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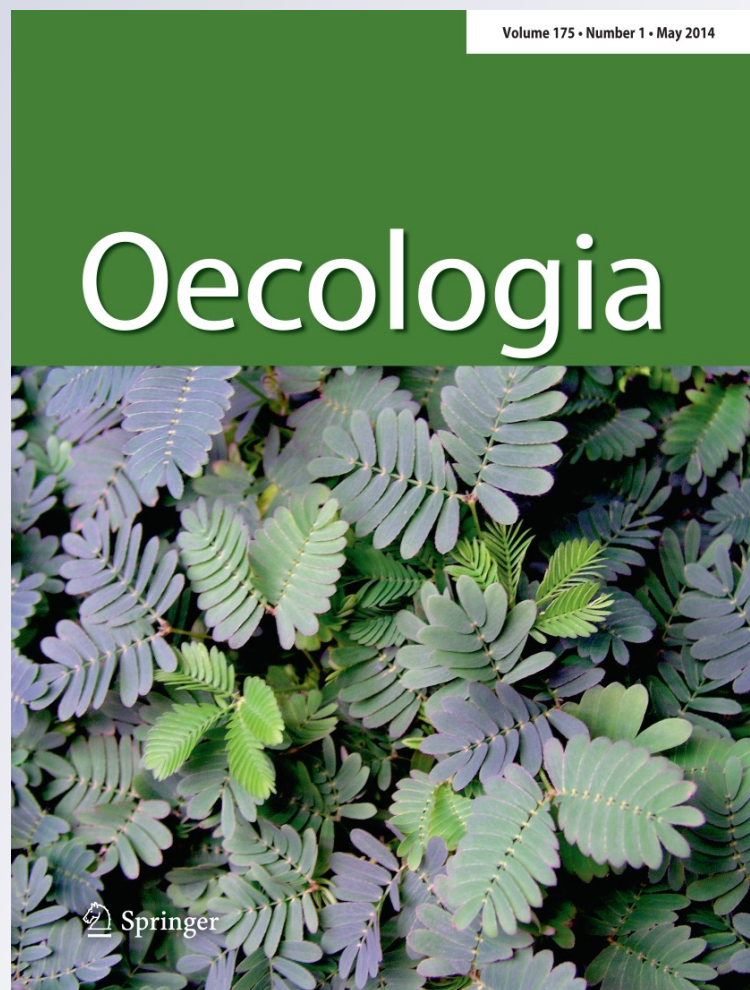
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Native insect herbivory limits population growth rate of a non-native thistle

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Abstract The influence of native fauna on non-native plant population growth, size, and distribution is not well documented. Previous studies have shown that native insects associated with tall thistle (*Cirsium altissimum*) also feed on the leaves, stems, and flower heads of the Eurasian congener *C. vulgare*, thus limiting individual plant performance. In this study, we tested the effects of insect herbivores on the population growth rate of *C. vulgare*. We experimentally initiated invasions by adding seeds at four unoccupied grassland sites in eastern Nebraska, USA, and recorded plant establishment, survival, and reproduction. Cumulative foliage and floral herbivory reduced *C. vulgare* seedling density, and prevented almost any reproduction by *C. vulgare* in half the sites. The matrix model we constructed showed that this herbivory resulted in a reduction of the asymptotic population growth rate (λ), from an 88 % annual increase to a 54 % annual decline. These results provide strong support for the hypothesis that indigenous

herbivores limit population invasion of this non-native plant species into otherwise suitable grassland habitat.

Keywords Biotic resistance · Ecosystem service · Invasion · *Cirsium vulgare* · Bull thistle

Introduction

The majority of introduced plants fail to establish or remain at low density in their novel environment, while only a few have become highly invasive (Williamson and Fitter 1996). The influence of native fauna as a factor contributing to cases of failed plant invasions remains under-evaluated experimentally (Eckberg et al. 2012). The two dominant hypotheses have focused on how either escape from herbivores (enemy release) or accumulation of new herbivores (biotic resistance) affect the outcome of plant invasions (Maron and Vila 2001; Levine et al. 2004; Williams et al. 2010). We know that biotic factors, such as native herbivores, can reduce survival, growth, and fecundity of non-native plants (Maron and Vila 2001; Levine et al. 2004; Eckberg et al. 2012; Suwa and Louda 2012). However, we still need quantitative, demographic assessment of the consequences of such effects by native biota on the population dynamics and density of non-native plants in their new environments. Herbivory effects on specific plant life stages, even when severe, may have limited influence on population growth, density, or distribution (Parker 2000; Maron and Vila 2001; Levine et al. 2004). Furthermore, there is often large spatial variation in the effect of herbivory on plant population dynamics (Louda 1982, 1983; Eckberg et al. 2012). Thus, population-level experiments at multiple sites or habitats are required, as has been done for native plant populations (Miller et al. 2009). Matrix models

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are useful for illustrating how effects of herbivory on plant individual parameters (i.e., survival, growth, and fecundity) jointly affect the asymptotic rate of population growth (Caswell 2001). Population-level studies of herbivory on plant invasion will help us better understand if, and how much, herbivores provide meaningful biotic resistance to plant population invasions.

