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ORIGINAL PAPER

Priority resource access mediates competitive intensity between an invasive weevil and native floral herbivores

S. M. Louda · T. A. Rand · A. A. R. Kula ·
A. E. Arnett · N. M. West · B. Tenhumberg

Abstract Mechanisms underlying invasive species impacts remain incompletely understood. We tested the hypothesis that priority resource access by an invasive biocontrol weevil, *Rhinocyllus conicus*, intensifies and alters the outcome of competition with native floral herbivores over flower head resources of the non-target, native host plant *Cirsium canescens*, specifically with the predominant, synchronous tephritid fly *Paracantha culta*. Four main results emerged. First, we documented strong, asymmetric competition, with *R. conicus* out-competing *P. culta*. Second, weevil priority access to floral resources accelerated competitive suppression of *P. culta*. Evidence for competitive suppression with increased weevil priority included decreases in both the numbers and the total biomass of native flies, plus decreases in individual *P. culta* fly mass and, so, potential fitness. Third, we found evidence for three concurrent mechanisms underlying the competitive suppression of *P. culta* by *R. conicus*. Prior use of a flower head by *R. conicus* interfered with *P. culta* pre-oviposition behavior. Once oviposition occurred, the weevil also reduced fly post-oviposition performance. Preemptive resource exploitation occurred, shown by the significant effect of flower head size on the total number of insects developing and in the magnitude of *R. conicus* effects on *P. culta*. Interference also occurred, shown by a spatial shift of surviving *P. culta* individuals away from the preferred receptacle resources as *R. conicus* priority increased. Finally, fourth, using an individual-based model (IBM), we found that the competitive interactions documented have the potential for imposing demographic consequences, causing a reduction in *P. culta* population sizes. Thus, priority resource access by an invasive insect increased competitive impact on the predominant native insect in the invaded floral guild. This study also provides the

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first experimental evidence for non-target effects of a weed biological control agent on an associated native insect herbivore.

Introduction

While studies examining the ecological consequences of insect invasions have increased over the last decade, experimental tests of the impacts of invasive insect herbivores on native populations and communities remain rare, particularly in non-forest systems (Kenis et al. 2009; Gandhi and Herms 2010). Some of the strongest case studies come from investigations of the impact of deliberately introduced biological control herbivores that have become invasive within natural ecosystems. These studies have revealed that biocontrol herbivores can have significant negative effects on native plant growth and population dynamics (Howarth 1990; Simberloff and Stiling 1996; Louda et al. 1997, 2003a, b, 2005a, b; Rose et al. 2005). However, to our knowledge, no published experiments to date have assessed the impact of such host-range expansions on the native insect herbivores associated with the new, non-target host plant. Further, relatively few studies actually identify the mechanisms underlying impacts of invasives on interacting native species, or model the potential demographic consequences of such interactions (Parker et al. 1999; Levine et al. 2003).

Identifying and quantifying the ecological factors underlying variation in the outcome and intensity of species interactions has been highlighted as a key area in need of further ecological research (Agrawal et al. 2007). Competition is one, often invoked, and potentially important mechanism by which invasive species may impact native species with whom they share resources (Levine et al. 2003). Competition is known to be an important force structuring herbivorous insect communities in general (Denno et al. 1995; Kaplan and Denno 2007), and some evidence suggests that invasive herbivores can negatively impact native insects (Kenis et al. 2009). Additionally, escape from natural enemies may confer a

competitive advantage to introduced herbivores over native species (Maron and Vila 2001; Wolfe 2002). Thus, weed biocontrol agents that have moved onto native host plants may impose strong competitive effects on native insect herbivores when they share limited, discrete resources, such as fruits or seeds.

One prominent factor often mediating the intensity and outcome of competition among herbivores is relative timing of host plant resource use, the *priority access effect* (Denno et al. 1995). Earlier arrival to a resource may confer a competitive advantage through a variety of direct (interference) and indirect (exploitation) mechanisms. For example, priority access may allow early-arriving herbivores to distribute semiochemicals that inhibit colonization, feeding, or oviposition by later-arriving insects (Nufio and Papaj 2001), or later-arriving species may avoid plant resources that have been previously modified or damaged. Also, a later-arriving species may be excluded from higher quality resources by space pre-emption of the earlier-arriving species, an interference effect (Denno et al. 1995).

Further, even when pre-oviposition interference mechanisms are incomplete, resulting in spatial co-occurrence on a shared plant resource, the outcome of the competitive interaction can be tipped in favor of earlier-arriving species through a pre-emption of resources that creates size (developmental) or numerical advantages (Denno et al. 1995). Interference and exploitative competition are not necessarily mutually exclusive processes. Both may operate, sequentially or additively, to determine the net outcome. The role of priority resource access, as a mechanism underlying the magnitude and outcome of the competitive effects of an invasive herbivore on native herbivores, is unknown.

We evaluated the putative competitive interaction between *Rhinocyllus conicus* Frölich, a biological control weevil that has become invasive on flower heads of the non-target native host plant, *Cirsium canescens* Nutt. (Platte thistle), and the tephritid fly *Paracantha culta* Wiedemann, the predominant native floral herbivore on *C. canescens*. We asked: Does competition occur? If so, does priority of access to flower head resources by the invasive biocontrol weevil, *R. conicus*, influence the outcome and intensity of its interactions with *P. culta*? Given evidence of a strong interaction, what are the mechanisms underlying the negative competitive

effects? Finally, are any demographic consequences likely for the native fly population?

Methods

Site and natural history

The experimental site was Arapaho Prairie, Arthur County, NE, a nature preserve in the Sand Hills of Nebraska, upper Great Plains, USA. The Sand Hills vegetation is a distinctive mix of tallgrass and midgrass prairie species (Kaul 1989; Keeler et al. 1980). No exotic thistle species occur at the site.

The non-target native host plant, *C. canescens*, is a characteristic thistle species of the Sand Hills prairie ecosystem (Kaul et al. 2007). It is monocarpic, growing as a rosette for 1–5 year to a threshold flowering size (Rose et al. 2005). It then flowers and sets seed, in late May to early June, dying afterward (Lamp and McCarty 1981; Louda and Potvin 1995). Seed production is reduced by the native floral feeders (Lamp and McCarty 1982b); and, flower heads with floral herbivores, especially with *R. conicus,* have few, if any viable seeds left in them (Louda et al. 1997, Louda 1998b and unpublished data). This seed loss has been shown to limit plant life-time maternal fitness (Louda and Potvin 1995) and plant density (Rose et al. 2005).

The native floral herbivores on *C. canescens* include two native picture-winged flies [Tephritidae: *Paracantha culta* Wiedeman, *Orellia occidentale* (Snow)]; a native weevil (Curculionidae: *Baris* nr. *subsimilis* Casey), and three native moths (Pyralidae: *Pyrausta subsequalis* subsp. *plagialis* Haim., *Homoeosoma impressale* Hulst, and *H. ardaloniphas* Goodson & Neunzip) (Lamp and McCarty 1982a; G. Balogh, personal communication). We quantified evidence of all floral insects, but focus here on the interaction of *R. conicus* with *P. culta,* since this fly was the most common of the native internal flower head feeders (Lamp and McCarty 1982a; unpublished data). Adult *P. culta* appear on *C. canescens* in May. Males are temporarily territorial, while attempting to attract a female; fertilized females oviposit preferentially into the top of small (10–20 mm), immature flower heads, with or without a male present (Lamp and McCarty 1982a; personal observation). Oviposition scars are sometimes evident, but not always. The larvae feed through a floral tube and its undeveloped ovary, preferentially attaching mouth hooks into the nutritive receptacle tissues under the floret. Mature larvae pupate in position within the flower head, leaving the pupal case as evidence of successful emergence (Lamp and McCarty 1982a). Fly parasitism rates are low (<3% of flies in dissected flower heads over 20 years: S. Louda, unpublished data).

The invasive Eurasian flower head weevil, *R. conicus*, was first recorded on *C. canescens* in 1993 (Louda et al. 1997; Louda 1998a). This biological control weevil was introduced into North America to control exotic thistles, particularly *Carduus* spp. (Goeden et al. 1974; Zwölfer and Harris 1984; Gassmann and Louda 2001). However, it now occurs in the majority of *C. canescens* flower heads initiated at this site (e.g., 78.9% of all flower heads dissected in 2007). Post-oviposition occurrence and feeding by *R. conicus* significantly overlaps that of *P. culta* (e.g., 57.8% of all flower heads dissected in 2007: S. Louda, unpublished data). Over-wintered *R. conicus* adults emerge in May, often aggregating on plants and deposit multiple eggs onto the full range of immature flower heads available; each egg is covered by a case of masticated plant tissue (Rees 1982), making weevil oviposition relatively easy to quantify. The larvae hatch, burrow into the flower head receptacle from below, and feed on the phloem-rich receptacle tissues and on the attached florets and developing ovaries and ovules. After 25–40 days, the mature larvae pupate in a distinctive chamber. The chamber remains, allowing quantification of weevil developmental success even after emergence.

Pre-oviposition fly behavior in relation to *R. conicus* oviposition priority

To quantify *P. culta* behavioral response to flower heads with versus without prior access to the heads by *R. conicus,* we ran a laboratory experiment. For each replicate ($n = 20$), we matched two flower heads by size, one collected with five *R. conicus* egg cases and one without any evidence of *R. conicus* use. Immediately upon return to the laboratory, we inserted the stem of each flower head through parafilm into a 500 ml flask with nutrient solution. The flasks were next to each other inside the screen cage (30 × 30 × 30 cm) with a sugar-water soaked cotton ball, and held at 20–25°C under ambient light conditions from

the windows. We introduced two pairs of *P. culta* per cage (2 females, 2 males), and recorded fly location and activity every 4 h (0800–2200 hours) over 8 days. We analyzed relative fly position and activity using ANOVA on arcsine-transformed proportions.

Post-oviposition test of priority access to floral resources

To test the magnitude of the interaction between *R. conicus* and *P. culta* and the effect of priority access on it, we did a field experiment. We found and measured the flower heads available at Arapaho Prairie 5–8 May 2000, recording evidence of any early weevil and fly oviposition, and covering each head with a mesh sleeve cage ($\sim$20 cm $\times$ 10 cm). After removing all heads with unambiguous evidence of early insect use from further consideration, we assigned the remaining flower heads within and between plants to one of five treatments, by first establishing blocks based on head size and position across plants (so head size was a covariate) and then randomly assigning treatments within blocks (initial $n = 48$–52 heads per treatment; final n by treatment in Table 1). The five treatments for *P. culta* flies were, in the order of increasing *R. conicus* weevil priority: flies only added (F0); flies added first (F1); flies and weevils added simultaneously (FW); weevils added first (W1); and, weevils only added (W0). Treatments were initiated 10–19 May 2000. This experimental design built on the results of our pilot experiment in 1999 (Online Resource 1).

