the plants (other than *C. solstitialis*) were at what we estimated to be peak biomass at each site (Coast and Interior: early May; Sierra: late May).

#### Statistical analyses

We used general linear models (GLM) and the Bonferroni post hoc test with site, seed addition level and granivore

treatment (caged and uncaged) as fixed independent factors and  $\ln(\text{total seedlings} + 1)$  as the dependent variable. Because granivores had no effect on seedling recruitment (see “Results”), we combined the data from caged and uncaged plots for all further analyses.

We regressed  $\ln(\text{seedlings} + 1)$  against  $\ln(\text{seed input} + 1)$  for both observational and experimental plots separately at each site. We used the 95% CI for the regression lines from the experimental and observational data to determine how well results from the two approaches correspond. We used binomial GLM to test for an effect of seedling density on the probability of mortality prior to flowering. Data from the observational and experimental datasets were analyzed separately for the three sites.

We tested for an effect of seed addition on the number of flowering plants after the effects of density-dependent mortality using GLM with the Bonferroni post hoc test in which site and seed addition level were fixed effects and the dependent variable was  $\ln(\text{flowering plants} + 1)$ . To determine if density had an effect on fecundity we regressed  $\ln(\text{mean seeds per plant} + 1)$  against  $\ln(\text{flowering plants per plot})$  for each site separately. To determine how the number of seeds added to the plots affected the number of seeds produced one year later, we used GLM with site and seed addition level as fixed effects, and the dependent variable was  $\ln(\text{seeds produced per plot})$ . All statistical tests described above were conducted using Systat v.10.2 (SAS Institute).

To quantify the degree of similarity in the community of plant competitors between the sites, we used the Jaccard similarity coefficient. The Jaccard coefficient is calculated as

$$\frac{A}{A + B + C},$$

where  $A$  is the number of species found in both sites,  $B$  is the number of species found in Site 1 but not in Site 2, and  $C$  is the number of species found in Site 2 but not in Site 1; i.e., it is the proportion of species any two sites have in common. We also compared percent cover for all species combined in each plot using ANOVA with site as the independent variable.

## Results

### Patterns of emergence in experimental seed addition plots

In California, *C. solstitialis* seedlings recruit episodically in response to rainfall events. In our experimental plots, 11 seedlings emerged in five of the 18 control plots (seed addition = 0) at the three sites, and all but one survived to



flower. No recruitment was recorded in any plot at any site after the third week of March 2008. Plants reached peak flowering in June, and by late July all plants were dead. We followed the fate of every marked seedling in our experimental plots (Coast: 610 seedlings; Interior: 559 seedlings; Sierra: 1,992 seedlings) and our observational plots (Coast: 636 seedlings; Interior: 1,231 seedlings; Sierra: 2,454 seedlings).

#### Insect and vertebrate seed predation

The proportion of inflorescences attacked by the biocontrol agents was consistent within and among sites, but not especially high. Site, plot type (observational or experimental) and plant density had no effect on the per plant attack rates. On average, at the Coast site, 11% of the inflorescences on each plant were attacked by *Eustenopus* and 5% were attacked by *Chaetorellia*; at the Interior site, 21% were attacked by *Eustenopus* and 6% were attacked by *Chaetorellia*; and at the Sierra site, 20% were attacked by *Eustenopus* and 5% were attacked by *Chaetorellia*. Using the same inflorescences that we used to estimate natural seed rain and fecundity, we calculated the mean number of viable seeds produced by unattacked inflorescences and those attacked by *Eustenopus* and *Chaetorellia* and estimated mean per plant reduction in fecundity due to attack. On average, viable seed production was reduced by 17% per plant at the Coast, 20% at the Interior and 19% at the Sierra (Swope, unpublished data).

Vertebrate granivores were actively feeding at the plots but were unable to enter the caged plots. Between 22 and 70% of the millet and barley seeds were removed from Petri dishes located between plots after each 48 h period at all sites; no seeds were missing from Petri dishes in caged plots. If post-dispersal seed predation is important to *C. solstitialis*, we ought to see increased seedling recruitment in plots from which granivores have been excluded, especially at the highest seed addition level(s) because they are more likely to attract the attention of granivores. We saw no such effect. Vertebrate granivores had no consistent or significant effect on the cumulative number of seedlings that emerged at any site or any seed addition level (see Table S2 in the “Electronic Supplementary Material” for statistics).