Most studies of the influences of herbivory by native species on exotic plant performance and density are conducted in areas where the invader is already established and abundant (Levine et al. 2004). This approach precludes a rigorous assessment of the effects of native insect herbivory (biotic resistance) on the critical initial phase of invasion: plant population establishment. Our previous study showed that herbivory significantly reduced the rate at which the Eurasian *C. vulgare* seedlings invaded new grasslands, an effect that intensified with lower propagule pressure (Eckberg et al. 2012). However, the effect of herbivory on population growth rate for newly established *C. vulgare* invasions remains unknown.

In this study, we experimentally evaluated whether biotic resistance contributes to the regional sparseness of *C. vulgare* [(Savi) Ten.] (bull or spear thistle) in the tallgrass prairie region of Nebraska, as hypothesized by Andersen and Louda (2008). This thistle has been recorded in Nebraska for over 105 years (Kaul et al. 2006). While it is classified as a noxious invasive weed in three neighboring states, Iowa, Minnesota, and Colorado (USDA 2012), it is relatively sparse in eastern Nebraska (Andersen and Louda 2008). These observations lead to the question: what limits this species in establishing populations in uninvaded areas? We know that native insects that specialize on the co-occurring native tall thistle [*Cirsium altissimum* (L.) Spreng] in tallgrass prairie spillover and cause a 50–88 % reduction in the seed production of *Cirsium vulgare* (Louda and Rand 2002; Takahashi 2006; Suwa et al. 2010), leading to our further investigation of plant population level effects of insect herbivory.

Previous work to model the population growth rate of *C. vulgare* relied heavily on data from its native range or the native congener, *C. altissimum* (Tenhumberg et al. 2008). Those model results suggested a growing *C. vulgare* population that was inconsistent with quantitative field observations (Andersen and Louda 2008). Our first experiment showed that greater propagule pressure and experimental reduction of herbivory led to the highest initial *C. vulgare* seedling density (Eckberg et al. 2012); however, in that study, we did not evaluate the effects of herbivory on plant population growth. In this study, we experimentally introduced *C. vulgare* into multiple currently uninvaded grassland sites in Lancaster and Saunders Counties, NE, USA. Using insecticide treatments, we quantified the parameters affected by native thistle-feeding insect herbivory on

the initial phases of population establishment and growth. Using these parameters, we then developed a matrix model to assess the role of such herbivory in population growth rate (λ).

Materials and methods

Natural history context

Cirsium vulgare is a short-lived, monocarpic perennial (Bullock et al. 1994). In our study region of eastern Nebraska, *C. vulgare* remains in the rosette form for at least 1 year, grows to flowering size, and then flowers late July into November (personal observation). Native thistle specialist insects spillover from the phenologically synchronized native *C. altissimum* (tall thistle); for example, 84.3 % of the 129 insect species found feeding on *C. altissimum* here were also recorded feeding on *C. vulgare* (Louda and Rand 2002; Takahashi 2006). The herbivores on *C. vulgare* belonged to several feeding guilds: stem borers and floral, phloem, and leaf feeders, and were primarily of native origin (Takahashi 2006). For example, timed observations conducted throughout the growing season at two sites in Lancaster County, Nebraska revealed that 97 % of the individuals identified on *C. vulgare* were native species (ESM 1). The primary non-native insect herbivore on *C. vulgare* in eastern Nebraska is *Trichosiromus horridus* (ESM 1); specifically, in a survey of 13 sites in Lancaster County adult *T. horridus* were found in fewer than 10 % of *C. vulgare* shoots (Takahashi et al. 2009).

Study sites

We initially established this experiment at nine grasslands in central Lancaster and southern Saunders Counties, NE, USA. However, five sites were removed from further consideration because *Cirsium vulgare* seedling density and/or size in the insecticide treatment were substantially lower (ESM 2). Therefore, this study quantifies the effects of insect herbivory on population growth in sites that were shown to be favorable to *C. vulgare* growth and survival. The average size of surviving seedlings at sites 8 and 9 was much greater than that at all other sites. However, this reflected very few individuals concentrated in 20 % of the plots positioned on the far west side of the site, and so sites 8 and 9 were deemed to have poor growing conditions and were excluded from further data collection. The four remaining grassland sites were in southern Saunders County (sites 1–3) and central Lancaster County (site 4). In these four sites, native thistle *C. altissimum* was present but *C. vulgare* was absent. Plots at sites 1 and 2 were in the same field (separated by >45 m) which was dominated by

the native tallgrass *Andropogon gerardii*, the subshrub *Apocynum cannabinum*, and the forb *Calystegia sepium*. Site 3 was separated from sites 1 and 2 by a forest stand and dominated by three grasses, *A. gerardii*, *Spartina pectinata*, and *Phalaris arundinacea*, and by the subshrub *A. cannabinum*. Sites 1–3 were hayed regularly in the decade prior to the study, and all flooded temporarily in September 2006 and August 2007. In 2007, we washed soil off of *C. vulgare* plants as a result of the flood. Site 4 grassland was located 44 km away from sites 1–3 and was dominated by the Eurasian grasses *Bromus inermis* and *Poa pratensis*.

