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Temporal- and density-dependent impacts of an invasive plant on pollinators and pollination services to a native plant

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Abstract. Pollinators and pollination services are under threat globally, and invasive plants have been implicated in their decline. Results of previous studies suggest that consequences of invasion for pollinators and plant–pollinator interactions are context specific. Investigating factors such as the density of an invasive plant and its phenology may provide a nuanced understanding of invasive species impacts. We conducted a 2-yr study in Montana to investigate how local pollinator abundance, richness, community composition, and visitation patterns varied with invasive *Centaura stoebe* density and phenology, and whether *C. stoebe* altered the reproduction of a co-flowering native plant, *Heterotheca villosa*, through changes in pollinator visitation. In an observational study, we found that during its peak bloom in August, *Centaurea stoebe* provided abundant floral resources to late-season pollinators. However, prior to *C. stoebe* bloom, native floral density and pollinator abundance and richness of these plots were lower compared to plots where *C. stoebe* was low or absent. Pollinator community composition in plots without *C. stoebe* was different compared to plots with *C. stoebe* (both high and low *C. stoebe* density), and these differences in pollinator composition strongly depended on the time of season. In an experimental study, we found that there was little evidence of competition between *C. stoebe* and *H. villosa* for pollinators at low relative densities of *C. stoebe.* Using experimental pollen supplementation, we observed no evidence of pollen limitation of seed set in *H. villosa* with increasing density of experimentally added *C. stoebe.* Our results suggest that the impact of an invasive plant on pollinators and plant–pollinator interactions depends on the relative density of the invasive plant and the timing of its bloom. Differences in pollinator visitation patterns over the growing season suggest that although *C. stoebe* provides abundant resources to late-season pollinators, displacement of native plants at high *C. stoebe* density may indirectly harm pollinators that are active before *C. stoebe* blooms or that prefer native plants. Based on our results, restricting *C. stoebe* to low densities may help mitigate negative repercussions to native plant reproduction and may even be beneficial to some pollinators.

Key words: *Centaurea stoebe*; competition; *Heterotheca villosa*; Montana; mutualism; plant–pollinator networks; spotted knapweed.

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Introduction

Invasive plants are both an instrument and a consequence of human directed environmental change (Vitousek et al. 1997). Invasive plants are of concern because they are associated with altered disturbance regimes (Brooks et al. 2004), decreases in native plant diversity (Kedzie-Webb et al. 2001), changes in trophic dynamics (Bezemer et al. 2014), and altered abundance (Vilà et al. 2011) or behavior of other organisms (Ortega et al. 2014). Like native plants, invasive plants often rely on mutualistic interactions with other organisms, such as pollinators, for their persistence and propagation. The integration of invasive plants into pre-existing mutualistic networks can have both direct effects on the organisms with which they interact (e.g., pollinators) (Fiedler et al. 2012, Davis and Cipollini 2014) and indirect effects on native plants in the ecosystem that rely on the same mutualistic partners (Muñoz and Cavieres 2008).

Both native and commercial pollinator species are currently experiencing population declines (e.g., Potts et al. 2010, Cameron et al. 2011, Burkle et al. 2013), and invasive plants have been implicated as one possible factor. However, studies documenting species-specific effects of invasive plants on pollinators range from being toxic to pollinator larvae at one extreme (Davis and Cipollini 2014) to boosting pollinator populations at the other by providing additional floral resources (Stout and Morales 2009). In most cases, invasive plants are visited by many native pollinators (Stouffer et al. 2014), but some research has found higher pollinator abundance and diversity in areas where an invasive plant is absent or has been removed compared to areas where it is present (Hanula and Horn 2011, Fiedler et al. 2012). Given that 87.5% of all angiosperms are animal pollinated (mostly by insects, but also birds, bats, reptiles, etc.) (Ollerton et al. 2011), invasive plants can have indirect impacts on native plant reproduction by altering pollinator population dynamics or behavior. Although two recent meta-analyses have found that invasive plants decrease visitation to and reproduction of co-flowering native species on average (Morales and Traveset 2009, Montero-Castaño and Vilá 2012), individual studies have found that some invasive plants facilitate pollinator visitation (McKinney and Goodell 2010, Woods et al. 2011) or have little or no impact (Dietzsch et al. 2011, Thijs et al. 2011, see Stout and Morales 2009 and Montero-Castaño and Vilá 2012 for a comprehensive list of papers).

Results of previous studies suggest that consequences of invasion for pollinators and plant–pollinator interactions are context specific, and investigation into factors that influence the outcome is ongoing. For instance, Morales and Traveset (2009) found that not only the presence, but the relative density of the invasive plant may influence pollinator populations and pollination services to co-flowering native plants. Since invasive plants have been found to compete with native plants for pollinators, it is expected that increasing density of an invasive plant would further decrease pollinator visitation to native plant neighbors (Stout and Morales 2009). However, the majority of previous studies on plant–pollinator interactions in invaded systems only tested the effect of the plant's presence/absence.

In addition to density-dependent consequences of invasion, the impact of an invasive plant could change over the course of the growing season. For instance, during the period when the invasive plant is in bloom it provides new floral resources to pollinators. However, since most flowering plants have a limited blooming period and pollinator species turnover can be rapid (Simanonok and Burkle 2014), only some pollinator species may benefit from the introduction of a new flowering plant. Therefore, it is important to consider the time period during the season when the invasive plant is not in bloom. If the invasive plant displaces native plants through competition, then those native floral resources (which might have bloomed at a different time) would no longer be available to pollinators (Stout and Morales 2009). The consequences of limited temporal availability of floral resources on pollinators have been investigated with mass flowering crops (Westphal et al. 2003, Persson and Smith 2013) but have been largely overlooked with other non-native plants.