#### Seed versus microsite limitation: experimental plots

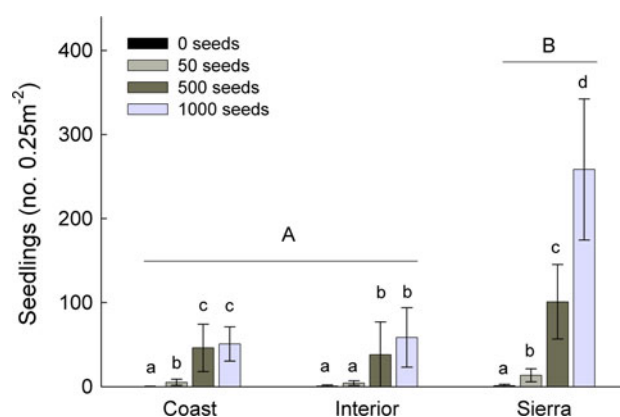
When recruitment is limited by seed availability, the cumulative number of seedlings that establish will increase as the number of seeds added increases. Alternatively, when recruitment is limited by the availability of germination microsites, higher levels of seed addition will not result in greater seedling establishment. The number of

seeds added had a significant effect on seedling recruitment at the Coast and Interior sites ( $F_{3,57} = 111.41$ ,  $MS = 56.52$ ,  $P = 0.0001$ ; Fig. 1). However, there was no difference in the number of seedlings that recruited in plots with 500 seeds and those with 1,000, indicating that recruitment into the populations at the Coast and Interior sites had become microsite limited.

We found a different pattern at the Sierra site. Seedling establishment continued to increase with increasing levels of seed addition, indicating that the threshold between seed and microsite limitation is greater than 1,000 seeds per 0.25 m<sup>2</sup> plot and is higher at the Sierra site than at the other two sites ( $F_{2,57} = 16.06$ ,  $MS = 8.36$ ,  $P = 0.0001$ ; Fig. 1). Further, at any given seed addition level, the total number of seedlings that established was higher at the Sierra site than at the Coast and Interior. The interaction between site and seed addition level was not significant ( $F_{6,57} = 1.47$ ,  $MS = 0.52$ ,  $P = 0.21$ ).

#### Seed versus microsite limitation: observational plots

Many of the observational plots at the Coast and the Interior sites had seed rain below 500 seeds per 0.25 m<sup>2</sup> plot (Fig. 2a, b), the density below which our experimental data indicate that recruitment is seed limited, i.e., seed limitation occurs frequently in these established invasions. Observational data were particularly revealing at the Sierra site, where natural seed rain was much higher than in the experimental plots, ranging up to 21,000 seeds per 0.25 m<sup>2</sup> plot. Even at these very high natural seed input levels, seedling recruitment showed a stronger response to seed input than did plots



**Fig. 1** *Centaurea solstitialis* seedling recruitment (mean  $\pm$  SD) per plot across four seed addition levels at the three sites. Data were transformed for analysis as described in the text; untransformed data are shown. Different lower-case letters indicate significant differences among seed addition levels; Upper-case letters indicate significant differences among sites (both  $P < 0.01$ , Bonferroni post hoc test)

at either the Coast or Interior sites (Coast:  $\ln(y + 1) = 0.578 + 0.442\ln(x)$ ,  $r_{adj}^2 = 0.26$ ,  $df = 32$ ,  $P = 0.001$ ; Interior:  $\ln(y + 1) = 0.325 + 0.572\ln(x)$ ,  $r_{adj}^2 = 0.76$ ,  $df = 33$ ,  $P = 0.0001$ ; Sierra:  $\ln(y + 1) = -1.28 + 0.675\ln(x)$ ,  $r_{adj}^2 = 0.56$ ,  $df = 18$ ,  $P = 0.0001$ ).