Experimental design

At each of our four study sites in 2005 (14–18 April), we introduced ~536 *C. vulgare* seeds into each of five insecticide and control (no insecticide) plots (2 × 2 m) ($n = 40$ plots; 5 plots/herbivory treatment/site). Within each study site, plots were established along two parallel transects (insecticide versus control) at five distances (0, 7.5, 15, 30, and 60 m) from a local *C. altissimum* patch. However, because we detected no significant distance effects (all $P > 0.11$) or distance × insecticide interactions (all $P > 0.20$) for any response variables, distance to *C. altissimum* patch was removed from the final analysis (ESM 3). The seeds introduced into each plot were collected from naturalized *C. vulgare* in eastern Nebraska (2001–2004). To estimate the seeding rate, we recorded the mass of 20 subsamples containing 100 filled, undamaged seeds (0.2476 g/100 seeds ± 0.0024 g SE). In a similar study, we observed an 87.1 % germination rate among filled, undamaged seeds (Eckberg et al. 2012). The 536 total seeds added to each plot included a mass equivalent to 486 seeds plus 50 seeds (~9 %) that were first exposed to 5 weeks of fluctuating spring air temperatures (stored in an unheated building) as such conditions can increase germination (Huarte and Benech-Arnold 2005). We disturbed (raked) the soil of each plot prior to seeding (plot establishment details in ESM 3).

Insecticide treatment

To reduce herbivory, we sprayed with bifenthrin, a non-systemic, pyrethroid insecticide (Control Solutions, Pasadena, TX, USA), using the recommended concentration of 0.06 %. Spraying began shortly after emergence in the spring (end of May) and continued through the end of August–mid-September. We sprayed plots at 3-week intervals, except that bolting plants were sprayed at 1- to 2-week intervals when flowering shoots were elongating rapidly (late June–early August). To minimize effects on pollinators, we did not directly spray open flowers. Control treatments were not sprayed with water only. Although water

addition could have enhanced growth of insecticide-treated plants, it is unlikely, as only an estimated 1.2–1.9 mm of water was added to the leaf surface during each application of insecticide. In total, only 7.1–15.1 mm of water was added annually to each insecticide plot, representing a 2.0, 3.7, and 2.0 % increase in rainfall from April–September for 2005, 2006, and 2007, respectively.

Bifenthrin application did not affect survival or growth of *C. vulgare* in the greenhouse (Eckberg et al. 2012). We treated entire plots with insecticide because large plot sizes and thistle densities made it logistically difficult to treat individual plants. Further, background vegetation was clipped and maintained at a height of ~25 cm in both treatments to minimize possible positive effects of insecticide on heterospecific competitors. Despite the clipping, however, the insecticide treatment increased heterospecific plant cover (ESM 4). Heterospecific competitors can reduce growth and seed production of *C. vulgare* (Suwa et al. 2010; Suwa and Louda 2012). Therefore, the increase in heterospecific cover associated with the insecticide treatment makes our estimate of the effect of herbivory on *C. vulgare* population growth a conservative one.

Measurements

Seedlings and juvenile rosettes

In 2005, seedling densities established were quantified at the end of the first growing season (end of September–early October) in all 40 plots. On up to 16 randomly selected *C. vulgare* seedlings in each plot at the end of the first growing season, we visually estimated the percent leaf chewing/removal by insect herbivores for each leaf and then recorded the percent leaf removal for the whole plant as the average removal from each leaf (total $n = 593$ seedlings). In the subsequent years, we recorded *C. vulgare* density in mid-May (2006) or end of June (2007) to estimate annual survival of the cohort. However, for site 4, we quantified *C. vulgare* density in 2008 (early June), rather than in 2007.

Fecundity

In years 2 (2006) and 3 (2007), we recorded the number of plants flowering in all plots, and we estimated seed production for each flowering *C. vulgare* ($n = 151$; 18.9 flowering *C. vulgare* on average/site/treatment). To estimate seed production, we collected all flowers that opened and matured from all flowering plants (end of July–October 2006; August–November 2007). Of the total 1,822 heads collected, only 14 (0.77 %) had dispersed seed partially or entirely before collection (12.1 heads on average/flowering *C. vulgare*; 97.7 seeds on average/flower head). In 2006, we collected flower heads after florets turned dark

brown and developing seeds were likely to be mature. In 2007, we covered the flower heads with a fine mesh once the florets turned brown, allowing complete seed maturation on the plant prior to collection. Seed numbers per flower head did not significantly differ between years (linear mixed effect model: $t_{145} = 1.30$, $P = 0.19$), suggesting that different collection procedures did not affect the seed production estimate. We determined the total seed mass for each flowering individual, and we then counted the seeds in a sub-sample that represented on average 10 % of the total seed mass. We used the seed number to seed mass ratio to estimate whole plant seed production. Cumulative seed production was calculated as the total seed production among all flowering plants per plot. The cumulative measurement included the fate of 94.9 % of the initial seedling cohort, those that either died or flowered by spring 2008 (insecticide 93.6 % vs. control 96.2 %), leaving only 5.1 % of the cohort alive at the end of the experiment.

Statistical analyses

We analyzed the data using linear mixed effect (LME) models in R (R Development Core Team 2005). Spray treatment (insecticide vs. control) was treated as a fixed effect. Site and plot within site were incorporated as random effects for each relevant response variable (ESM 5). We included a combined site by year random effect for variables with repeated measures (flowering plant density, fecundity) across 2 years. We also included a spray treatment by site interaction term to account for site-level variation in treatment effects. Response variables were: seedling density (number per plot), number of flowering individuals per plot (2006 and 2007), seed production per flowering individuals (2006 and 2007), and cumulative seed production per plot. We log-transformed ($\log+1$) number of flowering individuals and cumulative seed production. Proportion leaf damage ($+0.01$) was logit-transformed (Warton and Hui 2011). The performance data quantified from the experimental plots were used to parameterize the matrix model.