The purpose of this 2-yr study was to investigate on a local scale how the density and phenology of *Centaurea stoebe*, one of the most widespread and problematic invasive plants in the western United States and Canada (Watson and Renney 1974, DiTomaso 2000), affected pollinator foraging choices, plant–pollinator interactions, and pollination of native plants. This work contributes information to existing knowledge of invasive plant impacts on pollinators and plant–pollinator interactions by combining observational and experimental studies to gain a more insightful picture of a single invasive plant in situ and to be able to make causal inferences. We tested not just the presence/absence of *C. stoebe*, but also investigated the role of density in influencing the outcome of competition for pollinators with native plants. Furthermore, we documented plant–pollinator interactions in *C. stoebe* invasions across the growing season, which adds a rarely studied temporal perspective to these interactions. Our specific questions included: (1) how do pollinator abundance, richness, visitation, and community composition vary with *C. stoebe* density in invaded areas, and do these relationships change over the course of the growing season, and (2) how does experimentally manipulated density of *C. stoebe* influence pollinator visitation to and pollen limitation of reproduction in *Heterotheca villosa,* a native plant commonly found co-flowering with *C. stoebe*?

Methods

We conducted our investigation over two years, beginning with an observational study of pollinator visitation across an invasion gradient in summer 2012. On the basis of results from this study, we manipulated invader floral density and experimentally assessed pollen limitation of reproduction in a co-flowering native plant in a field experiment in 2013. The methods and results of these two studies are presented separately and synthesized in the discussion.

Study species

Centaurea stoebe L. (Fam: Asteraceae) (spotted knapweed) is an exotic perennial forb currently listed as a noxious weed in 14 states and four Canadian provinces (Rice 2014). It reproduces by seed with output ranging from 5000 to 40,000 seeds per m^2, depending on moisture (Sheley et al. 1998). A study in Montana investigating the pollination biology of *C. stoebe* found that it is an obligate outcrosser visited most frequently by *Apis mellifera* L. (Fam: Apidae) and *Bombus bifarius* Cresson (Fam: Apidae) (Harrod and Taylor 1995).

To investigate the impacts of *C. stoebe* on native plant reproduction, we selected *Heterotheca villosa* (Pursh) Shinners (Fam: Asteraceae) (hairy false goldenaster) as the focal native plant for our 2013 experiment because it co-flowered and shared pollinators with *C. stoebe* at a majority of sites during the 2012 study (see Results section). This species is widely distributed in the United States and southern Canada (United States Department of Agriculture 2015). *Heterotheca villosa* reproduces by seed, and seed output is much reduced in the absence of pollinators (R. E. Irwin, *personal communication*).

2012 observational study

Field sites.—We chose nine sites in western Montana where *C. stoebe* was present (Figure S1 and Table S1). Sites were chosen based on several criteria: accessability, variation in location and abiotic conditions, sufficient area of infestation (c. 1000 m^2 or larger), presence of native flowering plants, and spatial separation of at least 900 m to ensure minimal movement of pollinators among sites (Gathmann and Tscharntke 2002).

Study design.—At each of the nine sites, we established three permanent 4 m × 4 m plots: one plot each in an area of high (High) and low (Low) *C. stoebe* density and one plot outside of the *C. stoebe* infested area (Out). The criteria used for plot selection were based on plant density with High containing >15 plants, Low containing 5–10 plants, and Out containing zero *C. stoebe* plants per square meter. Plots within a site were located at least 6 m apart, reflecting the scale at which pollinators make foraging decisions (Klinkhamer et al. 2001). Our study design focused on a small scale (investigating pollinator visitation within a field) for reasons of feasibility and to obtain a high level of detail. We recognize this limits our scope of inference to pollinator movement and behavior, and we can only speculate how our results on a small scale apply to a population or landscape scale.

Pollinator observations and flowering plant surveys.—We visited each of the nine sites once per week from 8 July to 21 August to conduct pollinator observations. Observations were performed during hours of peak pollinator activity (~09:00–17:00) and sunny, calm weather. During each visit we censused the flowers and inflorescences of all forbs by species (including *C. stoebe*) in each plot. Hereafter, we refer to each composite inflorescence as a "flower". This allows us to use consistent terminology and facilitates calculation of a meaningful metric of floral density. We observed each plot for two 15-min time periods (30 min total) and recorded frequency and identity of all plant-pollinator interactions. We defined "pollinator" as any insect contacting the reproductive parts of a flower. If the identity of a pollinator was unknown, it was captured using a hand net and kept for later identification. Insects were identified to lowest taxonomic unit possible.