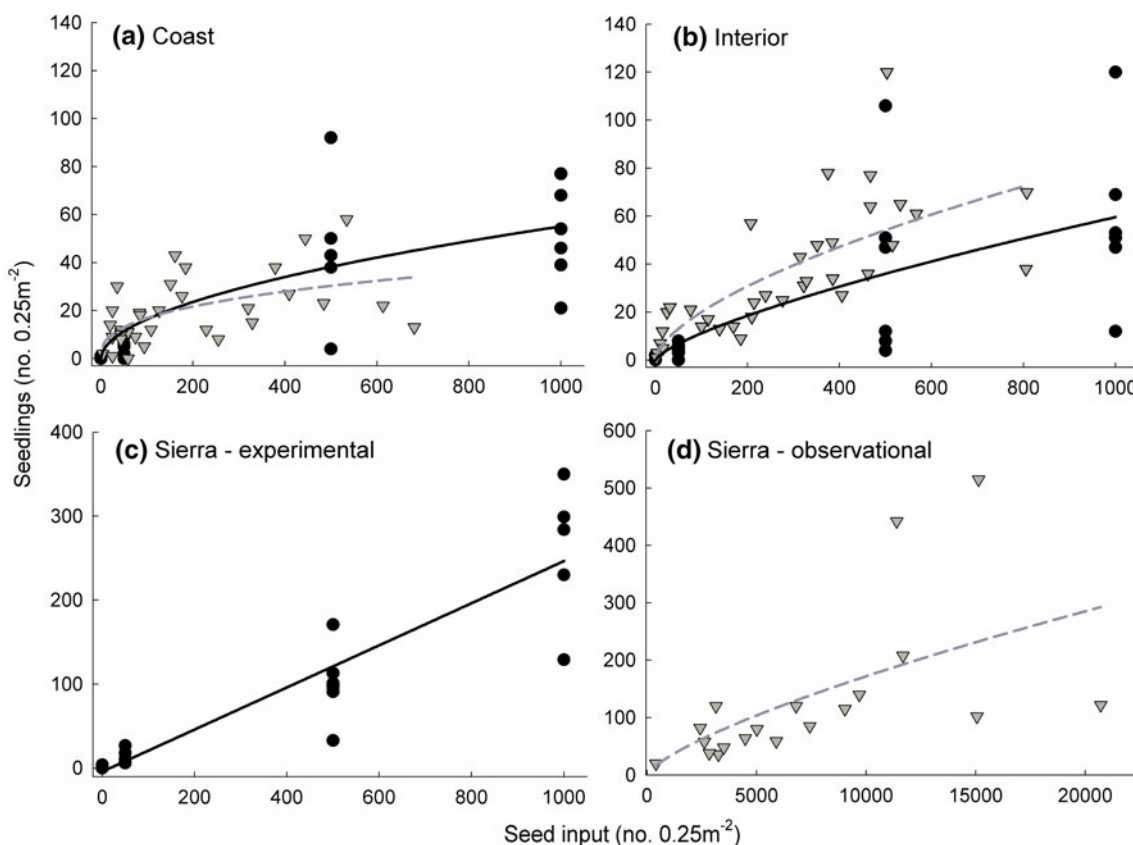
Comparing experimental and observational results

The relationship between seed input and seedlings established was similar between experimental and observational plots at the Coast and Interior. The 95% CI for regression lines for experimental and observational data overlap at both the Coast and Interior sites (Fig. 2a, b; 95% CI not shown for the sake of visual clarity). At the Sierra site, with the exception of one observational plot (406 seeds), there was no overlap between our experimental seed addition levels and natural seed rain (Fig. 2c, d). Nevertheless, despite a much higher seed input in the observational plots, the relationship between seed input and seedling recruitment (the slope of the line)

was nearly identical to that of the experimental plots, a remarkable finding given that natural seed rain was an order of magnitude higher than our seed addition levels (experimental plots: Coast:  $\ln(y + 1) = -0.090 + 0.557\ln(x + 1)$ ,  $r_{adj}^2 = 0.80$ ,  $df = 23$ ,  $P = 0.0001$ ; Interior:  $\ln(y + 1) = 0.134 + 0.492\ln(x + 1)$ ,  $r_{adj}^2 = 0.67$ ,  $df = 22$ ,  $P = 0.0001$ ; Sierra:  $\ln(y + 1) = 0.351 + 0.685\ln(x + 1)$ ,  $r_{adj}^2 = 0.90$ ,  $df = 22$ ,  $P = 0.0001$ ; see previous paragraph for results from observational plots).

Density-dependent mortality

If seedling survival is regulated by density-dependent processes, we ought to see an increased probability of dying in plots with higher seedling density, which we generally did (Table 1). The relationship between density and mortality was weak (observational plots) or nonsignificant (experimental plots) at the Coast, where density was lowest, and strongest in the observational plots at the Sierra, where density was highest.