Matrix model

We constructed a population matrix model using two stages: seeds (S) and plants (P). The model considered four transitions, $S \rightarrow S$, $S \rightarrow P$, $P \rightarrow S$, and $P \rightarrow P$. We chose a time step of 1 year, and the annual population census occurred in the fall after seed development. So, the model is a birth pulse, post-breeding model. All matrix model analysis was performed using R (R Development Core Team 2005).

Seed–seed transition ($S \rightarrow S$)

To transition from the seed stage to the seed stage, the seeds that do not germinate in the first season must survive in the seed bank into the second season. The seed bank reported for *C. vulgare* is very small, with little effect on population growth rate (Klinkhamer et al. 1988; Tenhumberg et al. 2008). In this study, we found that 0.22 % (± 0.08 % SE) of the seeds germinated in the second year implying a very low seed to seed transition. Further, any new and surviving recruits were counted in subsequent censuses. We therefore set the seed–seed transition rate to zero in our model; and, we evaluated the effect of this parameter by varying it from 0 to 0.20 (ESM 6).

Seed–plant transition ($S \rightarrow P$)

To transition from the seed stage to the plant stage, the seed must survive the winter, germinate in the spring, and then survive until the population census in the fall. We estimated rate of seedling establishment (a) as the number of new live plants at the end of the first growing season (2005) divided by the number of sown seeds (April 2005). This estimate does not include the potential post-dispersal predation of seeds, which could be a major factor reducing the number of seeds from seed production to germination in the following spring. If the probability of surviving post-dispersal seed predation is q , the seed–plant transition is

$$S \rightarrow P = aq$$

In the literature, post-dispersal predation of seeds in the native range varied between 18.5 % (The Netherlands) and 67 % (United Kingdom) (Klinkhamer et al. 1988; Bullock et al. 1994); thus, in our model, we assumed that post-dispersal seed predation was 0.4, near the middle of the observed range. So, $q = 1 - 0.4 = 0.6$. However, due to the high uncertainty of q in the introduced range, we also examined the effect of varying q from 0.1 to 1.0 on model predictions.

Plant–seed transition ($P \rightarrow S$)

To transition from the established plant stage to the seed stage, the juvenile plant must survive the winter, flower, and produce viable seeds. We followed a cohort of plants from 2005 to 2008 after which almost all plants either died or flowered and died (*C. vulgare* is monocarpic). We counted the number of plants in the spring, and the number of flowering plants each fall, and calculated the average probability of plants surviving and flowering in a single year as the geometric mean of survival and fecundity over the two annual periods as follows:

$$s_f = \sqrt{wgbh}$$

where w denotes survival from the end of growing season 1 (fall 2005) to beginning of season 2 (spring 2006), g is the proportion of plants alive in spring that flowered in the fall, b is the survival of the remaining immature plants until the beginning of season 3 (spring 2007), and h is the proportion of plants that flowered at the end of season 3 (fall 2007). Although using the geometric mean over two observation periods ignores annual variation in s_f , there were too few data to estimate s_f for each year separately. We determined the number of seeds produced by a flowering plant, F , as the arithmetic average of seed production per plot in 2006 and 2007. So,

$$P \rightarrow S = s_f F$$

At site 4, spring plant numbers were recorded only in spring 2006 and 2008. Assuming that both proportion of plants flowering and mortality rate were constant over these 2 years, we estimated survival, b^* , as the square root of the proportion of 2006 plants that were alive and did not flower by 2008. Given the increased uncertainty in survival at site 4, we varied b^* from 0.1 to 0.9 (ESM 7).

Plant–plant transition ($P \rightarrow P$)

To transition from plant stage to plant stage (persist in the plant stage as a rosette), the immature plant must survive for a year and not flower:

$$P \rightarrow P = \sqrt{w(1-g)b(1-h)}$$

This transition was observed during the 2nd to 3rd year for the cohort (or 2nd to 4th year at site 4) when plants were generally in rosette developmental stage.

Population growth rate (λ)

The dominant eigenvalue of the population matrix gives the population growth rate, λ . We estimated the effect of parameter uncertainty using Monte Carlo simulations. We created 10,000 sample matrices assuming that fecundity was log-normally distributed and the probability of surviving and flowering followed a beta distribution. The log-normal distribution was specified by the mean (μ) and the variance (σ^2) that were calculated as

$$\mu = \log(\bar{F}) - 0.5 \log\left(1 + \frac{\sigma_{\bar{F}}^2}{\bar{F}^2}\right), \text{ and } \sigma^2 = \log\left(1 + \frac{\sigma_{\bar{F}}^2}{\bar{F}^2}\right)$$

where \bar{F} is average seed production per plant for each treatment and site. The beta distribution was specified by the scale parameters α and β .

$$\alpha = s \left(\frac{s(1-s)}{\sigma_s^2} - 1 \right), \text{ and } \beta = (1-s) \left(\frac{s(1-s)}{\sigma_s^2} - 1 \right)$$

where s depicts the proportion of successes (i.e., mean survival), and σ_s^2 its variance. We used the mean and the sampling variance of the data to specify the distributions to construct the sample matrices. In some cases, the transition rates were calculated using several parameter estimates, e.g., calculating s_f involved four parameter estimates w , g , b , h ; in these cases, we used the Delta Method (Powell 2007) to estimate the sampling variance. For each of the 10,000 sample matrices, we calculated the dominant eigenvalue. We report the median and the 5th and 95th percentiles of the resulting distribution of λ values.