Data analysis.—All statistical analyses were conducted using R statistical software version 2.15.1 (R Core Team 2012). Response variables of interest included: pollinator abundance, pollinator richness, pollinator visitation (total pollinator visits per flower over the 30 min observation), floral abundance, and floral richness. To assess changes in plant and pollinator metrics over time, we first calculated an average value of each response variable during four ecologically relevant phenological phases: pre-*C. stoebe* bloom, early *C. stoebe* bloom, peak *C. stoebe* bloom, and late in the *C. stoebe* blooming period (Fig. S2). This was done to avoid problems with missing data using repeated measures (some observation periods had to be eliminated due to bad weather), and to make the results more ecologically meaningful (weekly observations are biologically arbitrary). Observation periods were assigned to these phenological phases post hoc, with all plots within a site receiving the same designation during one visit. To make these assignments, we identified the week at each site where the high-density plot achieved its greatest number of blooming *C. stoebe* flowers (between 244 and 649 depending on the site). Weeks before the high- or low-density plots at that site had reached half this number were considered early bloom. Weeks after which the high- or low-density plots dropped below half this number were considered late bloom. Weeks that fell between were considered peak bloom. This strategy allowed each of the phases to occur on different dates at each site, which was desirable because simply grouping them by date misleadingly grouped observations that were experiencing different conditions (Fig. S2).

We employed generalized linear mixed effects models using the lme function with a Gaussian identity link in the nlme package (Pinheiro et al. 2013) to determine (separately) whether floral density and species richness, as well as pollinator abundance, richness and visitation differed among plots with varying *C. stoebe* densities and during different phenological phases. In each model, we included *C. stoebe* density levels, phenological phase, and their interaction as fixed effects and plot nested within site as a random effect. We transformed all response variables to natural log scale to meet the assumption of homoscedasticity.

Differences in pollinator community composition among invasion levels and phenological periods were analyzed using Permutational Multivariate Analysis of Variance (PERMANOVA). This technique is not meant for hierarchical study designs and although it can "nest" structures to account for repeated measures, it will use the incorrect degrees of freedom to estimate *P*-values for the higher level variable. To investigate significance (i.e., *P*-values) using the correct degrees of freedom, we performed analyses in two stages. First, we ln-transformed pollinator abundances to obtain a convex distribution of abundances which emphasizes differences in small values (rare species) and de-emphasizes small differences in large values (common species). We then averaged pollinator species abundances over the whole season for each plot and calculated the pairwise similarity in species composition among all plots using both an abundance-based (Bray–Curtis) and incidence-based (Jaccard) metric. We tested for differences in pollinator community composition among *C. stoebe* densities using the "adonis" function in the R vegan package (Oksanen et al. 2013). We used ordination (Nonmetric Multi-Dimensional Scaling) to visualize these patterns in pollinator communities at each of the *C. stoebe* densities using the labdsv package (Roberts 2012). Next, to investigate differences in pollinator community composition over time and the interaction between time and *C. stoebe* density, we performed PERMANOVAs on community similarities (based on both Bray–Curtis and Jaccard) with species abundances at each plot averaged within each of the four phenological phases rather than over the whole season. Explanatory factors included *C. stoebe* density, phenological phase, and their interaction. Site was also included as an explanatory factor and permutations were constrained within sites (using the "strata" argument) to account for nestedness and repeated measures. We obtained similar results for both Bray-Curtis and Jaccard metrics and therefore present results for the Jaccard metric in the Appendix (Fig. S4).

2013 manipulative study

Field site.—We conducted the manipulative study ~6 km southeast of Bozeman, Montana (Fig. S1). The vegetation is a *Festuca idahoensis*/*Agropyron caninum* plant community type (Mueggler and Stewart 1980). The site has a history of being grazed and is dominated by native forbs. *Centaurea stoebe* was not present at the site.

Study design.—We simultaneously implemented (1) an addition experiment with greenhouse-grown *C. stoebe* plants, and (2) a supplemental-pollen experiment to test the effects of *C. stoebe* density on *H. villosa* pollinator visitation and degree of pollen limitation of reproduction. We tested four *C. stoebe* density treatments: 0:1 (*C. stoebe* plant: *H. villosa* plant), 1:1, 2:1, and 4:1. These treatments were implemented in a complete randomized block design with replication at the field site. We chose five sampling "blocks" between 75 m and 2 km apart at the field site where *H. villosa* was abundant. In each of the five blocks, we selected eight pairs (2 replicates for each of the four density treatments) of *H. villosa* for experimentation, with a minimum of 6 m between pairs (Klinkhamer et al. 2001). Each *H. villosa* pair was randomly assigned to one of the four treatments (5 blocks × 4 *C. stoebe* density treatments × 2 replicates = 40 pairs total). One *H. villosa* plant of each pair was randomly assigned to be pollinated naturally while the other received supplemental hand pollination to determine if *H. villosa* reproduction was pollen limited and to account for spatial variability in environmental conditions.

We placed potted *C. stoebe* plants within 1 m of selected *H. villosa* plant pairs according to their assigned density treatment. Since *H. villosa* plants often grew very close together, all *H. villosa* flowers within 1 m of target *H. villosa* plants were clipped to distinguish target *H. villosa* plants from surrounding plants and to maintain treatment ratios. To estimate local floral density and diversity, we censused all flowers by species within a 3 m radius of each *H. villosa* pair ($28.3\ m^2$) once during peak *H. villosa* bloom (between 30 July and 18 August). We harvested and removed all *C. stoebe* seedheads from the site before they matured.

Pollinator observations.—We conducted observations during peak *H. villosa* flowering. Each *H. villosa* pair and their associated *C. stoebe* (if applicable) were observed in 30–60 min segments over a 2- or 3-d period for a total of 90 min per pair (40 pairs × 90 min = 60 total hours of observations). Pollinators were identified into groups in the field (i.e., honey bee, bumble bee, other native bees, fly, butterfly, wasp, beetle, or unknown if the insect moved too quickly to identify) instead of being captured so as not to alter their visitation patterns.