**Fig. 2** Seed input and seedling recruitment per plot at the (a) Coast, (b) Interior and (c, d) Sierra sites. *Black dots and lines* represent experimental data; *inverted gray triangles and dashed gray lines* represent observational data. Experimental and observational data are not graphed together at the Sierra site because of differences in the

scales of experimental seed addition levels (0–1,000) and natural seed rain (406–21,000). Data were transformed as described in the text for analysis with linear regression; untransformed data with power curves are shown to facilitate site-to-site comparisons

**Table 1** Results of binomial GLMs of *C. solstitialis* mortality prior to flowering as a function of seedling density in observational and experimental plots

Site–plot type	Intercept (SE)	$\beta$ (SE)	<i>P</i>	Wald $\chi^2$	% Mortality <sup>a</sup> (seedlings, flowering plants) <sup>b</sup>
Coast–exp	0.831 (0.2373)	0.001 (0.0039)	0.74	110	71.2 (614, 177)
Coast–obs	0.551 (0.6315)	0.518 (0.2022)	0.01	6.554	89.8 (636, 65)
Interior–exp	0.296 (0.1961)	0.012 (0.0027)	0.001	18.532	74.6 (610, 155)
Interior–obs	1.655 (0.3661)	0.1359 (0.0939)	0.001	14.650	56.9 (1,231, 531)
Sierra–exp	1.100 (0.1125)	0.004 (0.0005)	0.001	76.343	55.4 (1,992, 889)
Sierra–obs	−5.109 (0.2887)	0.950 (0.950)	0.001	308.053	46.9 (2,454, 1,303)

<sup>a</sup> Percentage of seedlings that died prior to flowering

<sup>b</sup> The total number of seedlings and flowering plants in each plot type at each site

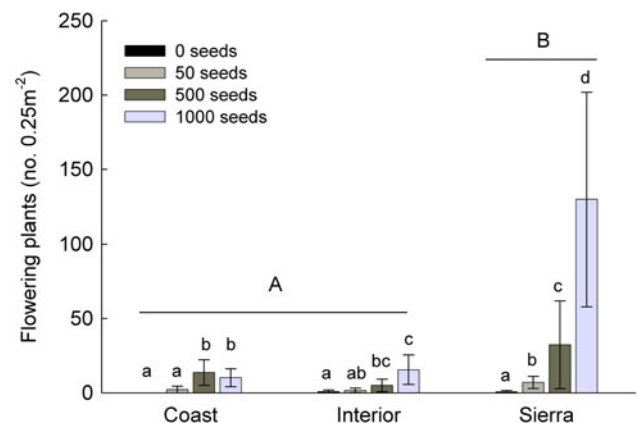
### Flowering plant density

The number of plants that survived to flower in experimental plots followed the same pattern as seedling recruitment ( $F_{3,57} = 52.31$ ,  $MS = 52.31$ ,  $P = 0.0001$ ; Fig. 3). The number of flowering plants continued to increase as the number of seeds added increased at the Sierra site but not at the Coast and Interior sites. The mean number of flowering plants per plot was much higher at the Sierra site, and there was no difference between the Coast and Interior sites ( $F_{2,57} = 16.31$ ,  $MS = 8.91$ ,  $P = 0.0001$ ). The interaction between site and seed addition was significant ( $F_{6,69} = 3.74$ ,  $MS = 0.55$ ,  $P = 0.003$ ). A total of ten plants survived to flower in four control plots (0 seeds added). Two control plots in the Sierra had one flowering plant each and two control plots in the Interior had four flowering plants each.

### Density, fecundity and total seed production

Higher flowering plant density led to a reduction in the mean number of seeds plants produced at all three sites (Coast:  $r_{adj}^2 = 0.51$ ,  $df = 7$ ,  $\ln(y + 1) = 3.712 - 0.041\ln(x)$ ,  $P = 0.029$ ; Interior:  $r_{adj}^2 = 0.36$ ,  $df = 10$ ,  $\ln(y + 1) = 4.339 - 1.007\ln(x)$ ,  $P = 0.03$ ; Sierra:  $r_{adj}^2 = 0.73$ ,  $df = 22$ ,  $\ln(y + 1) = 5.282 - 0.958\ln(x)$ ,  $P = 0.0001$ ; Fig. 4). The effect of density on fecundity was generally greater than its effect on survival.