Using insects caught in the field, we added two mating pairs of weevils to the mesh sleeve cages of three treatments (FW, W1, W0). Counting *R. conicus* egg cases every 1–2 days, we allowed weevils to remain in the cage until the mean number of eggs expected for a head that size were laid ($\leq$8 days). The eggs expected were based on field data (Louda and Arnett 2000; Louda et al. 2005b; Rand and Louda 2006, S. Louda, unpublished data); so, the time interval used allowed densities of *R. conicus* comparable to observed field densities. At the same time, we added two pairs of *P. culta* flies to the mesh sleeve cages of the first three treatments (F0, F1, FW). These flies remained in the treatment mesh sleeve cages for 6 days; missing or dead flies were replaced daily. For the two asynchronous priority treatments with both insects (F1, W1), we allowed the first species to establish (as above) before adding the second species, and then allowed them to remain for 2–8 days (as above). We kept all flower heads covered with the mesh sleeve cage during and after the manipulation, except when the flowers were in anthesis (2–3 days) to allow natural pollination. Flower heads were collected as they matured; all heads had matured or aborted by 14 July.

In the laboratory, we dissected all of the experimental flower heads, recording the number, developmental stage and condition of all insects found by species, as well as *P. culta* fly position relative to the receptacle resources. For fly position, the location of each individual *P. culta* fly was scored as: *in* the receptacle (deeply embedded), *on* the receptacle (contact, but little penetration), or *above* the receptacle in either the lower, or the middle, or the upper third of the volume of the matured flower head. The timing of *R. conicus* development, analyzed as proportion immature weevils in total weevils per head, did not differ significantly among treatments with weevils added (ANOVA, arcsine-transformed proportions: mean 3.6%, $F_{3,183} = 1.295$, $P = 0.278$). We also recorded: individual insect mass (on an analytical scale, to 0.1 mg); receptacle damage type and damaged area; and, the number of filled, unconsumed seed resources remaining. We calculated the proportion of seeds eaten or damaged as: $1 -$ [(number of intact seeds remaining)/(total number of seeds expected)]; the total number expected was determined from a regression of the counts of intact seed from undamaged flower heads by size and by position (data from Louda and Potvin 1995; Rose et al. 2005).

Oviposition in 2000 started early. Already by 6–8 May, at least a week earlier than usual (S. Louda, personal observation), some flower heads had either *P. culta* oviposition, evidenced by oviposition scars (often, but not always detectable), or *R. conicus* oviposition, evidenced by fresh weevil egg cases. To allow the largest sample size given the plants available, we recorded such ovipositions and assigned these flower heads to the appropriate initial treatment on 10–19 May. In the analysis, we first treated these heads as a separate sub-treatment within each treatment. The effect of natural colonization versus experimental colonization on key response variables (numbers of flies, weevils, and filled undamaged seeds) was not significant (one-way

Table 1 Parameters (X, SE) in the priority experiment relevant to understanding treatment response, with the treatments ordered (left to right) by increasing influence (priority) of the exotic invasive weevil, *Rhinocyllus conicus*; differences among treatments were evaluated using ANOVA (*F* test with orthogonal contrasts among treatments, *F*), or Kruskal–Wallis ANOVA on ranks (Tukey's HSD, *H*) on non-normal variables

	Fly Alone (F0)			Fly-first (F1)			Together (FW)			Weevil-first (W1)			Weevil alone (W0)			Test	*P*
	N	*X*	SE	*N*	*X*	SE	*N*	*X*	SE	*N*	*X*	SE	*N*	*X*	SE		
Initial conditions																	
Number heads per plant	12	7.1	1.77	15	5.7	1.00	12	0.2	1.22	13	5.7	0.64	11	5.7	1.79	*F*	>0.200
Stem height (cm)	12	20.8	3.40	15	17.1	4.09	12	11.5	3.11	13	10.7	1.81	11	7.9	2.29	*F*	0.081
Head width (mm)	45	18.4	1.00	67	18.2	0.68	54	15.9	0.93	30	18.4	1.32	36	12.7	1.01	*F*	<0.001
R. conicus eggs	45	0.0		61	20.6	2.04	50	15.3	1.88	30	19.5	3.27	33	15.4	1.82	*F*	<0.001
Outcome, per head																	
Individual *R. conicus* (mass, mg)	45	–	–	7	5.0	0.18	53	5.0	0.21	30	5.1	0.21	36	5.5	0.26	*F*	0.013
Proportion fly mass	45	1.00	0.000	65	0.48	0.029	54	0.23	0.029	30	0.09	0.021	36	0.0	–	*H*	<0.001
Individual *P. culta* (mass, mg)	45	2.64	0.057	65	2.74	0.070	39	2.61	0.069	22	2.53	0.138	36	–	–	*F*	<0.001
Proportion damaged	45	0.60	0.05	67	0.96	0.012	55	0.94	0.02	30	0.88	0.038	36	0.90	0.037	*H*	<0.001
Final head width (mm)	45	24.5	0.67	67	23.7	0.51	55	22.0	0.91	30	24.6	1.11	36	19.0	1.07	*F*	<0.001
Seeds remaining	45	23.2	7.28	66	0.3	0.17	55	2.3	1.65	30	1.6	1.19	36	0.3	0.28	*H*	<0.001

Flower heads with no insects averaged 189.0 (18.8) filled seeds (*N* = 12)

ANOVA, *P* > 0.10 in all cases). Thus, we combined the data within treatment in the analysis here. Final sample size was decreased by the loss of 19 heads: nine heads on plants killed by the Plains pocket gopher (*Geomys bursarius*), seven heads lost to severe stem-mining and feeding damage by the early moth (*P. s. plagialis*), and three heads disappeared. Thus, the final sample sizes of flower heads per treatment were: 45 fly-only (F0), 67 fly-first (F1), 54 simultaneous addition of fly and weevil (FW), 30 weevil-first (W1), and 36 weevil-only (W0), for a total of 232 heads.

For statistical analyses, when possible we used parametric ANOVA or ANCOVA with initial flower head size as the covariate, to evaluate the experimental outcome. In this case, significant models were followed by orthogonal contrasts to compare among specific treatments (see figures). Transformations used were: ln(numbers), square-root(counts) and arcsine(proportions). When, however, the assumptions of normality and homogeneity of variances were violated even after transformation, we used nonparametric Kruskal–Wallis ANOVA on ranks and Tukey's HSD test for multiple comparisons.

Individual based model development

To evaluate if the *R. conicus* priority effect quantified in this experiment could lead to the observed declines

in *P. culta* populations (Louda and Arnett 2000), we constructed an individual based model (IBM) to predict the stochastic carrying capacity for the fly through time, both in the presence and the absence of an *R. conicus* priority effect. The model represents dynamic oviposition behavior by the fly, one mechanism that has been suggested to reduce tephritid competition effects (i.e., Headrick and Goeden 1990; Lalonde and Roitberg 1992). We assumed that the probability of a female *Paracantha* ovipositing in a flower head, P_E, decreases with the number of eggs already present, and that this probability can be modeled with the following sigmoidal function:

$$P_E = \exp(-(E/\eta)^{\beta}), \tag{1}$$

where E represents the number of eggs already present in the flower head, η denotes the scale parameter, and β denotes the E-dependent shape parameter. Note, η and β together specify the strength of oviposition avoidance (see Figures I and II of On-Line Resource 2). Oviposition probability was equal to one when zero larvae were in a flower head, followed by a decreasing probability of fly oviposition with increasing number of larvae in a flower head until the probability asymptotes to zero. We chose values for η and β that would result in number of flies per flower head that was within the range of naturally occurring flower head infestations (field data: 0–0.8 flies per head, simulations: 0–0.24 flies per head). The range in fly infestation is expected to vary more in the field because head sizes vary in the field but are identical sizes in the simulations.

The model assumes that at the beginning of each annual generation of flies, there were 20 flower heads available per unit area of prairie, which is a likely density in the typical thistle patch (unpublished field data). We started the model with 10 female flies per unit area. We assumed each female fly emerged with 100 eggs, and could visit 10 flower heads before she died (10 oviposition opportunities), including potential return visits to an already visited flower head. At the start of the simulation, the first fly randomly chose a flower head and oviposited a clutch of eggs, with probability P_E. Fly clutch size was drawn from a truncated Poisson distribution and varied between one and five eggs, consistent with empirical observations (Lamp and McCarty 1982a, b). The model then updated the egg load of the fly and the number of

eggs in the flower head. Over time, the egg load of the fly declined and the numbers of eggs in the flower heads increased. The model sequentially provided all flies with 10 opportunities to oviposit a clutch of eggs. Then the model counted the number of eggs in all flower heads to determine the population size of the next generation. In our model, we assumed all eggs survived to become adult flies; model predictions did not change even if only a fraction of the eggs survived (unpublished data). We simulated fly populations for 10 consecutive generations; however, after only 2–3 generations the fly population stabilized and fluctuated around the stochastic carrying capacity, K_s. We ran the model 100 times for 10 generations and calculated the average value for K_s. Note, K_s specifies the average number of flies that, in the long run, can be sustained by the resources available ($N = 20$ flower heads in the model). The predicted K_s was not influenced by the initial egg load of female flies (Figure III, On-Line Resource 2) and only slightly increased with the number of oviposition opportunities (Figure IV, On-Line Resource 2).

In the simulations with the weevil priority effect, we randomly distributed *R. conicus* eggs among the flower heads before each new fly generation emerged. We assumed that each early oviposited *R. conicus* larva consumed more resources than a fly larva. If the priority strength was 9, each *R. conicus* egg had the same deterrent effect on fly oviposition probability as nine fly larvae (Eq. 1: one fly egg + one *R. conicus* egg → $P_{E=10} = 0.37$). In our simulations we explored the effect of priority strength, the proportion of heads infested with weevil eggs, and resource availability (number of flower heads) on the average stochastic carrying capacity K_s.