Reproductive output of Heterotheca villosa.—We visited each focal *H. villosa* plant every 1 to 3 d to hand pollinate all open *H. villosa* flowers in the supplemental pollination treatment. Flowers were hand pollinated using a small paintbrush to transfer pollen from flowers of non-target plants. When there was little pollen available on surrounding flowers (such as after a rain event) we hand pollinated by plucking non-target flowers from plants at least 5 m away from the target plants and gently rubbing their anthers against stigmas of target flowers. This method was generally avoided so as not to alter the local floral abundance. After anthesis and seed maturation, we collected all capitula on target *H. villosa* plants. We randomly subsampled 10 capitula per *H. villosa* plant in all replicates of 3 (of the 5) randomly chosen blocks. Seeds from individual capitula were counted and weighed to the nearest 0.0001 mg. Seeds less than 2 mm long were assumed to be aborted and were not included in the count or weight.

Data analysis.—We tested the effects of *C. stoebe* density on total pollinator visits per flower, solitary bee visits per flower, and fly visits per flower (the later two being the most frequent pollinator groups that visited *H. villosa*) to *H. villosa* simultaneously with Multiple Analysis of Variance (MANOVA) because of correlations among response variables. Treatment (number of *C. stoebe* plants) was the explanatory variable and block was a blocking variable. In addition, to assess differences in pollinator visitation between plant species, we compared pollinator visits per flower to *H. villosa* vs. *C. stoebe* using an ANOVA with plant species, treatment and their interaction as explanatory variables and plant pair nested within block to account for the fact that experimental plants were paired.

To determine whether the degree of pollen limitation of *H. villosa* reproduction varied with experimental *C. stoebe* densities, we first calculated effect sizes (log response ratio) for the various measurements of reproductive output (i.e., number of seeds per capitulum, total mass of all seeds per capitulum, and mass per seed) (Kelley and Preacher 2012). Since these response variables were correlated, we performed a single MANOVA with *C. stoebe* density as the explanatory variable and block as a blocking variable.

RESULTS

2012 observational study

Plant community.—Twelve native and two non-native forb species co-flowered with *C. stoebe* across the nine sites in 2012 (Table 1). In general, floral species richness was low at all sites: in any given week, no plot had more than four species in bloom. Floral richness differed significantly across *C. stoebe* density plots with the highest richness occurring at intermediate *C. stoebe* density, but was not strongly influenced by phenological phase (Table 2). Low density plots had on average 1.4 times more flowering species than both High and Out density plots (95% CI between 1.1 and 1.7).

Over the course of the season, total floral abundance (per 16 m^2) ranged from zero to 650 flowers. Differences in total floral abundance among High, Low, and Out plots greatly depended on the time of season (Table 2, Fig. 1). Native floral abundance differed across *C. stoebe* density, with Out plots having twofold more native flowers than Low plots and 10-fold more native flowers than High plots. Native floral abundance also varied over time, but there was no interaction between density and time (Table 2), suggesting that *C. stoebe* drove the interaction between density and time in total floral abundance. On average, all plots had the highest native floral abundance pre-*C. stoebe* bloom and decreased by 38%, 66% and 76% during early, peak and late *C. stoebe* bloom, respectively (Fig. 1).

Pollinator community.—Sixty-two insect taxa were observed visiting flowers of the 14 plant species in the 2012 observation plots (Figure S3).

Table 1. Plant species that co-flowered with *Centaurea stoebe* at the 2012 field sites, their native/non-native status, number of sites where they were present (n = 9), and the number of weeks they co-flowered with *C. stoebe*.

Latin name	Common name	Native/non-native	No. sites present	No. weeks co-flowered with *C. stoebe*
Achillea millefolium	Common yarrow	Native	3	1
Allium cernuum	Nodding wild onion	Native	2	3
Berteroa incana	Hoary alyssum	Non-native	1	2
Chrysothamnus viscidiflorus	Yellow rabbitbrush	Native	1	2
Epilobium brachycarpum	Tall willowherb	Native	4	5
Erigeron divergens	Spreading fleabane	Native	2	6
Erigeron speciosus	Aspen fleabane	Native	4	3
Heterotheca villosa	Hairy false golden aster	Native	6	6
Hieracium scouleri	Scouler's woolyweed	Native	3	3
Liatris punctata	Dotted blazing star	Native	1	3
Lupinus spp.	Lupine	Native	6	5
Sedum lanceolatum	Spearleaf stonecrop	Native	2	1
Symphyotrichum falcatum	White prairie aster	Native	2	4
Tragopogon dubius	Yellow salsify	Non-native	3	1

Table 2. Generalized linear mixed model results for the observational study in 2012 examining the effect of phenological period and *Centaurea stoebe* density on floral richness and abundance, and pollinator richness and abundance.