This effect of density on fecundity yielded an interesting result. When we compared the number of seeds produced on a per area basis (0.25 m<sup>2</sup> plot), we found no significant effect of seed addition level on the number of seeds produced per plot ( $F_{3,58} = 1.14$ ,  $MS = 4.90$ ,  $P = 0.34$ ; Fig. 5). In other words, seed production in the plots to which we added 50 seeds was not statistically different from seed production in the plots to which we added 500 or 1,000 seeds. As for comparisons among sites, again we found that there was no difference in the number of seeds produced per plot between the Coast and Interior



**Fig. 3** Flowering plants (mean  $\pm$  SD) per plot across four seed addition levels at the three sites. Data were transformed as described in the text for analysis; untransformed data are shown. Different lower-case letters indicate significant differences among seed addition levels; upper-case letters indicate significant differences among sites (both  $P < 0.01$ , Bonferroni post hoc test)

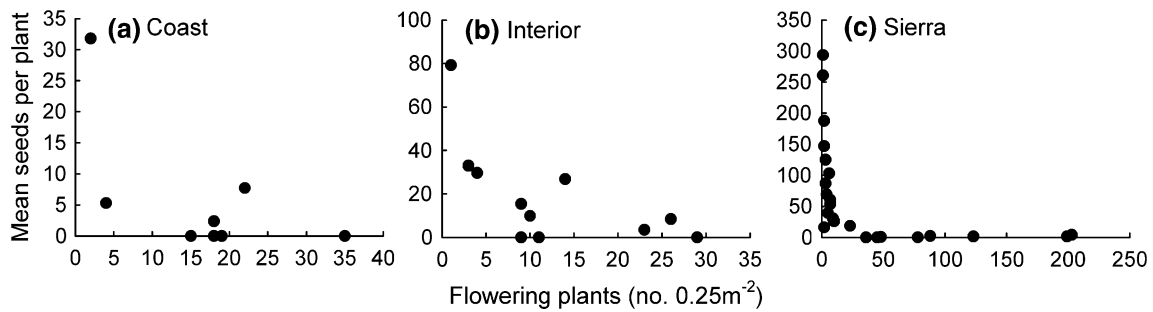
populations, and that seed production in the Sierra population was significantly higher ( $F_{2,58} = 13.79$ ,  $MS = 59.26$ ,  $P = 0.0001$ ). The interaction was not significant ( $F_{3,58} = 0.74$ ,  $MS = 3.19$ ,  $P = 0.62$ ).

### Competitive environment

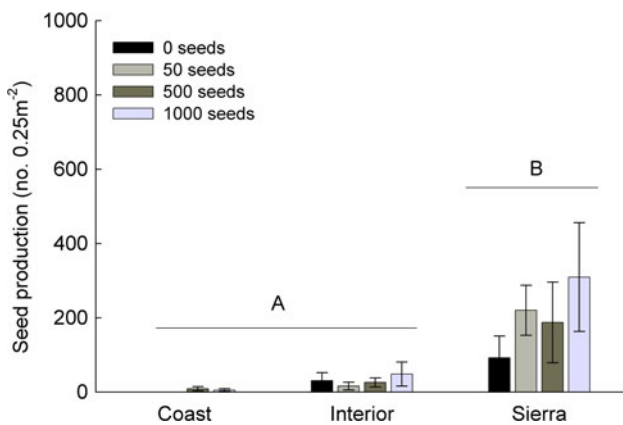
Exotic grasses dominated in plots at all three sites. At the Coast and Interior, *Bromus diandrus*, *B. hordeaceus*, *Avena fatua*, and *Lolium perenne multiflorum* were most abundant. At the Sierra site, *B. tectorum* and *A. fatua* were most abundant, *B. diandrus* and *B. hordeaceus* were present in lower numbers than at the Coast and Interior, and *L. p. multiflorum* was absent. *Erodium cicutarium*, and to a lesser extent *E. botrys*—both exotic annual forbs—were common (together occurring in nearly 50% of the subframes at each site).