Results

Pre-oviposition fly behavior in relation to prior weevil oviposition

In the laboratory test of fly behavior, while native *P. culta* used heads with and without prior weevil exposure, it clearly preferred both plants and flower heads without prior oviposition by *R. conicus*. More *P. culta* occurred on plants without egg cases deposited by *R. conicus* in prior access (51.6%, SE

2.96) than on plants with *R. conicus* egg cases (39.3%, SE 2.65) (ANOVA on arcsine-transformed proportions: $F_{1,57} = 9.59$, $P = 0.003$). Further, more *P. culta* females observed probing flower heads for oviposition were on heads without *R. conicus* egg cases (44.4%, SE 2.87) than with *R. conicus* egg cases (27.5%, SE 3.41) (ANOVA, arcsine-transformed proportions: $F_{1,57} = 14.376$, $P < 0.001$). Thus, we found that prior exposure to and use of a plant or flower head by *R. conicus* interfered to some degree with the pre-oviposition behavior of *P. culta*, reducing the acceptability of the flower head resources to the native fly.

Post-oviposition response to priority floral resource access in the field

Total numerical response

In the field experiment, the total number of insects developing per flower head varied significantly among treatments (Fig. 1a; $P < 0.001$), consistent with the results of our initial, smaller-scale experiment the previous year (Online Resource 1). The total number of insects was highest in the fly-first (F1) treatment, intermediate in three treatments—fly-only (F0), simultaneous (FW) and weevil-first (W1)—and lowest in the weevil-only (W0) treatment (Orthogonal contrasts, all $P < 0.002$). More insects occurred in the fly-first (F1) treatment than any of the other three treatments with weevils added (Fig. 1a). The total number of insects that developed decreased as *R. conicus* priority increased (Fig. 1a). Flower head size, a measure of floral resource availability, was a significant covariate in explaining the total number of insects that developed per flower head within treatment ($F_{1,226} = 83.862$, $P < 0.001$). Thus, when the two insects co-occurred, priority access by *P. culta* allowed more total insects to be packed onto the floral resources, whereas priority access by *R. conicus* led to fewer total insects developing on the flower head resources.

Total biomass response

Since insect sizes vary, we also asked how total insect biomass changed in response to increased weevil priority. Total insect biomass per flower head (Fig. 1b) showed the same pattern as total insect numbers (Fig. 1a) (ANCOVA, square-root transformed flower head diameter as covariate, on square-root transformed biomass, $P < 0.001$). Insect biomass per flower head was higher in the fly-first (F1) treatment, when *R. conicus* was added after a week delay, than in the other treatments (124.2 mg vs. 38.0–76.1 mg; orthogonal contrasts, all $P < 0.001$), again consistent with the results of the preliminary experiment (Online Resource 1: Results). Flower head diameter also helped explain the total biomass of insects per flower head within treatment ($F_{1,226} = 99.537$, $P < 0.001$). Thus, when the two insects co-occurred, total insect biomass decreased as *R. conicus* priority on floral resources increased.

Weevil numerical response

As planned, more *R. conicus* developed in the four treatments with weevils than in the fly-only treatment, where *R. conicus* densities were near zero, indicating a successful execution of the treatment (Fig. 1c; ANOVA, $P < 0.001$, $R^2 = 0.708$). No significant differences in number of weevils per head occurred among the four treatments with weevils added (Fig. 1c; orthogonal contrasts, all $P > 0.20$), although the number trended toward being higher in the weevil-first (W1) treatment (Fig. 1c). Finally, flower head size, representing floral resource availability, again was a significant covariate, helping explain variation in the number of *R. conicus* that developed per flower head within treatment ($F_{1,226} = 49.148$, $P < 0.001$). Overall, these results suggest a carrying capacity of $\sim$10–13 *R. conicus* per Platte thistle flower head, and no significant effect of co-occurring *P. culta* on weevil numbers.

Weevil biomass response

The total biomass of *R. conicus* per flower head (Fig. 1d) was higher in the weevil-first (W1) treatment than in the other treatments with weevils added (Tukey's HSD, $P = 0.025$). Exclusive *R. conicus* access (W0 treatment) led to the largest weevils (5.8 mg per weevil vs. 4.9–5.0 mg in the other three treatments with weevils added); individual *R. conicus* size (mass) was significantly greater in the weevil-only (W0) treatment than in the other weevil added treatments (ANCOVA treatment main effect, $F_{3,181} = 3.665$, $P = 0.013$). Flower head size was
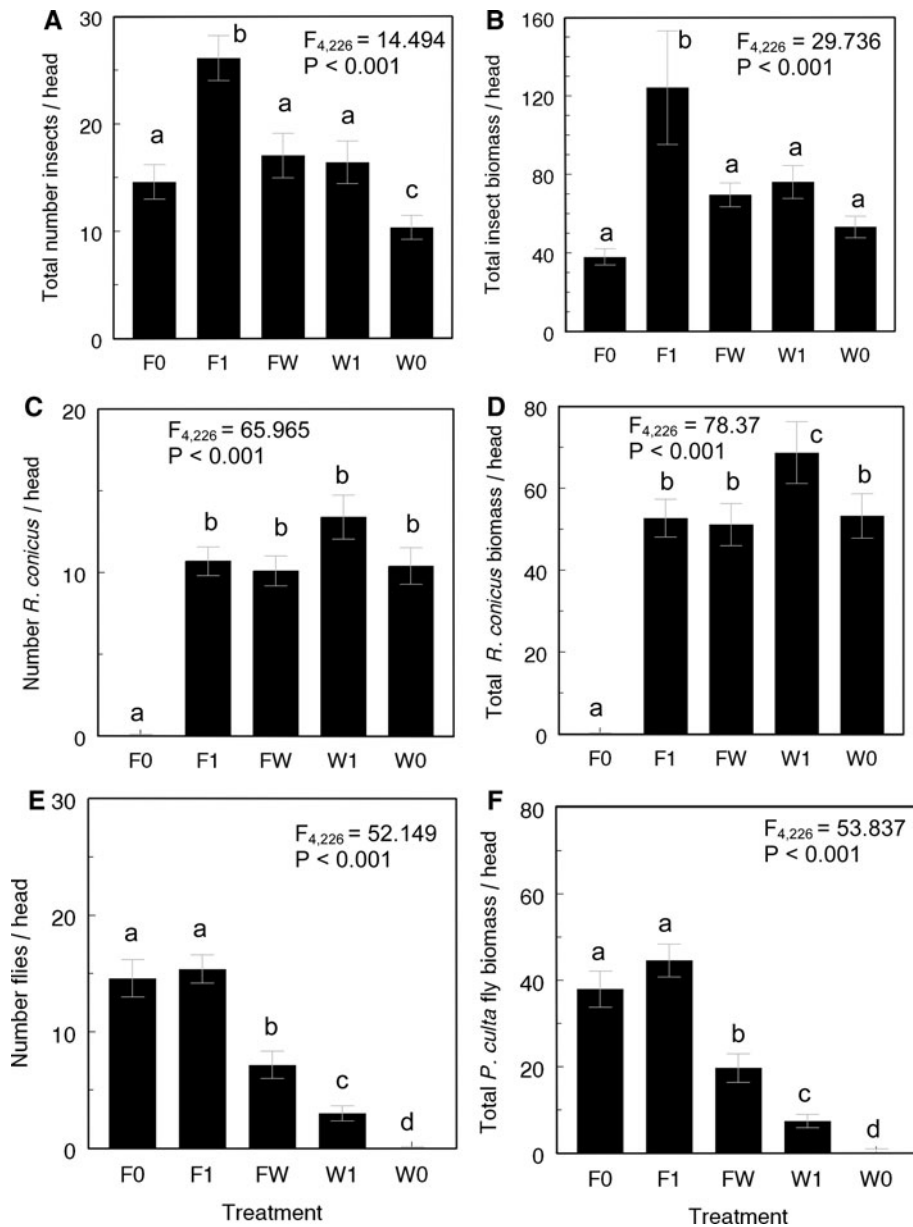
Fig. 1 Number and biomass of insects per flower head overall and by species in the field priority access experiment (least squares mean, SE), by treatment: **a** total number of insects; **b** total mass of insects; **c** number *Rhinocyllus conicus*; **d** mass of *R. conicus*; **e** number of the tephritid fly *Paracantha culta*; and **f** mass of *P. culta*, per flower head; treatment effects evaluated by ANOVA/ANCOVA (*F* test, orthogonal contrasts among treatments). The treatments were: *F0* fly-only, *F1* fly-first, *FW* fly and weevil simultaneously, *W1* weevil-first, and *W0* weevil-only, with the treatments ordered (*left to right*) by increasing priority of access by the exotic invasive weevil, *R. conicus*, relative to the native fly, *P. culta*

significant in predicting individual weevil size (mass) within treatment ($F_{1,181} = 24.109$, $P < 0.001$). These results, where weevils with priority access were largest, suggest that the native *P. culta* did have a negative effect *R. conicus*, decreasing individual weevil mass somewhat when the two species co-occurred, even though it had no effect on the number of successful weevils.
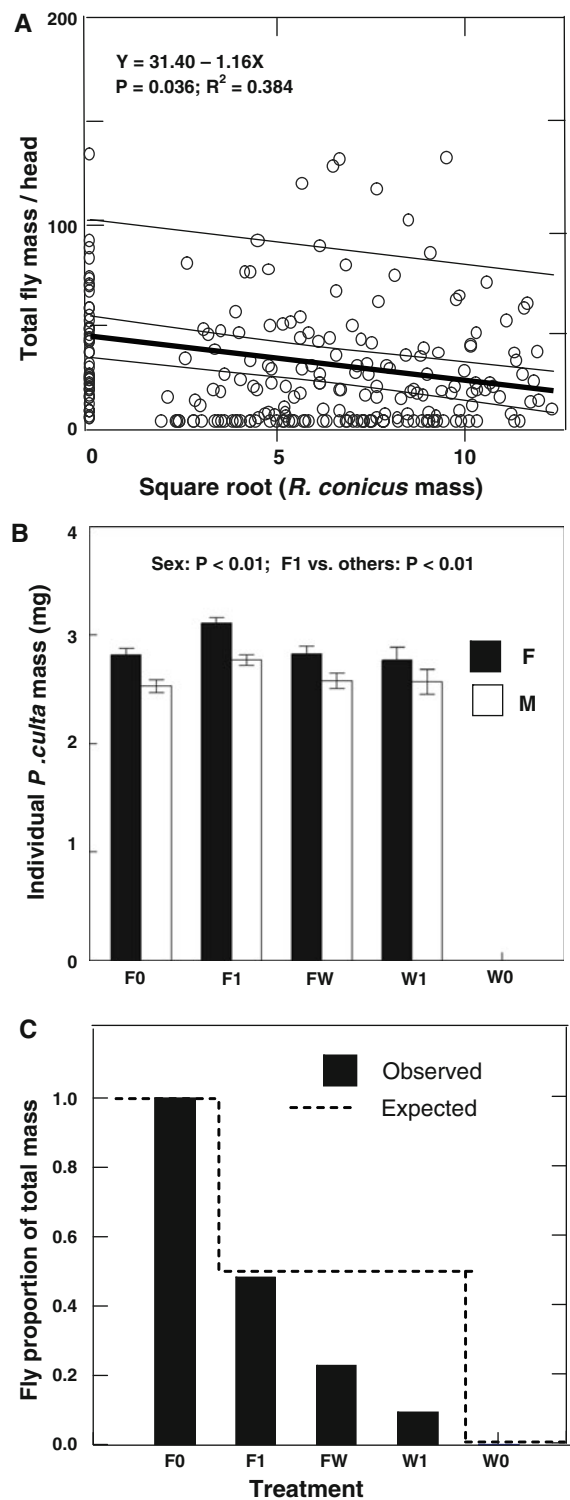
Responses of the tephritid fly, *Paracantha culta*, to increasing *Rhinocyllus conicus* biomass, correlated with increasing priority of the introduced weevil shown in Table 1 (*F1*, *FW* to *W1* and then to *W0*) in the field priority access experiment: **a** least squares linear regression showing relationship of total fly biomass per head to *R. conicus* biomass per head; **b** individual female (*F*) and male (*M*) fly size (mg) by treatment (treatment effect: $P < 0.001$ for both sexes); and **c** *P. culta* biomass as a proportion of total insect biomass, by treatment ($P < 0.001$), where the *dashed line* represents the expected value under null hypothesis of equal, reciprocal competition between native fly (*P. culta*) and exotic weevil (*R. conicus*). The treatments were: *F0* fly-only, *F1* fly-first, *FW* fly and weevil simultaneously, *W1* weevil-first, and *W0* weevil-only, with the treatments ordered (*left to right*) by increasing influence of the exotic invasive weevil, *R. conicus*