Results

Seedling herbivory and density

Insecticide treatment of seedlings significantly reduced foliage herbivory on new seedlings in their first year (2005: $t_{35} = -8.26$, $P < 0.001$; ESM 5). Overall, leaf area damaged was reduced from 15.5 to 1.5 % per plant (Fig. 1a). Among sites, the treatment effect was generally consistent, and there was low variation in leaf damage (Fig. 1a).

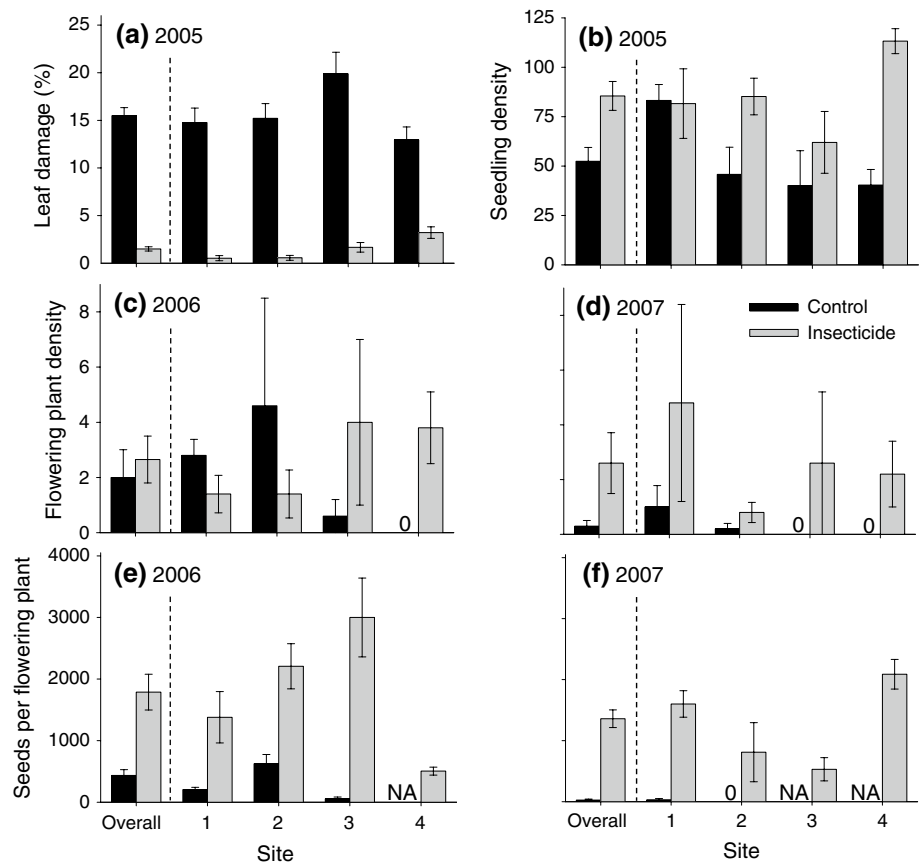
The insecticide treatment led to significantly higher seedling density by the end of the season ($t_{35} = 2.11$, $P = 0.042$; ESM 5). Herbivory reduced seedling density by 39 % overall; however, the magnitude of the effect varied by site, ranging from virtually no effect at site 1 to large (35–64 %) reductions in seedling density at the other three sites (Fig. 1b).

Flowering plant density and fecundity

Insecticide treatment increased the density of flowering plants (2006, 2007), but this effect was only marginally significant overall ($t_{71} = 1.96$, $P = 0.054$; ESM 5). The magnitude of the effect varied among sites and years (Fig. 1c, d). Herbivory reduced flowering plant density by 25 % in 2006 and by 89 % in 2007 (Fig. 1c, d). Even more striking, herbivory prevented all flowering at site 4 in both 2006 and 2007 and at site 3 in 2007.

Herbivory caused a significant reduction in fecundity of the *C. vulgare* plants that succeeded in flowering ($t_{24} = 3.53$, $P < 0.002$; ESM 5). Depending on the site and year, herbivory reduced fecundity between 72 % (site 2 in 2006) to 100 % (site 2 in 2007) (Fig. 1e, f). The decrease in fecundity was caused by a 79 % reduction in seed production per flower head ($t_{24} = 8.92$, $P < 0.001$) while the average number of mature flower heads per plant did not differ

Fig. 1 Differences in performance of *Cirsium vulgare* in response to insecticide treatment across sites and overall: **a** percent leaf area damage on seedlings; **b** number of seedlings per plot at the end of the first growing season (2005); **c, d** number of flowering plants per plot in 2006 and 2007; **e, f** number of viable seeds produced per flowering plant in 2006 and 2007. Bars mean \pm SE; NA indicates that no seed production occurred because *C. vulgare* did not produce mature flowers; a 0 indicates *C. vulgare* flowered, but did not produce any viable seeds. Gray bars insecticide treatment, and black bars unsprayed control treatment



significantly between the insecticide and control treatments ($t_{24} = 1.27, P = 0.220$).