Coast plots had significantly more total foliar cover than did Sierra plots, but neither the Coast nor the Sierra site





**Fig. 4** The effect of flowering plant density on fecundity in the experimental plots at the (a) Coast, (b) Interior, and (c) Sierra sites. Data were transformed as described in the text for analysis with linear regression; untransformed data are shown



**Fig. 5** Seed production (mean  $\pm$  SD) per plot across four seed addition levels at the three sites. Data were transformed as described in the text for analysis; untransformed data are shown. Upper-case letters indicate significant differences among sites (both  $P < 0.01$ , Bonferroni post hoc test)

differed from the Interior site ( $F_{2,52} = 6.19, P = 0.004$ ). Total mean foliar cover at the Coast was 92% ( $\pm 2\%$  SE), 78% ( $\pm 5\%$ ) at the Interior site, and 69% ( $\pm 5\%$ ) at the Sierra site. The Coast–Interior comparison had a Jaccard coefficient score of 0.57; the Coast–Sierra comparison had a Jaccard coefficient score of 0.50, and the Coast–Sierra comparison had a Jaccard coefficient score of 0.50. Unique species (those found at only one site) occurred in few plots and provided very little cover ( $\leq 2$  subframes on average per plot).

**Discussion**

Our goal was to quantify the relationship between seed input and recruitment in the invasive plant *C. solstitialis*, and to determine whether granivory or density-dependent processes modified the population-level consequences of increased seed input. We also studied the generality of these results across different environments in the introduced range in California. Our results address the potential of seed-feeding specialist enemies (biocontrol agents) to

reduce plant density and change the trajectory of the invasions by reducing local seed production.

Seed limitation occurred in all of our populations. Many plots (both observational and experimental) fell along the portion of the curve in which recruitment was still increasing with increasing seed addition. The results from our experimental seed additions do not decisively identify the point at which seed limitation gave way to microsite limitation at the Coast and Interior sites, but it appears to be around 500 seeds (Fig. 1). Results from our observational study show that natural seed rain within established invasions at these two sites ranged from well below to well above this threshold, indicating that both seed and microsite limitation occurred. Recruitment at the Sierra site remained seed limited at all seed addition levels and showed no evidence of microsite limitation, even in the observational plots where seed rain was substantially higher.

Post-dispersal granivory has the potential to decouple seed input from seedling emergence by removing seeds that are likely to germinate and reach reproductive maturity. However, we found no effect of post-dispersal granivores on *C. solstitialis* recruitment at any of the three sites despite the fact that they were actively feeding at all sites. The biotic resistance hypothesis posits that attack by native enemies will reduce the likelihood of exotic species establishment. Generalist granivores may be especially important in reducing exotic plant establishment because generalists can have large impacts on their prey populations (Brown et al. 1996; Parker et al. 2006), and unlike specialists, they are more likely to feed on novel species (e.g., Blaney and Kotanen 2001; Agrawal and Kotanen 2003; Parker and Hay 2005; Nuñez et al. 2008). It is possible that there is temporal variation in granivore effects (Maron and Simms 2001), or that at extremely high densities the seeds might attract vertebrate granivores (Hulme 1994). However, our highest experimental seed densities were higher than the natural densities at two of our sites, and the lack of granivore effect was consistent across a broad range of environmental conditions. We think that, as

a general rule, vertebrate granivores do not contribute to population regulation of *C. solstitialis* in California.

Density-dependent mortality can reduce or eliminate gains in recruitment that are the result of higher seed inputs. Seedling mortality in our study was high (47–90%) but the influence of density was weak (relative to its effect on fecundity), except in the observational plots at the Sierra site where seedling density was highest (up to 515 seedlings per plot). *C. solstitialis* seedling survival is influenced by the amount and timing of winter rainfall and precipitation totals were below average in the year of this experiment (Table S1 of the “[Electronic Supplementary Material](#)”). The abiotic stress imposed by drought may account for the high mortality and relatively small effect of density in this study. It is possible that density will have a larger effect on seedling mortality in wet years when more plants survive long enough to compete with one another, similar to what we documented in the observational plots at the Sierra site, where seedling density was highest and its effect on survival greatest. Because the effect of density on mortality was not strong, the pattern of recruitment in response to seed addition persisted to the flowering stage. At the Coast and Interior, the number of flowering plants increased as seed input increased, but only up to a point, after which there were no further gains in the number of plants. At the Sierra site, the number of flowering plants continued to increase across all seed addition levels.