Fly numerical response

As *R. conicus* priority access to flower head resources increased, the number of *P. culta* developing decreased significantly and systematically from fly-only (F0) and fly-first (F1) treatments, which did not differ significantly from each other, to the simultaneous treatment (FW) and, finally, to the weevil-first (W1) treatment (Fig. 1e; ANCOVA, square root-transformed head size as covariate, on square root-transformed counts, $P < 0.001$). The interaction reduced *P. culta* numbers as *R. conicus* priority increased. Among treatments with both species (Fig. 1e), fly success decreased 52.0% from the fly-first (F1) treatment to the simultaneous (FW) treatment and 58.9% from the simultaneous treatment (FW) to the weevil-first (W1) treatment. In the dissections, we found no evidence to suggest that intra-guild predation by *R. conicus* occurred and caused mortality of *P. culta* larvae in the flower head. In sum, in treatments with both *P. culta* and *R. conicus*, the numbers of successful flies decreased severely as weevil priority access to floral resources increased, without any evidence of direct weevil-induced fly mortality.

Fly total biomass response

The response in fly biomass paralleled the pattern in fly numbers (Fig. 1f), with decreased fly biomass as *R. conicus* biomass increased in co-occurrence (Fig. 2a), and increased weevil biomass correlated with increased weevil priority (Table 1). Total biomass of *P. culta* flies per flower head differed significantly among the five priority treatments (ANCOVA, square-root transformed head size as covariate, on square-root transformed proportion total insect biomass: $F_{4,226} = 53.837$, $P < 0.001$). Mean number of flies per head did not differ between fly

only (F0) and fly first (F1), nor between weevil first (W1) and weevil only (W0); however, among treatments with both species, fly biomass declined significantly as *R. conicus* priority increased (Fig. 1f); the decreases amounted to 51.5% and 73.7% in total fly biomass from F1 to FW and from FW to W1 respectively, even though the treatments started with comparable numbers of insects and differed only in timing of access. Results in the earlier, smaller experiment were comparable (Online Resource 1).

Further, when the two species co-occurred, the relative contribution of *P. culta* flies to total insect biomass decreased significantly as *R. conicus* priority increased (ANOVA on arcsine-transformed proportions: $F_{4,224} = 442.672$, $P \ll 0.001$, $R^2 = 0.894$). In fact, the fly contribution was much lower than expected in the concurrent (FW) and weevil first (W1) treatments, with the expected value based on the null hypothesis of equal, reciprocal competition between *P. culta* and *R. conicus* (observed = bars, expected = dashed line: Fig. 2c). When the two species co-occurred, the negative effects of the interaction on the native fly increased as the introduced weevil priority increased; the proportionate contribution of flies to total insect biomass declined 51.7% from fly-first (W1) treatment to the simultaneous (FW) treatment, and 58.4% from FW to the weevil-first (F1) treatment. Thus, in co-occurrence, stepwise increases in *R. conicus* priority led to significant absolute and relative decreases in *P. culta* fly biomass, as well as in the fly numbers.

Individual fly size response

Since insect fecundity is generally related to insect size, we examined change in individual insect size (mass) by sex across the priority treatments. Females were larger than males on average (Fig. 2b). Individual fly mass by sex varied among treatments (Fig. 2b; Kruskal–Wallis ANOVA: female flies, $H_3 = 21.194$, $P < 0.001$; male flies, $H_3 = 11.861$, $P < 0.01$). Both female and male *P. culta* were significantly larger in the fly-first (F1) treatment (females: 3.01 mg, males: 2.73 mg) than in the other treatments with flies (females: 2.59, 2.70 and 2.94 mg; and males: 2.28, 2.59 and 2.61 mg, in the F0, FW and W1 treatments, respectively), consistent with the earlier experiment (Online Resource 1:

Results). The smaller individual sizes of *P. culta* in the fly-only treatment (F0) than in the fly-first treatment (F1) could not be explained by any of the measurements we took (Table 1). However, critically, among the treatments where the two species co-occurred and varied only in timing of access (F1, FW, W1), both female and male individual fly sizes, and so potential fecundity and lifetime fitness, were lower when *R. conicus* had either equal (FW) or earlier access (W1) to flower head resources than in the fly-first treatment (F1), where *P. culta* had the temporal advantage. Comparable results occurred in the preliminary experiment (On-Line Resource 1).

Spatial displacement of P. culta by R. conicus

Foraging niche of *P. culta* within the flower head, measured as fly position in relation to the highly nutritive receptacle resources (see "Methods"), was severely and progressively disrupted as *R. conicus* priority increased (Fig. 3). For example, among treatments with both species, the number and proportion of *P. culta* deeply buried into the receptacle resources was inversely related to the degree of *R. conicus* priority on a head (Fig. 3a, b). These results are consistent with the larger individual female fly size in co-occurrence when *P. culta* had priority access (F1) versus when the *R. conicus* had equal or higher priority access (FW, W1) to floral resources (Fig. 2b). Overall, in co-occurrence, *P. culta* was increasingly excluded from the nutritious receptacle resources with increasing *R. conicus* priority access to those resources.

Feeding damage

The proportion of seeds eaten or severely damaged per flower head increased significantly when *R. conicus* was present, from 60% in the fly-only (F0) treatment to 88–96% in the treatments with the weevil added (Table 1; Kruskal–Wallis ANOVA on arcsine-transformed proportion damaged, $P < 0.001$). Also, the number of intact seeds remaining, representing unexploited resources, decreased significantly when *R. conicus* occurred (Table 1): from an average of 23.2 undamaged seeds per flower head in the fly-only (F0) treatment to only 0.3–2.3
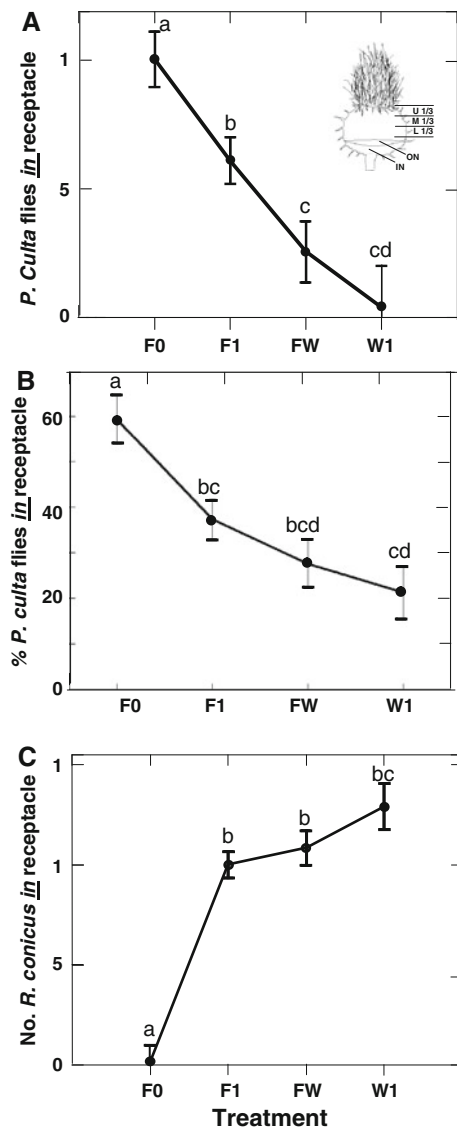
undamaged seeds in the treatments with *R. conicus* data recorded in the field ($P < 0.001$), results which parallel the reported field data (Louda and Potvin 1995; Louda et al. 1997, 2005b). Thus, the resource depletion caused by *R. conicus* feeding reduced the seed resources remaining dramatically (tenfold) in co-occupied heads. Overall, the co-occurrence of and priority access by the introduced weevil *R. conicus* with *P. culta* led to significant decreases in the total fly numbers, total fly biomass and individual fly sizes, and so potential fitness, on its native non-target host plant, *C. canescens*.

Projected fly population sizes with versus without the weevil

The individual based model (IBM) shows that populations of *P. culta* flies are likely to be negatively affected, by both *R. conicus* co-occurrence and flower head resource limitation. The long-term stochastic carrying capacity (K_s) of the fly population declined as the level of *R. conicus* infestation increased (Fig. 4a). Further, K_s for the fly population declined as *R. conicus* priority strength increased (Fig. 4b). Finally, K_s also declined as flower head resource availability decreased; specifically, fly populations with *R. conicus* present had lower overall population numbers relative to fly-only populations at any given resource level (Fig. 4c). As a result, the additive effects of *R. conicus* and resource limitation should drive fly populations even lower than the presence of *R. conicus* alone (Fig. 4c). Overall, the demographic inference from the model is that *R. conicus* co-occurrence and priority access, in the context of resource constraint, has the potential of leading to decreased population sizes of the native tephritid fly *P. culta* on its co-evolved native host plant.