Response variable	Explanatory variable	df	*F*	*P*
Total floral richness	*C. stoebe* density	2,16	5.519	0.015
	Phenological phase	3,66	1.644	0.188
	C. stoebe density × phenological phase	6,66	2.099	0.065
Total floral abundance	*C. stoebe* density	2,16	1.868	0.187
	Phenological phase	3,66	14.739	<0.001
	C. stoebe density × phenological phase	6,66	15.349	<0.001
Native floral abundance	*C. stoebe* density	2,16	12.261	<0.001
	Phenological phase	3,66	9.012	<0.001
	C. stoebe density × phenological phase	6,66	0.442	0.848
Pollinator richness	*C. stoebe* density	2,16	0.766	0.481
	Phenological phase	3,66	8.713	<0.001
	C. stoebe density × phenological phase	6,66	10.775	<0.001
Pollinator abundance	*C. stoebe* density	2,16	0.321	0.730
	Phenological phase	3,66	10.504	<0.001
	C. stoebe density × phenological phase	6,66	6.582	<0.001
Pollinator visits per flower	*C. stoebe* density	2,16	0.115	0.892
	Phenological phase	3,66	3.890	0.013
	C. stoebe density × phenological phase	6,66	0.917	0.488

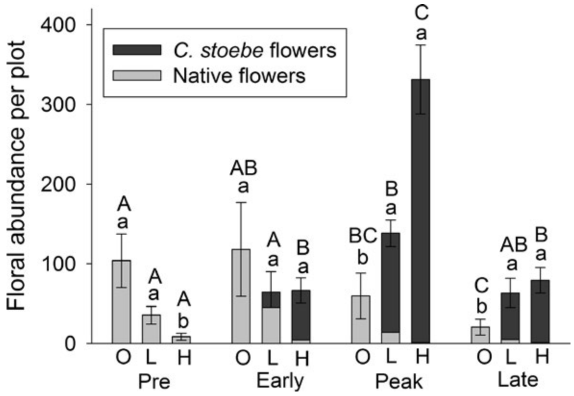

Fig. 1. Mean number of flowers per plot during the four phenological time periods in Out (O), Low (L), and High (H) *Centaurea stoebe* density. Error bars are for total floral abundance (representing ±1 SE), and the different shades represent *C. stoebe* and native floral abundance. Lowercase letters indicate significant differences ($P < 0.05$) in total floral abundance among *C. stoebe* densities within a time period, and uppercase letters indicate significant differences in total floral abundance within the same density across time periods.

Only 19 of these were bee taxa, but they constituted 68% of the 1506 individuals observed. Other flower visitors included butterflies (five taxa, 12% of total), flies (25 taxa, 11% of total), wasps (seven taxa, 7% of total), beetles (four taxa, 0.1% of total), and true bugs (Miridae, two individuals). Twenty-six pollinator taxa exclusively visited flowers of native plants. In comparison, 37 taxa visited *C. stoebe* flowers, of which nine were exclusive visitors to *C. stoebe*. *Apis mellifera* accounted for 22% of all visits to *C. stoebe* flowers and rarely visited flowers of other species. The other 28 taxa seen visiting *C. stoebe* also visited at least one native plant (Fig. 2). *Heterotheca villosa* shared the greatest number of pollinators (19 taxa) with *C. stoebe.*

Results of PERMANOVA indicate that pollinator communities differed among *C. stoebe* density plots ($F_{2,15} = 2.36$, $P = 0.001$), and visual NMDS representation shows pollinator communities observed visiting Out plots were different compared to High and Low plots, although there was some overlap between Out and Low (Fig. 3, NMDS stress 19.8). The differences in pollinator communities among plots of varying *C. stoebe* density strongly depended on phenological phase ($F_{6,64} = 1.34$, $P = 0.034$).

Pollinator abundance, richness, and visitation.— Both the abundance and richness of pollinators that visited the High, Low, and Out plots depended on the phenological phase of *C. stoebe* (abundance: $F_{6,66} = 6.58$, $P < 0.001$; richness:

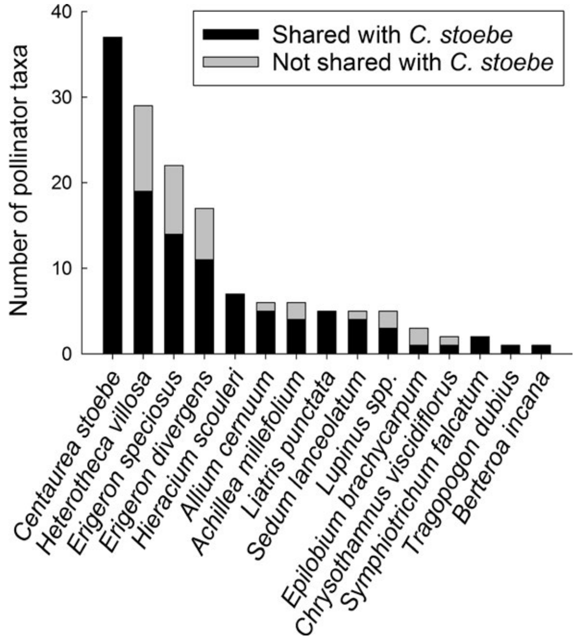

Fig. 2. Total number of pollinator taxa observed visiting flowers of each plant species across the whole season. Black portions of the bar represent pollinator taxa that are shared with *Centaurea stoebe* and gray portions of the bar are pollinator taxa not shared with *C. stoebe*.