Just as density can influence survival, it can also influence fecundity. Higher flowering plant density reduced the average number of seeds individual plants produced at all three of our sites. This produced an interestingly result in which a near-constant number of seeds were produced per plot by a highly variable number of flowering plants at each site. These results are consistent with other work on *C. solstitialis*. Garren and Strauss (2009) found that in an old field, a few large plants produced as much seed as many small plants, resulting in a constant final yield in seed production.

We might expect to find large differences in the community of plant competitors that might explain the large differences we documented in *C. solstitialis* performance at the Coast, Interior and Sierra sites. However, our data do not suggest that this was the case. Our experimental and observational plots were located in annual grasslands dominated by the same exotic species (*A. fatua*, *B. diandrus*, *B. hordeaceus*, *B. tectorum*, *E. botrys*, *E. cicutarium* and *L. p. multiflorum*). All three sites had  $\geq 50\%$  of their species in common, and the unique species provided  $\leq 6\%$  of the total cover in all plots at each site.

Although total foliar cover by competing species was lower at the Sierra site than at the Coast site, it was still quite high (mean cover = 69%) and not lower than at the Interior site. Further, no species were physiologically

active in any of the plots during the summer months when *C. solstitialis* was flowering so that it flowered without interspecific competition. Differences in total cover by competing species may account for some of the differences we measured in *C. solstitialis* success among sites, but we do not think it explains most of it.

There are large differences in climatic conditions at the three sites. It has been suggested that the cooler, wetter conditions in coastal areas may be suboptimal for this species (DiTomaso and Gerlach 2000; DiTomaso and Healy 2007). *C. solstitialis* is considered to be the most widespread exotic plant in the state (Pitcairn et al. 2006), due in large part to its success in the hot, dry interior grasslands. Until recently it was assumed that the climate in the higher elevations of the Sierra exceeded *C. solstitialis*' physiological limits, and so would prevent the plant from invading (Maddox and Mayfield 1985). However, *C. solstitialis* has been expanding its range into the mid- and high-elevations of the Sierra Nevada Mountains since the late 1990s (Pitcairn et al. 2006), and our data show that climate is not a barrier to invasion in that region.

The success of biocontrol agents can be measured by the degree to which they reduce flowering plant density and/or local seed production, and the latter is strongly linked to the long-term trajectory of the local population. Our experimental data show that seed limitation occurred at all sites and persisted through to the flowering stage. Therefore, biocontrol seed predators, or any control method that reduces seed production, has the potential to reduce flowering plant density at all three sites. Seed predators may also slow the spread of the plant into uninvaded areas at the edge of the current invasions where recruitment is expected to be seed limited (Kelly and McCallum 1995; Jongejans et al. 2008). Although our study was not designed to test this possibility, the frequency or intensity of the seed limitation that we observed may be attributable to seed predation by the established biocontrol agents *Eustenopus* and *Chaetorellia*. However, the magnitude of a seed predator's impact would vary among sites due to the presence of microsite limitation in the Coast and Interior but not the Sierra. For example, in plots with high seed input (1,000 seeds), seed predators that consumed half of the seeds would significantly reduce flowering plant density at the Sierra site but would have no effect at the Coast and Interior; however, in plots with low seed input, seed predators will reduce flowering plant density at all sites. On the other hand, increases in seed input (conversely, reductions in seed input) did not affect the total number of seeds going back into the plots. This decoupling of seed production from flowering plant density was driven by a compensatory response in the plants that survived to flower. Thus, in our populations, seed predation would be

expected to reduce flowering plant density but not to lead to overall population declines.

We know little about the prevalence of seed limitation in invasive plants and therefore the potential of seed-feeding biocontrol agents to reduce invasive plant density or the trajectory of an invasion. Nuñez et al. (2008) found that native generalist seed predators fed heavily on the seeds of exotic pines, reducing the number of seedlings that established, and this limited the invasion locally of species that were invasive elsewhere in the region. Seed predation ought to be important in expanding populations, because those populations will be the most seed limited (Maron and Vilà 2001; Parker 2001). We expect the population-level response to seed addition (or predation) to be highly sensitive to this gradient in background plant density, reflecting how far the population is from equilibrium density at any point in space. However, when exotic plant density is high, seed predation may have no detectable effect on population persistence if there is a large seedbank or if the invader is superabundant and fecund (Noble and Weiss 1989; Hoffmann and Moran 1998; Parker 2000), or if there is a strong compensatory response in the surviving plants, as in this study.

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