Discussion

Research on the non-target effects of invasive weed biocontrol agents is generally focused on inter-trophic

A

B

C

level effects of the introduced insect on the newly adopted native host plant. However, more complex pathways of interaction characterize communities (e.g., Pearson and Callaway 2003). The aim of this study was to experimentally evaluate one such interaction, the putative intra-trophic level competition between the invasive flower head weevil, *R. conicus*, and the native tephritid fly, *P. culta*, mediated through the floral resources of the weevil's newly adopted, non-target native host plant, Platte thistle, *C. canescens*. We quantified the competitive interaction and assessed whether priority resource access influenced the outcome and intensity of competition between the two insects. We also determined the effect of priority access by the weevil on *P. culta* pre-oviposition behavior and post-oviposition development, as potential mechanisms that could underlie the observed competitive effects. Finally, we developed an IBM to explore the demographic implications of the experimental data. The results provide strong evidence that biological control agents can exert significant non-target effects on native species through complex competitive mechanisms, potentially reducing numbers of the affected native species, and so producing unanticipated, non-target, "ripple" effects within communities.

Role of priority access in the intensity and outcome of interaction

Overall, *R. conicus* had strong competitive effects on the co-occurring native tephritid fly, *P. culta*. Both the total numbers and the biomass of the flies were significantly lower in the presence of *R. conicus* on the flower head resource. When the two species co-occurred within a flower head, mean individual fly size (mass) by sex, a parameter of fitness, was greater when the fly had priority than when *R. conicus* had simultaneous or prior access. Further, total fly mass decreased as total *R. conicus* mass increased in a

flower head (Fig. 2). These experimental results are consistent with our initial experiment (On-Line Resource 1) and with the available evidence suggesting that invasive insect herbivores have the potential of exerting negative effects on native competitors (reviewed in Kenis et al. 2009). The results here also are consistent with the evidence showing that competition often occurs among overlapping biological control agents (Berube 1980; Smith and Mayer 2005; Hunt-Joshi and Blossey 2005; Crowe and Bouchier 2006). To our knowledge, however, this study provides the first experimental evidence demonstrating strong negative competitive effects of an introduced weed biological control agent on a resident native insect herbivore associated with a newly adopted, non-target native host plant.

Priority access to flower head resources by *R. conicus* strongly increased the intensity of competition and the weevil's impact on the native tephritid fly, *P. culta*. The native fly escaped the competitive effects of the introduced weevil only when the flies had sole or priority access to a flower head. Fly numbers, total biomass and individual size (mass) were strongly competitively suppressed whenever oviposition by *P. culta* was either synchronized with, or followed, oviposition by *R. conicus*.

Further, the competitive effects were highly asymmetric. Priority access to the floral resources benefited the native fly more than the introduced weevil when the two species co-occurred. Both the numbers and the biomass of *P. culta* in flower heads with both species dropped dramatically as *R. conicus* priority increased (Fig. 1). Alternately, the only evidence that *R. conicus* was affected, even somewhat, by the presence or timing of *P. culta* attack was that individual weevil mass was highest in the weevil-only (W0) treatment, without any flies. Neither weevil numbers nor total weevil biomass per flower head were altered by weevil priority access to the floral resources. These results add to the evidence compiled in a meta-analysis (Kaplan and Denno 2007) showing that competition between insect herbivores is often highly asymmetric.

Other studies of floral herbivore interactions also have found strong asymmetries in floral competition between weevils and tephritid flies in co-occurrence. For example, two studies evaluated the interaction of two floral-feeding biocontrol insects on knapweed (*Centaurea* spp) flower heads. Crowe and Bouchier

(2006) found that both the rate of attack by, and the total numbers of, the weevil *Larinus minutus* Gyllenhal were decreased significantly in the presence of the gall-inducing tephritid fly, *Urophora affinis* Frauenfeld. However, Smith and Mayer (2005) found no evidence of a competitive effect of *U. affinis* on *L. minutus*; instead, the presence of the weevil led to significant decreases in the fly (*U. affinis*). Both studies document an interaction between the floral insects. The discrepancy between the two studies, however, likely can be explained by a difference in the timing of attack (Crowe and Bouchier 2006). In the field, *U. affinis* uses flower heads before *L. minutus* does and so has priority, potentially allowing the galling flies to successfully sequester floral resources before the weevil oviposits. In Smith and Mayer's (2005) study, however, both insects were released into cages simultaneously, establishing synchrony of attack and likely increasing the competitive effect of the weevil on the fly (Crowe and Bouchier 2006). These studies of interactions between biological control agents suggest that the timing, or priority, of insect attack actually altered the competitive hierarchy between the floral feeding insects. In contrast, in our study the invasive weevil was generally the superior competitor. We found that the numbers of *R. conicus* were not reduced by the presence of the fly, regardless of the relative timing of attack (Fig. 1c). Yet, the numbers and performance of the native tephritid *P. culta* (Figs. 1, 2, 3) were strongly and negatively suppressed by presence of the weevil, *R. conicus*, when weevil attack of flower heads preceded or coincided with that of flies.

Multiple mechanisms led to competitive outcomes

Priority access to a shared resource can confer an advantage through a variety of direct (interference) and indirect (exploitation) competitive mechanisms. When both species co-occurred, we found that flies were displaced from the receptacle, clear evidence for interference competition from the weevils that came from below; and, we found numerical and size differences related to the priority access treatment, highly suggestive evidence for exploitative competition as a factor in explaining the strong effects of *R. conicus* on *P. culta*.

The laboratory data on pre-oviposition behavior by the fly showed that prior oviposition by *R. conicus*

interfered at least partially with flower head use and oviposition by *P. culta*. The native flies spent less time actively exploring and ovipositing on heads to which *R. conicus* had had prior access than on heads with no evidence of weevil oviposition, although priority weevil access did not preclude fly use completely. Partial fly avoidance of flower heads with *R. conicus* eggs supports long-term field observations that show that as *R. conicus* numbers increased with invasion, *P. culta* numbers decreased (Louda and Arnett 2000; Louda et al. 2005b). The inference from the laboratory experiment and the related field data is that the demographic impacts emerging from the individual-based model include some shift in fly use to less preferred, later developing, smaller, and so inferior, flower head resources, which will depend upon the availability of other suitable floral resources. Quantitative evaluation of this inference remains to be done.

Results from the field experiment examining the effects of post-ovipositional interactions suggested that the competitive effects of *R. conicus* on *P. culta* in co-occurrence were mediated by both exploitative competition for limited resources in smaller flower heads or in heads with high insect densities, and by interference competition, via spatial displacement of flies to lower quality resources, in larger flower heads or at lower total insect densities. At least three lines of evidence suggest that exploitation competition contributed to the strong competitive effect of *R. conicus* on *P. culta*. First, flower head size, a measure of resource availability, was consistently a highly significant covariate in explaining within treatment variation of both numbers and biomass of insects, as well as numbers and performance of *P. culta* developing in a flower head; larger heads have more insects and greater insect success. These results suggest that decreased resources intensify the consequences of post-oviposition competition. This is consistent with field observations, where smaller heads tend to have only one of the two species developing, despite evidence of oviposition by both (S. Louda, unpublished data). Second, in co-occurrence both the number and biomass of successful *P. culta* flies decreased as the number of insects, especially *R. conicus*, increased. Finally, third, the remaining, unconsumed resources within a flower head, measured as the number of matured intact seeds remaining, decreased dramatically as *R. conicus* presence

and priority increased and *P. culta* abundance and biomass decreased. Together, these three lines of evidence suggest that the floral feeding insects are often resource-limited, and that the addition of *R. conicus* significantly decreases resource availability to *P. culta*.

Further, the evidence shows interference occurred in the larger heads, ones in which both insects developed. When the two species co-occurred, the number of *P. culta* in contact with the highly nutritive tissues of the receptacle decreased as the priority and number of *R. conicus* increased. Such competitively mediated spatial niche shifts, a form of interference competition in which the inferior or later-arriving competitor is physically displaced to poorer feeding sites, are quite common within insect communities (Denno et al. 1995). Here, the degree of fly displacement within flower heads was directly proportional to the degree of priority access by *R. conicus*, suggesting that simultaneously- or earlier-arriving weevils interfered directly with feeding by the native fly. Fly mass, recorded for the flies surviving in co-occupied heads, decreased as weevil mass increased (Fig. 1; On-Line Resource 1); and, weevil mass increased as weevil priority increased among the treatments (F1 $\leq$ FW $\leq$ W1: Fig. 1). Thus, fly displacement to increasingly distant, likely inferior feeding positions away from the nutrient-rich receptacle in co-occurrence provides an explanation for the significant declines in successful *P. culta* numbers, total mass, and individual fly size by sex observed as *R. conicus* priority, presence and mass increased.

Cumulatively, the results suggest that a combination of mechanisms underlie the overall competitive effects of the weevil *R. conicus* on the native tephritid fly *P. culta*. These mechanisms include: (1) pre-ovipositional interference, mediated by at least partial fly oviposition avoidance of flower heads previously colonized by the weevil; (2) post-oviposition resource use and depletion, mediated by flower head size and, so, resource availability; and, (3) spatial displacement from preferred feeding sites, resulting in interference with fly feeding, in larger flower heads.

Demographic implications of the experimental data

The individual based model (IBM) showed that the competitive interactions documented here can cause

decreases in the population size of *P. culta* flies. Both resource limitation and *R. conicus* priority and level of infestation affected the stochastic carrying capacity (K_s) for the fly population. Sustainable numbers of flies dropped as the *R. conicus* infestation was increased or as weevil priority was increased. Thus, the additive effects of resource limitation, now associated with declines in *C. canescens* populations (Rose et al. 2005), and *R. conicus* occurrence are likely to decrease populations of *P. culta*. Specific model predictions of demographic impacts and underlying mechanisms are open to test with further experimental and observational data. In sum, the individual based model results, which are consistent with extensive field data suggesting an inverse relationship between the numbers of the native fly and the invasive weevil (Louda and Arnett 2000; Louda et al. 2005b, Russell and Louda 2004, 2005, and S. Louda, unpublished data), implies that both occurrence and priority access of the weevil *R. conicus* could have a strong negative effect on *P. culta* population density.