Cumulative seed production

To integrate the cumulative effects of herbivory on density of seedlings, flowering plants and plant fecundity, we quantified the cumulative number of *C. vulgare* seeds produced per 4-m² plot over a 2-year period (2006 + 2007; Fig. 2). Herbivory in the control treatment caused significant reductions in cumulative seed production relative to insecticide-treated plots ($t_{35} = 3.19, P = 0.003$; ESM 5); the decrease averaged nearly an order of magnitude: from 8,314 to 878 seeds per 4-m² plot (Fig. 2). Because 60 % of control plots produced no viable seeds, median seed production was zero for the control treatment versus 4,154 seeds per plot for the insecticide treatment. The effect of herbivory was large at three of the four sites. The weak effect of herbivory at site 2 reflects one anomalous control plot that produced 13,821 seeds, compared to <1,250 seeds produced in all other control plots ($n = 20$). Overall, the insecticide treatment demonstrated that herbivory significantly reduced seedling and juvenile density, flowering plant density, and individual plant fecundity. This led to a highly significant reduction in cumulative seed production by *C. vulgare* under most conditions.

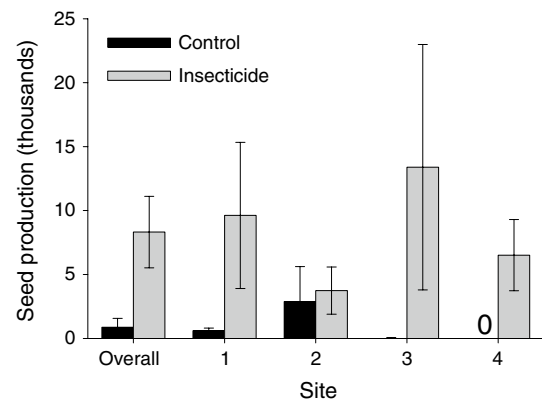


Fig. 2 Cumulative seed production per 4-m² plot over both seasons, overall and among sites. Bars mean \pm SE; $n = 40$ plots (5 plots/treatment/site)

Asymptotic population growth rate

The matrix model demonstrated that ambient levels of insect herbivory significantly reduced the median asymptotic population growth rate (λ) of *C. vulgare* from 1.88 to 0.46 (Fig. 3). Thus, herbivory led to an annual population decline of 54 % compared to an 88 % annual increase with experimentally reduced herbivory within the same sites.

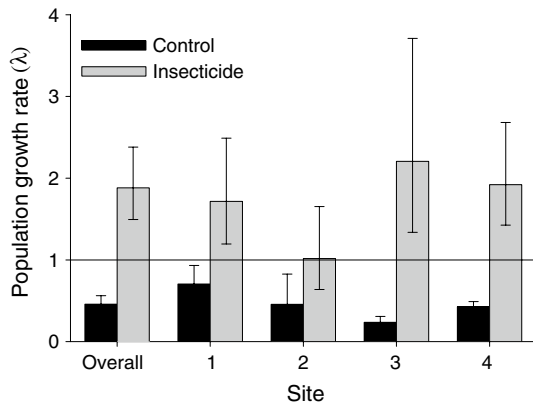


Fig. 3 Asymptotic annual rate of population growth (median $\lambda \pm 5$ th and 95th percentiles) for each site and herbivory treatment. Population stasis is indicated by the *reference line*, $\lambda = 1$

The median rate of decline in λ varied from 30 to 77 %, depending upon the site. With experimentally reduced herbivory, our model predicted that *C. vulgare* populations had the potential to be stable (λ not significantly different from 1) at site 2 and to increase by an annual rate of 72–121 % at the other sites (Fig. 3). Individual transitions ($S \rightarrow P$, $P \rightarrow P$, $P \rightarrow S$) by herbivory treatment and site are presented in ESM 8.

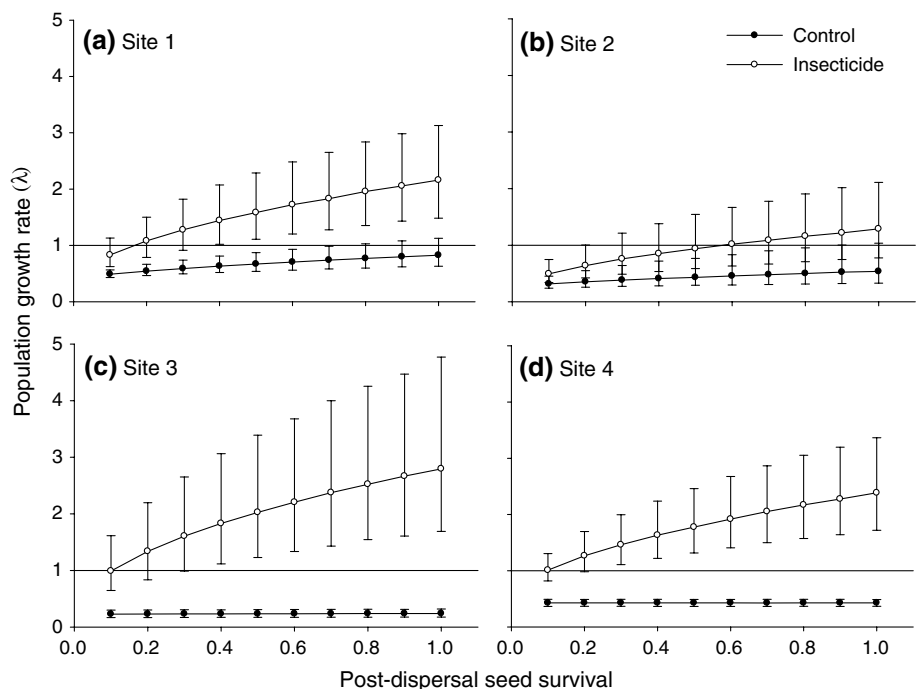
To evaluate uncertainty in post-dispersal seed survival (q), we varied q in the matrix and determined its effect on λ . The model suggested a median population decline for *C. vulgare* exposed to ambient levels of insect herbivory