$F_{6,66}$ = 10.78, $P < 0.001$; Fig. 4). Prior to *C. stoebe* bloom, Out plots had significantly greater pollinator richness and abundance than High plots (t_{16} = 4.11, P = 0.0008 and t_{16} = 3.45, P = 0.003, respectively). During early *C. stoebe* bloom, when there were few *C. stoebe* flowers, there was no significant difference in pollinator abundance or richness among *C. stoebe* density plots (Fig. 4, Table A2). In contrast, at peak *C. stoebe* bloom, High plots had significantly higher pollinator richness and abundance than Out plots (t_{16} = 3.52, P = 0.003 and t_{16} = 2.58, P = 0.02, respectively). This pattern continued into late *C. stoebe* bloom in pollinator richness but not pollinator abundance (Fig. 4, Table S2). In general, pollinator abundance and richness in Low plots were intermediate between Out and High plots. Pollinator abundance and richness in the Out plots were highest at the beginning of the observation period and decreased over time (Fig. 4). High and Low plots on the other hand, began the period of observation with low pollinator abundance and richness and peaked during full *C. stoebe* bloom.

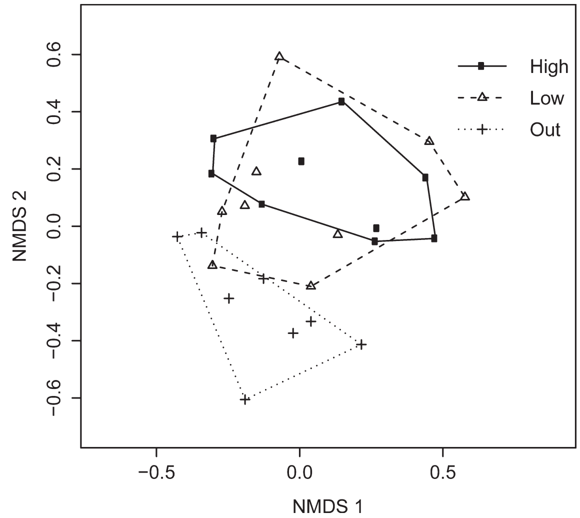

Fig. 3. Ordination of pollinator communities in each plot averaged over the whole season (stress = 19.8). Symbols indicate different *Centaurea stoebe* density classes, and polygons encompass all plots of the same *C. stoebe* density class.

The number of pollinator visits per flower to all plant species in a plot did not significantly differ across *C. stoebe* densities ($F_{2,16}$ = 0.12, P = 0.892). However, visits per flower varied over the season ($F_{3,66}$ = 3.91, P = 0.012), with flowers during early *C. stoebe* bloom receiving the highest number of visits per flower. All plots during early flowering received between 9% and 14% more visits per flower than during any other time period.

2013 manipulative study

Plant community.—*Heterotheca villosa* was the most abundant plant species in bloom during the census (Table S3). Mean floral species richness of each 28 m^2 circle surrounding the target plants was 3.45 (±0.22 SE), which is within (but on the high end of) the range of floral richness in the 2012 pollinator observation plots. The ratio of *C. stoebe* flowers to *H. villosa* flowers across all treatments ranged from 0.2 to 4.1. The mean ratio for low *C. stoebe* density treatment was 0.5 (±0.02 SE), the mean ratio for the intermediate treatment was 1.0 (±0.07 SE), and the mean ratio for high *C. stoebe* density treatment was 2.2 (±0.11 SE), all of which

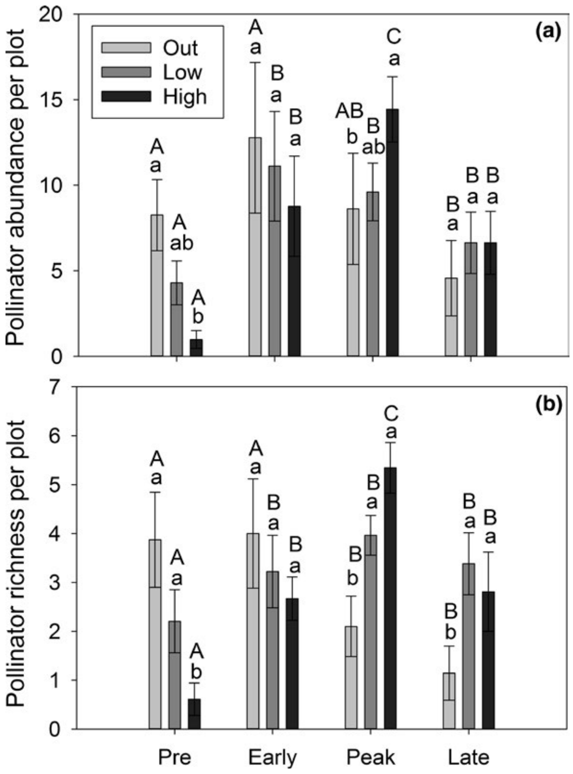

Fig. 4. Mean pollinator (a) abundance and (b) richness at High, Low, and Out plots during the four phenological phases of *Centaurea stoebe*. Lowercase letters indicate significant differences ($P < 0.05$) between density plots within a single phenological phase and uppercase letters indicate significant differences within a single density across time. Error bars represent ±1 SE.

Table 3. Pollinator groups observed visiting *Heterotheca villosa* and *Centaurea stoebe* in 2013 by percent.

	% of visitors	
Pollinator group	*H. villosa*	*C. stoebe*
Bumble bees	3.6	42.7
Butterflies	1.1	2.6
Beetles	1.6	0.5
Flies	31.3	5.4
Honey bees	0.7	2.1
Other native bees	60.4	46.2
Wasps	0.7	0.0
Unknown insects	0.7	0.5

fell below the plant ratios of 1, 2 and 4. This flower ratio was lower than we intended and expected, and may have been a factor of stunted growth or water stress of potted *C. stoebe* plants. The 2013 floral ratios were lower than those in the 2012 observational study, which had a range of 0.1 to 56 *C. stoebe* to *H. villosa* (mean of 4.2).