Inferences for invasion outcomes

The experimental results here demonstrate strong competitive effects of an invasive insect on the native fly, *Paracantha culta*, both before co-occurrence (pre-oviposition) and in co-occurrence (post-oviposition) within individual thistle flower head resource packets. Further, the IBM model results show that such interactions can have significant, negative demographic consequences for the native fly population. Although the direct trophic effects of biological control agents on non-targeted native hosts are increasingly documented (Simberloff and Stiling 1996; Louda et al. 1997, 2005b; Louda 2000; Louda and Arnett 2000), our understanding of the complex direct and indirect pathways and mechanisms by which such non-target interactions ripple through communities remains in its infancy (Pearson and Callaway 2003). The experimental results presented here unequivocally demonstrate that not only native plants, but also their associated native herbivores, can be significantly affected by the host range expansion of a weed biological control insect. Further, the IBM model demonstrates the potential for significant, negative effects on the native fly populations. More generally, the research provides experimental and

modeling support for the idea that competition can be an important mechanism underlying invasive species effects on native species within the same trophic guild.

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RESOURCE 1: SMALL-SCALE PRELIMINARY FIELD TEST

METHODS

As an initial test of the interaction between the invasive biocontrol weevil, *Rhinocyllus conicus,* and the native tephritid flies (*Paracantha culta*, *Orellia occidentale*), we measured all flower heads $\geq$ 12 mm diameter (n = 1 – 3 per plant, median = 1) on 60 plants on 25 - 29 May 1999. Then, we removed any smaller flower head buds and covered each experimental flower head with a large (1 l volume) clear plastic cage with mesh windows; the cage was supported by an adjacent pole. Flower heads within and between plants were assigned to one of four treatments by blocking on head size and head position within a plant; and, treatments were randomly assigned within blocks (n = 15 flower heads per treatment). The four treatments were, in the order of increasing *R. conicus* influence: flies-only (F0), flies-first followed by weevils (F1), weevils-first followed by flies (W1), and weevils-only (W0). We used insects caught in the field and held in the lab (< 7 d) until the treatment were established 4 – 6 June 1999. We added five flies of one tephritid species (50% fly-added cages received *P. culta;* 50% received *O. occidentale*) for the first two treatments (F0, F1); the flies remained in the cages for 4 d. At the same time, we added four weevils (2 mating pairs) to cages of the other two treatments (W1, W0); the weevils remained in the cage until a target number of eggs, determined from field observations and based on flower head size, had been laid ($\leq$ 6 d). In the two priority treatments (F1, W1), we gave the insect with priority 6 d to establish before adding the second insect species, which then was allowed to remain for 4 – 6 d, as above. Flower heads were kept covered by the cage for the rest of the season, except for ~48 h when the flowers were in anthesis to allow natural pollination. Upon maturation, we collected the flower heads, dissected them, and counted and weighed all insects and recorded feeding damage.

Dissection showed that the initial treatment assignment was compromised for eight flower heads. Two heads initially assigned to weevil only (W0) also produced *P. culta;* fly oviposition must have occurred prior to our caging and addition of weevils, suggesting these heads belonged in the fly first (F1) treatment. One head in the weevil first (W1) treatment produced no weevils but only flies, suggesting this head belonged in the flies only (F0) treatment. Finally, five heads to which *O. occidentale* had been added produced only *P. culta,* indicating that *P. culta* had oviposited earlier than our subsequent treatment and suggesting these heads belonged in the fly first (F1) treatment. We compared responses for these eight naturally-imposed treatments with the experimentally-imposed treatments, with using Kruskal-Wallis ANOVA on ranks. Since we found no statistical difference in the number of flies in these heads by treatment ($P = 0.367$), the eight heads were included in the treatment suggested by the dissection data. Given these re-assignments along with the death of several plants and their flower heads, final sample sizes by treatment were: 8 fly only (F0), 18 fly first (F1), 5 weevil first (W1), and 4 weevil only (W0) replicates.

We analyzed treatment effect on: numbers of insects, insect weight (mass), difference in proportion of the receptacle damaged, and numbers of intact seed resources remaining, using one-way analysis of variance (ANOVA) followed by Student's t test with the Bonferroni correction. However, when the underlying assumptions for parametric ANOVA were not met, we used nonparametric Kruskal-Wallis ANOVA on ranks.

RESULTS

The four treatments were effective in altering resource access between *R. conicus* and the native flies. No difference occurred between fly species (Kruskal Wallis ANOVA, $P > 0.20$), so

we combined the two flies in subsequent analyses. As planned, flower heads with *R. conicus* added (F1, W1, W0) had significantly more weevil egg cases (8.0 − 8.5) than did flower heads in the fly-only (F0) treatment (0.0) (Table 1; Kruskal Wallis ANOVA, P < 0.001).

Numerical Responses

The total number of insects developing per flower head was similar among treatments (Table 1, P = 0.322), although there was a trend toward higher total numbers in the fly first (F1) treatment.

The total number of *R. conicus* developing per flower head did not differ significantly among the treatments with weevils added (Figure 1A, P > 0.20 in all contrasts); however, as planned, the number of weevils developing was significantly higher in weevil-added treatments than in the fly-only (F0) treatment (Figure 1A; ANCOVA, flower head size as covariate: $F_{3,31} = 34.487$, P < 0.001, $R^2 = 0.770$). Timing of *R. conicus* development was similar among the three weevil-added treatments, since the proportion of immature weevils remaining as the plants senesced did not differ among weevil-added treatments (ANOVA: $F_{2,22} = 0.410$, P = 0.669).

The total numbers of flies developing in a flower head decreased progressively and significantly as *R. conicus* priority access increased (Figure 1C; ANOVA: $F_{3,32} = 3.485$, P = 0.027). Additionally, the relative contribution of native flies to the total number of insects in a flower head decreased as *R. conicus* priority increased: 41% in fly first (F1) vs. 15% in weevil first (W1) treatments. The number of flies developing in the treatment without weevils (F0) was significantly greater than in the treatment where weevils had priority (W1) (Tukey's HSD P = 0.034). The overall decrease represented a decrease in *P. culta,* the predominant native fly (from 9.4 to 0.0 per head), while numbers of *O. occidentale,* the later-occurring fly, actually increased:

from 0.2 in fly-only (F0), to 2.0 in fly-first (F1) and weevil-first (W1) treatments (Table 1), suggesting both that *P. culta* is more negatively affected by interaction with *R. conicus* than *O. occidentale* was and also that *P. culta* out-competed *O. occidental,* explaining the increase in *O. occidentale* with the decrease in *P. culta*. Differences in the number of flies developing among treatments could not be explained by variation in flower head size (floral resource availability), since average flower head size (diameter) was similar across all treatments (Table 1). Overall, the total number of native flies declined in flower heads shared with *R. conicus,* and the decline in *P. culta* in particular was greater when the weevil had earlier access to the flower head resources.

Biomass Responses

Since insects vary in size, we also examined biomass response. The patterns in biomass paralleled the patterns in numbers (Figure 1). The total biomass of insects developing per flower head did not differ among treatments (Figure 1E; ANOVA, $F_{3,32} = 0.836$, $P = 0.568$). Thus, shifting priority of access from native fly to introduced invasive weevil did not substantially alter the total floral insect biomass developing in each flower head.

Weevil biomass was significantly higher in all three weevil-added treatments (F1, W1, W0) than in the fly-only (F0) treatment, showing the treatments worked as expected (Figure 1B; Kruskal Wallis ANOVA on ranks: $Q = 3.554$, $P < 0.05$). Weevil biomass did not vary significantly among the three treatments with weevils added (Figure 1B; Kruskal Wallis ANOVA on ranks, $Q = 1.260$, $P > 0.05$), suggesting flies had little or no effect on weevil performance as measured by biomass.

Fly biomass, however, decreased significantly as *R. conicus* priority increased (Figure 1D; ANOVA: $F_{3,32}$ = 4.881, P = 0.007). Fly biomass was higher in the fly-only (F0) treatment than in each of the other three treatments (pairwise t-tests, Holm-Sidak correction for multiple comparisons: all t > 2.87, all P < 0.05). Total fly biomass also declined as total *R. conicus* biomass increased (Figure 2A; regression P < 0.01). The relative contribution of flies to total insect biomass per head dropped 88.9% from the fly-only (F0) treatment to the weevil-first (W1) treatment (Table 1). So, *R. conicus* priority decreased both the development of *P. culta* biomass, as well as decreasing fly numbers (above).

The individual size of *P. culta* adults that developed, especially females, appeared negatively affected by increasing interaction with *R. conicus* as weevil priority increased (Figure 2B, P < 0.001). Individual female *P. culta* tended to be bigger in the fly-only (F0) treatment (14.8 mg, SE 2.73) than in the fly-first (F1) treatment (10.6 mg, SE 0.82) (Mann-Whitney U = 27.0, P = 0.062). Individual male *P. culta* size did not differ statistically among treatments, although the trend also was for males to be bigger in the fly-only (F0) treatment (10.8 mg, SE 0.93) than in the fly-first (F1) treatment (8.6 mg, SE 0.90) (Mann-Whitney U = 22.0, P = 0.131). Reduced size of emerging flies, especially females, suggests a reduction in fitness, since egg production is generally related to insect body size.

Resource Use

Evidence of insect feeding damage to floral resources increased as the number of *R. conicus* increased, including fewer and fewer intact seed resources remaining (Table 1). Treatment differences were driven by much lower damage in the fly-only (F0) treatment than in the three treatments with weevils (ANOVA: $F_{1,30}$ = 6.370, P = 0.02). The number of intact seeds

remaining as unconsumed resources was significantly lower when weevils were present than when flies alone occurred (ANCOVA, head size as the covariate: Treatment $F_{2,11} = 4.356$, P = 0.011) . Overall, *R. conicus* dramatically reduced floral guild resources whenever it was present, helping explain the declines observed in the numbers, biomass and individual fly mass with increasing introduced weevil priority to flower head resources.

Table 1. Parameters (X, SE) potentially affecting treatment response in the preliminary priority experiment, with the treatments ordered (left to right) by increasing influence of the exotic invasive weevil, *Rhinocyllus conicus;* differences among treatments were evaluated using ANOVA (F test), or Kruskal-Wallis ANOVA on ranks (H test) on non-normal variables.