across all values of q and in all sites (Fig. 4). The 95th percentile overlapped with $\lambda = 1$ only in site 1 for $q \geq 0.8$ and site 2 for $q \geq 0.9$; in all other sites, the 95th percentile was below $\lambda = 1$ (Fig. 4). Further, we investigated other sources of uncertainty and found that herbivory reduced λ below 1 for a wide range (0–0.20) of seed-to-seed transitions (ESM 6) and, at site 4, for a very wide range of plant-to-plant transitions (0.1–0.9; ESM 7). Thus, it is clear that ambient herbivory can cause *C. vulgare* populations to decline even with virtually no decrease in seed survival from post-dispersal seed predation.

Discussion

A continuing challenge in ecological research is to better understand the extent to which native fauna limit the invasion success of non-native plants (Maron and Vila 2001; Levine et al. 2004; Mitchell et al. 2006; Eckberg et al. 2012; Suwa and Louda 2012). Our study demonstrated that native insect herbivory severely impacted *Cirsium vulgare* demographic rates, from seedling to adult seed production stage, and led to significant reductions in the estimated asymptotic rate of population growth (λ). With experimental reduction of herbivory by native insects, *C. vulgare* populations increased by 88 % annually; yet, with ambient levels of such herbivory, populations declined by 54 % annually. Population decline primarily resulted from a drastic reduction in the plant-to-seed ($P \rightarrow S$) transition, from 29.8 to 0.8. This transition included herbivory impacts on: (1) *C.*

Fig. 4 Asymptotic annual rate of population growth (median $\lambda \pm 5$ th and 95th percentiles) across sites in relation to herbivory treatment and post-dispersal seed survival. Population stasis is indicated by the *reference line*, $\lambda = 1$



vulgare flowering plant density (57 % less, Fig. 1c, d); (2) individual plant fecundity (87 % less, Fig. 1e, f); and (3) plant survival (12 % less; ESM 8). These results are based on a simple, two-stage matrix model; population growth rate is not influenced by the number of stages included in a matrix model (Tenhumberg et al. 2009; Salguero-Gómez and Plotkin 2010). These findings are consistent with experimental studies showing that insect herbivory often severely reduce the fecundity of thistles (Louda and Potvin 1995; Rose et al. 2005, 2011; Suwa et al. 2010). Similar to the native *C. altissimum*, the late flowering time of *C. vulgare* combined with the occurrence of late-season herbivores, such as *Homoeosoma eremophasma* (Pyralidae) (Takahashi 2006), likely prevent successful compensatory seed production (Rose et al. 2011).

The insect assemblage feeding on *C. vulgare* was shown to be highly similar to the native tall thistle, *Cirsium altissimum* (Takahashi 2006). These insects also reduce population growth of the native *C. altissimum* (Rose et al. 2011). However, the results here demonstrate that the magnitude of the effect was more severe for the non-native *C. vulgare*. We found that the $\Delta\lambda$ (λ herbivores absent – λ herbivores present) was 1.42 for *C. vulgare* (above), compared to 0.80 for *C. altissimum* (Rose et al. 2011). Further, the effects of native insect herbivory on *C. vulgare* population growth quantified here were more severe than those observed on average in other native plant populations (average $\Delta\lambda = 0.04$) (Maron and Crone 2006; Miller et al. 2009) and in populations of another related native thistle species, *Cirsium canescens* ($\Delta\lambda = 0.33$) (Rose et al. 2005). Thus, the data here provide strong support for the new associations hypothesis (Parker et al. 2006), since native herbivores caused greater population-level impacts on the non-native plant compared to the native congener.

We infer that intense herbivory on *C. vulgare* by thistle-feeding insects was facilitated by the presence of the native, phenologically synchronized congener, *C. altissimum* (tall thistle), at all study sites. Native *C. altissimum*, the most common congener in our system, has been shown to provide a source of native thistle-feeding herbivores which are hypothesized to contribute to the regionally sparse distribution of *C. vulgare* in eastern Nebraska (Louda and Rand 2002; Andersen and Louda 2008). Further, herbivory on *C. vulgare* intensifies with proximity to *C. altissimum* on the local scale (Andersen and Louda 2008). Also, native thistle-feeding insects reduce the survival, growth, and fecundity of individual *C. vulgare* across multiple sites in eastern Nebraska (Suwa et al. 2010; Eckberg et al. 2012; Suwa and Louda 2012). Finally, the current study extends these results by showing strong effects of herbivory on the *C. vulgare* population growth rate across multiple sites where we experimentally introduced this thistle.