Pollinator community.—Both *C. stoebe* and *H. villosa* were visited by insects in all pollinator groups, with the exception of no wasps visiting *C. stoebe* (Table 3). Bumble bees and other native bees were the most frequent visitors to *C. stoebe*, accounting for 89% of all observed visits. Bumble bees did not frequently visit *H. villosa*, but other native bees accounted for a large portion of total visits to *H. villosa*, and along with flies accounted for 92% of all observed visits to *H. villosa* flowers.

Pollinator visitation.—The mean number of pollinator visits per flower to *H. villosa* did not differ across *C. stoebe* densities ($F_{3,32}$ = 0.48, P = 0.88, Fig. 5). Visits per flower were greater to *C. stoebe* compared to *H. villosa*, but only when *C. stoebe* plants outnumbered *H. villosa* plants ($F_{1,27}$ = 8.33, P = 0.008, Fig. 5). In the 1:1 treatment there was no difference in the mean number of pollinator visits per flower to *C. stoebe* compared to *H. villosa*. However, in the 4:1 treatment, *C. stoebe* received on average 1.5 more visits per flower than *H. villosa* (T = 2.3, P = 0.029).

Reproductive success of Heterotheca villosa.—There was no evidence of a difference in pollen limitation of *H. villosa* reproduction (effect size) among *C. stoebe* density treatments as measured by seeds per capitulum, total seed mass per capitulum, and mass per seed ($F_{3,18}$ = 1.26, P = 0.28). Hand pollinated and naturally pollinated plants had (respectively) a mean (±SE) mass per capitulum of 13.131 mg (±0.925) and 11.641 mg (±0.827); a mass per seed of 0.333 mg (±0.014) and 0.326 mg (±0.011); and 29.5 seeds (±2.2) and 24.0 seeds (±1.9).

DISCUSSION

The findings of this study agree with previous research indicating that invasive plants integrate into pre-existing mutualistic networks and are utilized as a resource by many native pollinators (Morales and Traveset 2009, Stout and Morales 2009). Given that *C. stoebe* is a late-season bloomer and during peak bloom high

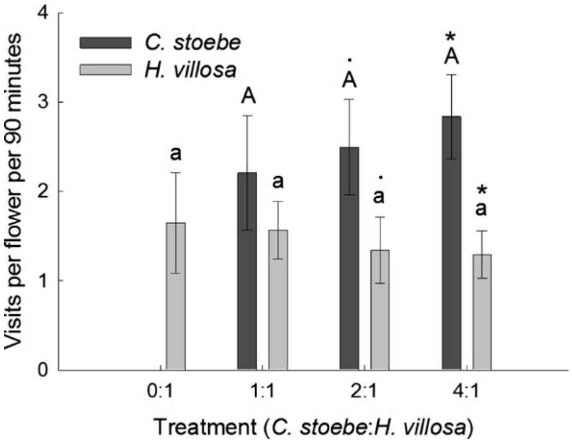

Fig. 5. Pollinator visits per flower per 90 min observations to *Heterotheca villosa* and *Centaurea stoebe* by treatment. Lowercase letters indicate differences in number of visits per flower to *H. villosa* across *C. stoebe* density treatments (significance at $P < 0.05$), and uppercase letters indicate differences in the number of visits per flower to *C. stoebe.* Symbols indicate differences in number of visits between *C. stoebe* and *H. villosa* within the same *C. stoebe* density treatment ("." indicates significance of $P < 0.1$ and "*" indicates significance at $P < 0.05$). Error bars represent ±1 SE.

C. stoebe density plots attracted a greater number of pollinators than nearby plots without *C. stoebe*, this study supports the hypothesis that some invasive plants could be boosting late-season pollinator populations (Bjerknes et al. 2007), assuming that the floral rewards offered by *C. stoebe* are comparable to those offered by native plants. However, the response of pollinators to *C. stoebe* that we observed was complex; depending on the time of season and/or the density of *C. stoebe*, this plant appeared to help, hurt or have neutral effect on pollinators and pollination services. Our results emphasize that the consequences of a plant invasion for pollinators and plant–pollinator interactions is context specific, and that certain factors such as density and phenology of the invader may play a role in the outcome.

The temporal perspective offered by this study of changes in the pollinator community and visitation patterns during a single season caused by plant invasions has generally been overlooked. Whereas previous studies have primarily investigated differences in pollinator visitation and composition averaged across the whole blooming period of a target invasive plant or during a snapshot in time, few have explored changes over the season including when the invader was not in bloom (but see Lopezaraiza-Mikel et al. 2007). During peak flowering, high-density *C. stoebe* plots attracted a greater abundance and richness of pollinators than plots without *C. stoebe*, which might lead to the conclusion that this invasive plant may benefit pollinators at the end of the season when native floral abundance is in decline. But these same high-density plots were visited by a lower abundance and richness of pollinators than plots without *C. stoebe* prior to *C. stoebe* flowering. This phenomenon is likely simply due to the presence of fewer plants other than *C. stoebe* in the high-density plots, which is evident in Fig. 1. Therefore, while *C. stoebe* might benefit certain late-season pollinators, if *C. stoebe* displaces native plants, then the late-season benefit might be at the expense of early season pollinators.