Per flower head:	Fly-only (F0)		Fly-first (F1)		Weevil-first (W1)		Weevil only (W0)		Test	P
	X	SE	X	SE	X	SE	X	SE		
Number of insects:										
R. conicus egg cases	0.0	--	8.3	0.72	8.5	1.56	8.0	2.42	H =	< 0.001
Total number flies + weevils	9.7	1.93	12.9	1.24	8.8	1.66	8.0	1.73	F =	0.322
Number *P. culta*	9.4	1.99	5.4	1.26	0.0	0.00	0.0	--	F =	< 0.001
Number *O. occidentale*	0.2	0.22	0.7	0.34	2.0	1.68	0.0	--	F =	0.322
Final status:										
Proportion fly biomass	1.0	0.00	0.34	0.07	0.111	0.09	0.0	--	H =	< 0.001
Final head width, mm	26.2	1.24	25.4	0.92	20.6	3.06	25.5	2.77	F =	0.264
Proportion receptacle damaged	0.42	0.118	0.98	0.11	0.89	0.02	0.99	0.01	F =	0.011
Seeds (resources) remaining	48.1	14.82	21.3	8.02	0.0	0.00	3.8	3.80	H =	0.20

Figure 1. Number and biomass of insects per flower head (least-square mean, SE): A, number *Rhinocyllus conicus;* B, biomass of *R. conicus*; C, number native tephritid flies *(Paracantha culta, Orellia occidentale);* D, biomass of tephritid flies; E, total insect biomass; and, F, proportion of the flower head receptacle surface damaged by insect feeding. The treatments were: F0 = fly-only; F1 = fly-first; W1 = weevil-first; and W0 = weevil-only, ordered (left to right) by increasing priority of the invasive weevil, *R. conicus*.
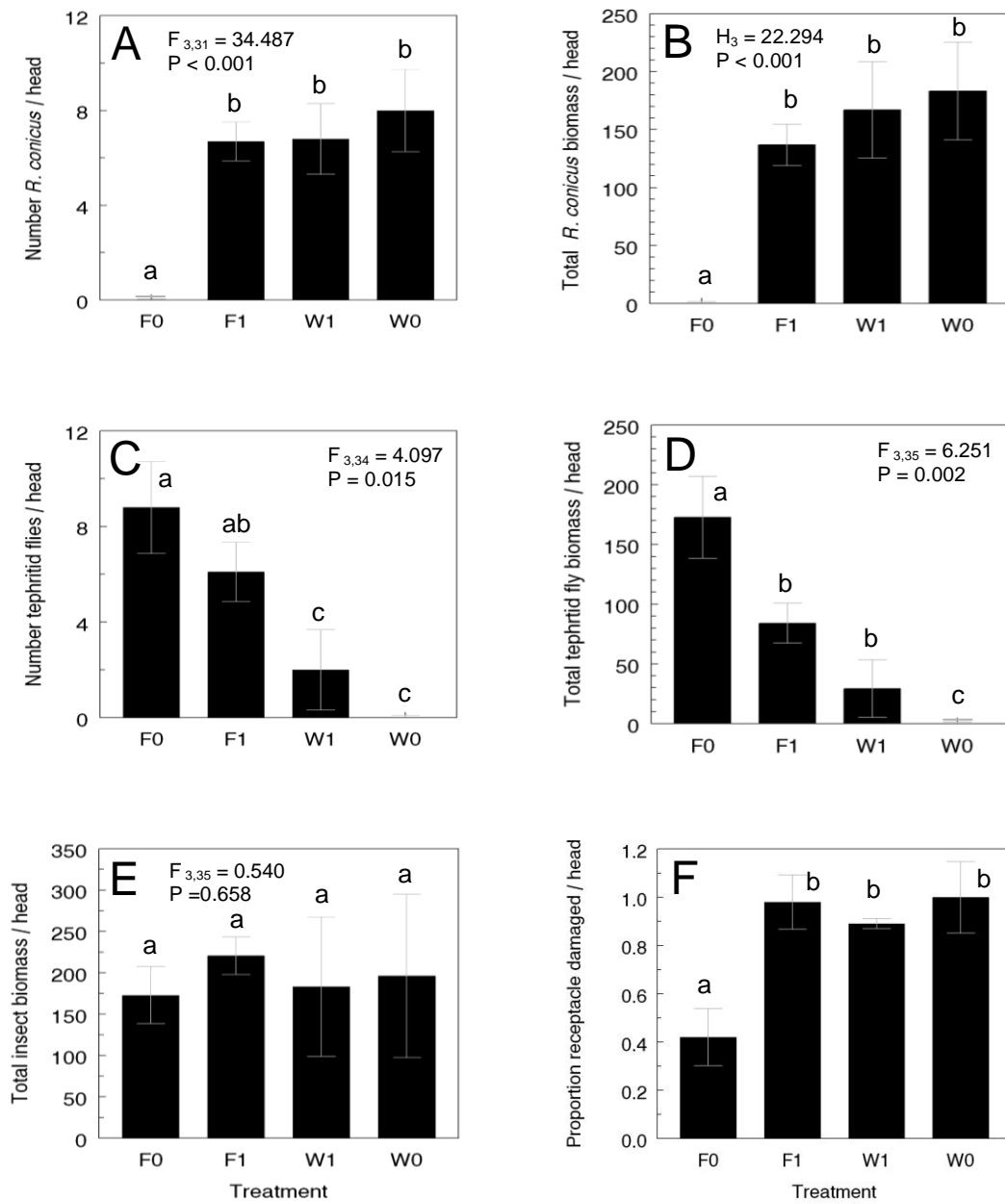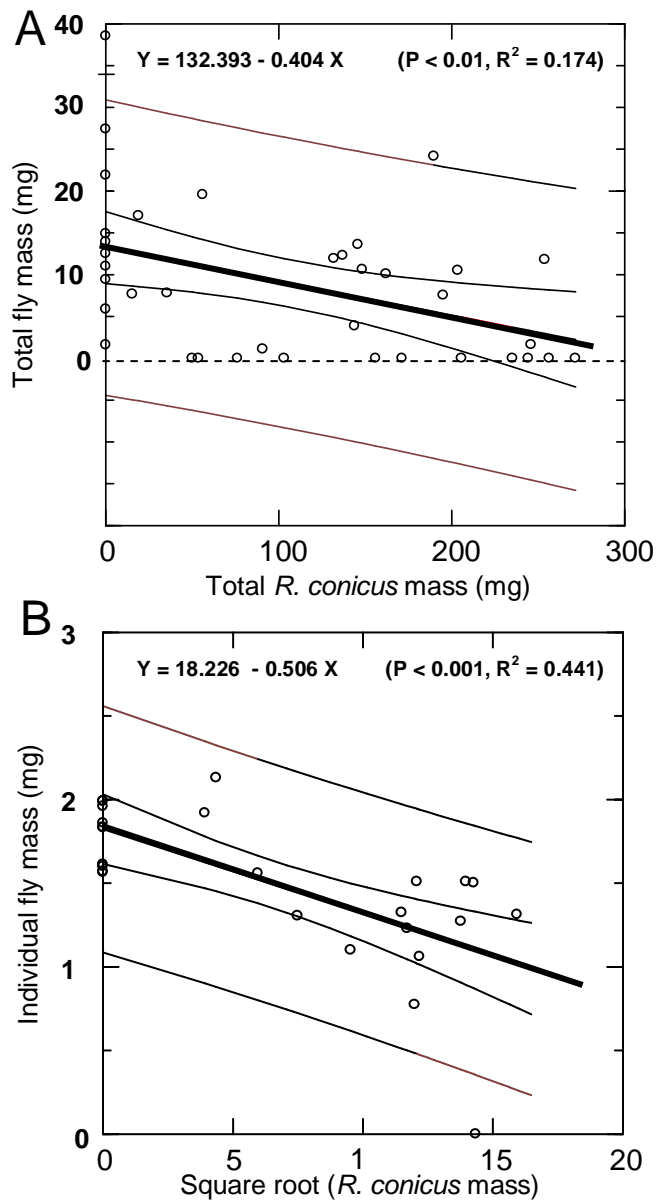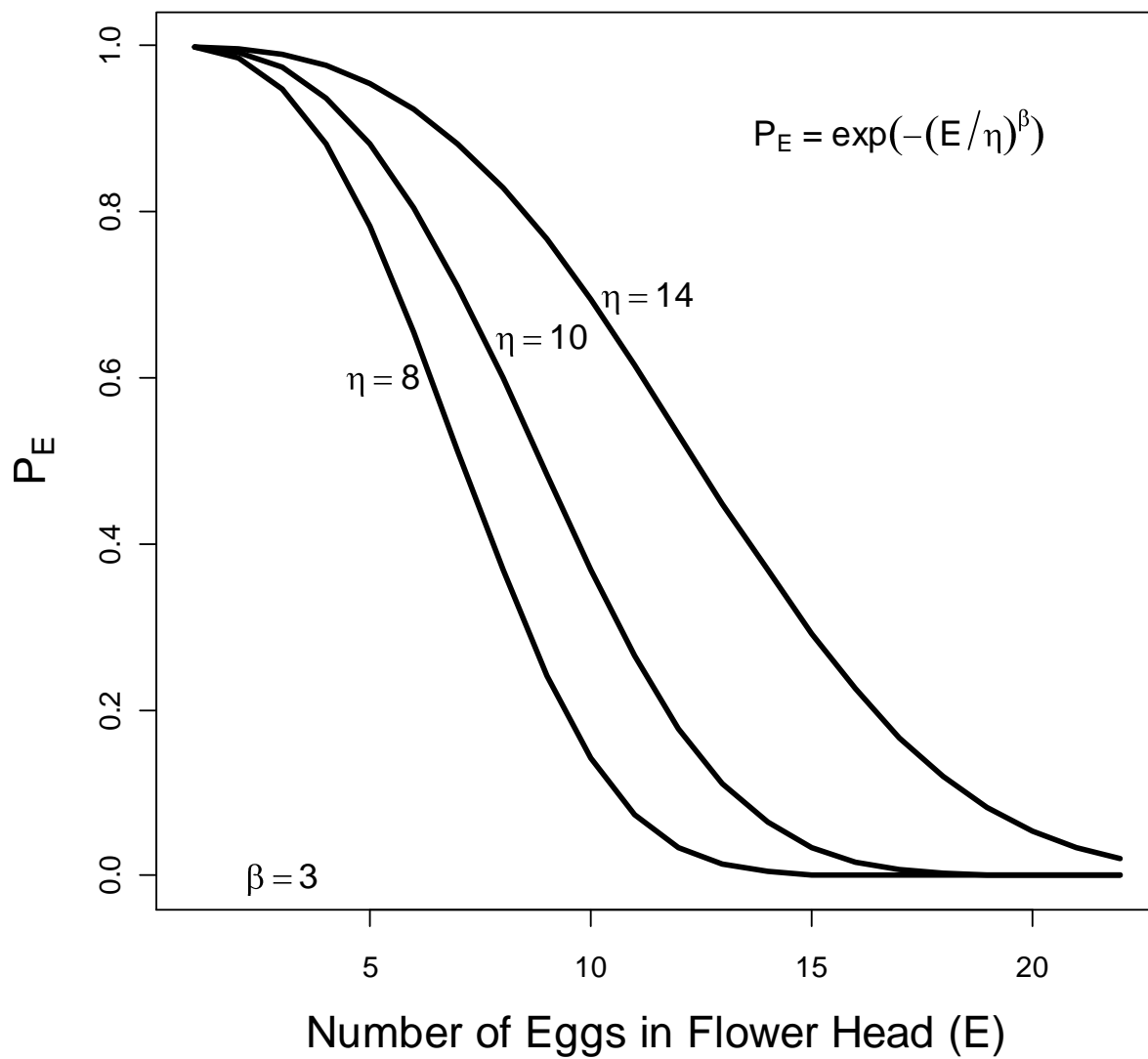
Figure 2. Least squares regressions showing the relationships for: A, total fly biomass to total *R. conicus* biomass per head; and, B, individual fly biomass to total *R. conicus* biomass per head (square root-transformed) in the preliminary insect priority access experiment on Platte thistle (*Cirsium canescens*). The priority access treatments were: F0 = fly-only; F1 = fly-first; W1 = weevil-first; and W0 = weevil-only.