The results also suggest that native insects can virtually exclude *C. vulgare* from otherwise suitable habitats. At each site, when insect pressure was reduced by insecticide, the mean population growth rate was above 1 ($\lambda = 1.02$ – 2.21), compared to below 1 ($\lambda = 0.24$ – 0.71) under ambient levels of insect herbivory (Fig. 3). Native herbivores also limit native plant density and distribution (Louda 1982, 1983; Louda and Potvin 1995; Louda and Rodman 1996; Miller et al. 2009; Rose et al. 2011). For example, Louda and Rodman (1996) experimentally demonstrated that native insects, not physiological constraints, virtually excluded the native crucifer *Cardamine cordifolia* from dry, open-sun environments in their native range. The current study documents an important role of insect herbivores in the site distribution and local density of a known invasive non-native plant. Thus, our study adds to a small but growing number of cases suggesting that biotic factors, rather than climate matching, can significantly influence the spatial dynamics of non-native plant invasions (Maron 2006; Gallagher et al. 2010).

We specifically focused parameter estimation on the four sites with favorable growing conditions. At these sites, *C. vulgare* seedling densities and/or sizes were higher in our insecticide-treated plots than at five other sites (ESM 2). Though the effect of herbivory on *C. vulgare* seedling survival is generally more severe with poor growing conditions (Eckberg et al. 2012), subsequent survival or reproduction in the 2nd and 3rd years are usually very low with poor growing conditions, even with the insecticide treatment (Eckberg, unpublished data). Population growth rates for both control and insecticide may be much lower at such sites, and *C. vulgare* populations may decline even in the absence of herbivory. Further, we reduced heterospecific competition in our experimental plots by mechanical disturbance and clipping, and we know that removal of heterospecific competition can enhance *C. vulgare* survival, biomass, and flowering (Suwa and Louda 2012). Thus, we clearly show that herbivores consistently suppress *C. vulgare* population growth in sites with favorable growing conditions and with experimentally lower competition. The combined results on *C. vulgare* now suggest that herbivory operates as a significant filter to population establishment and growth (Tenhumberg et al. 2008; Suwa et al. 2010; Eckberg et al. 2012; Suwa and Louda 2012), and contributes to the observed regional sparseness of *C. vulgare* in the tallgrass prairie ecosystem of eastern Nebraska, as previously hypothesized (Louda and Rand 2002; Andersen and Louda 2008).

The effects of foliage and floral herbivory on *C. vulgare* seed production were even more severe than previously observed on established *C. vulgare* plants in existing populations (Louda and Rand 2002; Suwa et al. 2010). Suwa et al. (2010) showed that established *C. vulgare*

rosettes exposed to herbivory still produced between 1,000 and 19,000 seeds, much higher than the 0–625 seeds observed in this study (Fig. 1e, f). Additionally, Tenhumberg et al. (2008), using demographic data reported in the literature to construct a matrix model, found that *C. vulgare* should increase by 53.8 % ($\lambda = 1.538$) annually in eastern Nebraska, even with observational data on herbivory included (Louda and Rand 2002). A major difference between this study and Tenhumberg et al. (2008) is that we used experimental data in situ to parameterize our model, compared to relying on the global literature to provide estimates of previously unknown parameters. A key difference that emerged was in seed production after losses from herbivory: we found viable seed production to be less than one-third (382 seeds) of the best estimate from the literature used in the previous matrix model (1,283 seeds) (Tenhumberg et al. 2008).

One implication of this finding is that the effects of herbivory on non-native plant populations may be underestimated by studies (most of them) that focus on populations that are already established and flourishing. Established plant populations must have already overcome some of the effects of herbivory (Levine et al. 2004). For example, herbivory had almost no effect on population growth rate of several highly invasive plants (Schutzenhofer et al. 2009; Williams et al. 2010). *C. vulgare* could become locally abundant in sites with lower herbivory effects (Eckberg et al. 2012) or high disturbance resulting from grazing (Bullock et al. 1994). By studying *C. vulgare* in our experimentally introduced populations, we found that herbivory can cause population decline in the early stages of a *C. vulgare* invasion, an effect that could prevent population establishment. Because the majority of studies on biotic resistance by herbivory focus on established non-native plant populations (Levine et al. 2004), further studies are needed to understand the extent to which herbivory limits the establishment and growth of non-native plant populations.

In conclusion, herbivory by resident insect herbivores substantially reduced the establishment and population growth of *C. vulgare*, a known worldwide invasive plant species, in the western tallgrass prairie region. These findings contribute to the growing body of evidence that herbivore-mediated biotic resistance can be particularly intense for non-native plants with ecologically similar congeners (Louda and Rand 2002). This study tests this hypothesis by experimentally evaluating demographic parameters, and it shows that native insect herbivores can cause a severe decline in population growth rate during the initial stage of a plant invasion, likely contributing significantly to the exclusion of this non-native plant from otherwise suitable habitat. Thus, the study provides strong support for the hypothesis that insect herbivores provide a valuable

ecosystem service—biotic resistance to *C. vulgare* invasion (Louda and Rand 2002). We infer that such severe effects of herbivory are often likely to go unnoticed if not studied experimentally at the very earliest stages of the invasion process.

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Conflict of interest The authors declare that they have no conflict of interest.

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