Pollinator communities visiting our small-scale plots also differed across *C. stoebe* density, and varied over the growing season. Plots without *C. stoebe* were visited by a different community of pollinators than plots with *C. stoebe*. These results were similar to pollinator responses observed following invasive plant removal (Hanula and Horn 2011, Fiedler et al. 2012). In part, these observations may be attributed to differences in pollinator phenologies, that is, different species are active at different times over the course of the season which may occur on short time scales (Simanonok and Burkle 2014). Pollinator turnover occurred simultaneous to shifts in floral resource availability among plots over the season (from natives in Out plots in early July to primarily *C. stoebe* in High plots in August). Thus, different pollinator species may have visited different plots simply because that was where the floral resources were located on the landscape during their lifetime. This would mean that although *C. stoebe* may have a direct positive effect on some late-season pollinator taxa (e.g., butterflies *Thymelicus lineola* Ochsenheimer (Fam: Hesperiidae), *Hesperia comma* L. (Fam: Hesperiidae) and *Neophasia menapia* Felder (Fam: Pieridae), Figure S3), it may have an indirect negative effect on early season pollinators through displacement of spring-blooming native plants by *C. stoebe*. However, not all the differences among pollinator communities can be attributed to seasonal variation in pollinator activity because all native plants shared at least some pollinators with *C. stoebe*. *Melissodes* sp. for instance was active the entire length of the study period, and visited a number of native species as well as *C. stoebe.* Thus, it was a combination of pollinator turnover through the season and foraging behavior of pollinators within an invaded area that contributed to differences in the community of pollinator visitors to plots of differing *C. stoebe* densities.

The suggestion that high-density *C. stoebe* areas are indirectly detrimental to early season pollinators rests on the assumption that *C. stoebe* displaces native plants, but this was not necessarily the case in our study. Although *C. stoebe* has been negatively associated with native floral abundance and richness (Kedzie-Webb et al. 2001, Ortega and Pearson 2005, May and Baldwin 2011), our 2012 study was observational so differences in the plant community across *C. stoebe* densities cannot be directly attributed to *C. stoebe*. Alternatively, it is possible that *C. stoebe* occupies places where few native plants are likely to grow (e.g., disturbed areas). For example, some of our sites clearly experienced grazing or urban use pressure that might favor invasive plants. If this is true, *C. stoebe* may provide extra floral resources to pollinators at the end of the season without compromising floral resources provided by native plants at the beginning of the season. Furthermore, pollinator abundance and richness in the low *C. stoebe* density plots never significantly differed from the plot with the highest pollinator abundance or richness in any time period. Thus, pollinators might benefit throughout the season in areas where *C. stoebe* abundance remains low. It is also important to keep in mind the scale of our study, which is only at the level of a field or meadow. If pollinators respond similarly at a landscape scale, we would expect the pollinator responses we found for each density category to represent landscapes where *C. stoebe* is present at that constant density.

Since *H. villosa* was the plant most commonly found co-flowering and sharing pollinators with *C. stoebe* in 2012, we expected that *H. villosa* had the highest likelihood of being indirectly affected by *C. stoebe* through interactions with shared pollinators. Although we found little evidence of competition between *C. stoebe* and *H. villosa* for pollinators at low relative densities of *C. stoebe*, we observed a trend (non-significant) of decreasing visits per flower to *H. villosa* with increasing density of *C. stoebe*, indicating that there may be competition at greater densities of *C. stoebe* than we tested. Other studies investigating the impact of the relative abundance of an invasive plant on pollinator visitation to a co-flowering native plant have found mixed results, including facilitative, competitive and neutral interactions (Muñoz and Cavieres 2008, Flanagan et al. 2010, Chung et al. 2014). A meta-analysis of seven studies found an increasing negative impact on visitation to and reproduction of native plants with increasing abundance of the invasive plant (Morales and Traveset 2009). The studies used in the meta-analysis had mean invasive:native plant ratios that were within the range employed in this study: 0.9 to 1.8 for low and high invasive plant abundance, respectively, suggesting that the range we tested was high enough to produce significant effects in other systems. The fact that it did not suggests that either *C. stoebe* is a weak competitor for pollinators, or *H. villosa* is a particularly strong competitor and able to attract pollinators despite the invasion of a new and attractive plant.

Implications for management

Weed control is often given high priority in both agricultural and natural systems (Randall 1996, Menalled et al. 2009) and imposes substantial management costs (Pimentel et al. 2005). If native plant and pollinator conservation is also a priority, invasive plant management may need to be more carefully evaluated given the context specific impacts of invasive plants on pollinators and native plant reproduction presented in this study and in other published literature. For instance, when evaluating the impact of an invasive plant on pollinators and plant pollinator interactions, it is important to consider the impacts on pollinators over the whole season, not just when it is in bloom. Managers should also recognize that the interaction outcome between an invasive and native plant vying for pollinators likely depends on the relative density of the invasive plant. Based on our results with *C. stoebe*, keeping this invasive plant at low density may be sufficient to prevent negative repercussions for native plant reproduction and may even be beneficial to some pollinators. This may come as good news to managers faced with a widespread and persistent invasive plant such as *C. stoebe* which is beyond eradication; maintaining ecologically tolerable thresholds may be a reasonable management goal in such cases.

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