A

$Y = 132.393 - 0.404 X$ ($P < 0.01$, $R^2 = 0.174$)

Total fly mass (mg)

Total *R. conicus* mass (mg)

B

$Y = 18.226 - 0.506 X$ ($P < 0.001$, $R^2 = 0.441$)

Individual fly mass (mg)

Square root (*R. conicus* mass)

**RESOURCE 2: Individual-Based Model (IBM) Parameter Estimation Details**

I. Effect of changes in the shape parameter (β) on the oviposition probability. As β varies, the shape of the oviposition curve changes, affecting the rate at which the probability of fly oviposition declines with increasing numbers of insects present in a flower head.

The figure shows oviposition probability P_E plotted against the number of eggs in flower head (E), with three curves for $\eta = 8$, $\eta = 10$, and $\eta = 14$, and $\beta = 3$.

$$P_E = \exp\left(-(E/\eta)^{\beta}\right)$$

II. Effect of changes in the scale parameter (η) on the oviposition probability. As η increases the probability of ovipositing asymptotes to zero at higher E-values).

$$P_E = \exp\left(-\left(E/\eta\right)^{\beta}\right)$$
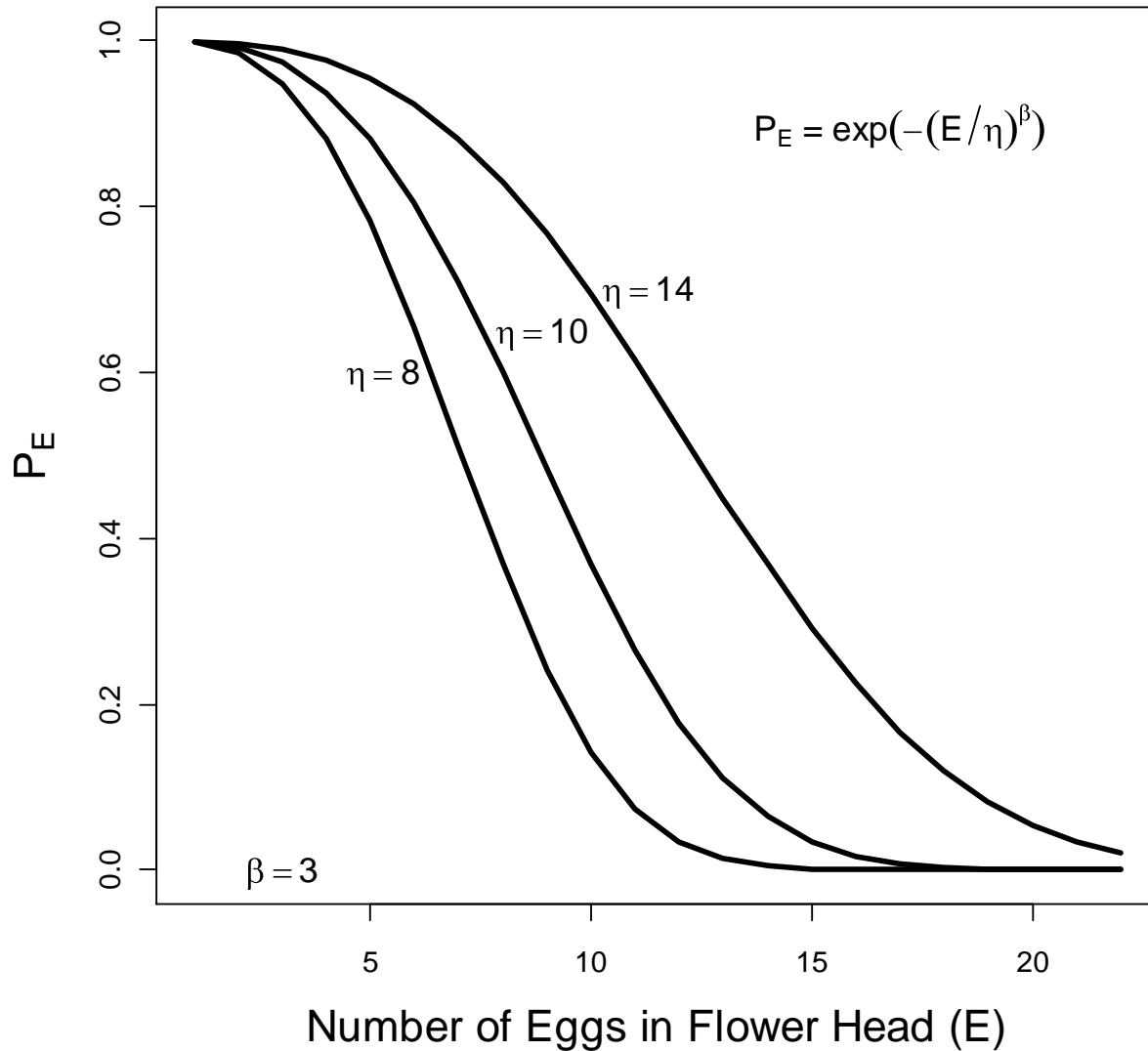
III. Effect of initial egg load of female flies on stochastic carrying capacity (K_s) per 20 flower heads. We found that K_s was not influenced by egg limitation in female flies, at any level of *R. conicus* infestation of flower heads. ($\beta = 3$, $\eta = 10$, number of flower heads = 20, priority strength = 9 [~ strength shown in the pre-oviposition experiment]).
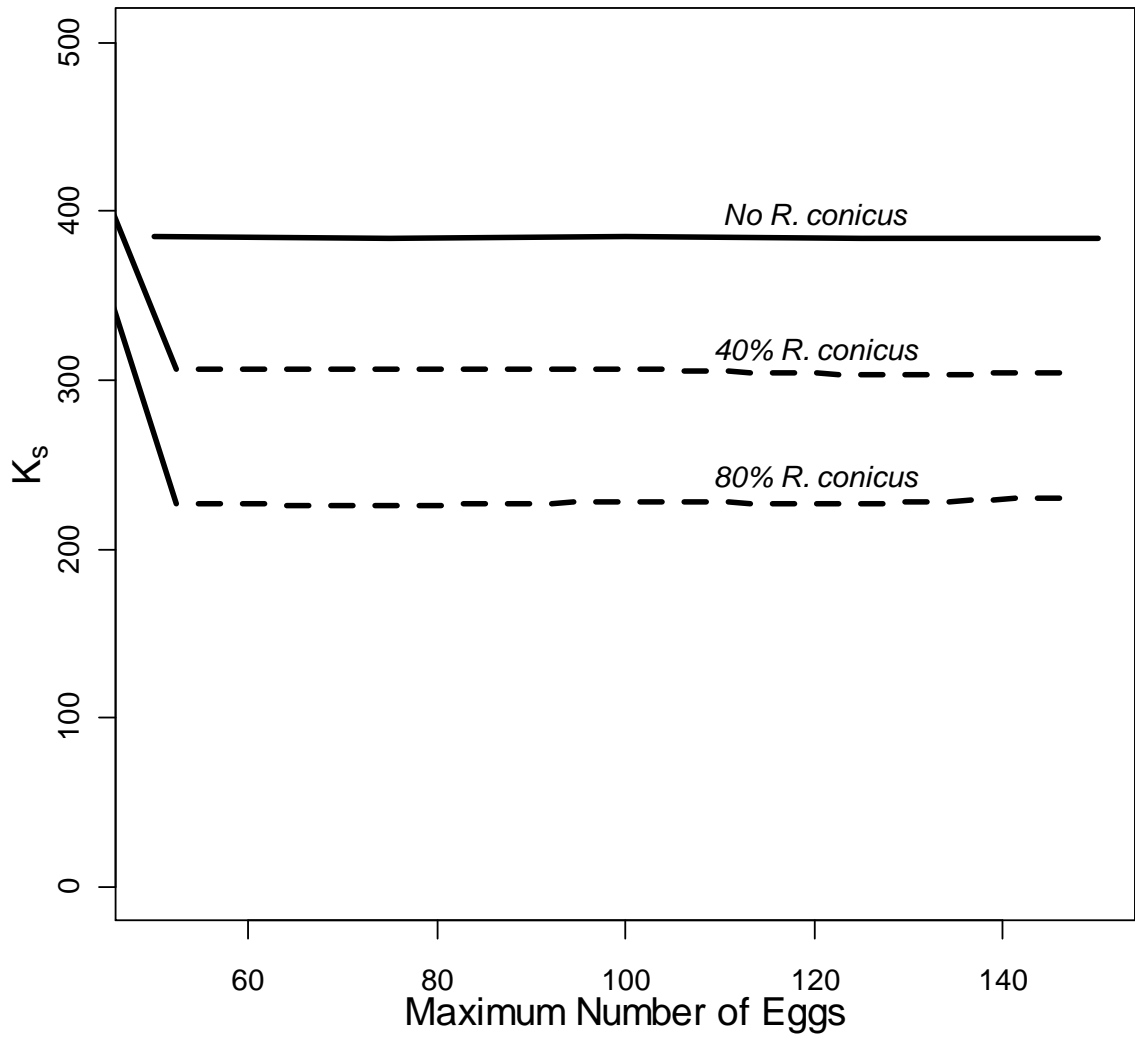
IV. Effect of the number of oviposition opportunities per female fly on the stochastic carrying capacity (K_s) per 20 flower heads. We plotted the effect for three levels of *R. conicus* infestation of flower heads. ($\beta = 3$, $\eta = 10$, number of flower heads = 20, priority strength = 9 [~ strength shown in the pre-oviposition experiment]).