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VARIATION IN HERBIVORE-MEDIATED INDIRECT EFFECTS OF AN INVASIVE PLANT ON A NATIVE PLANT

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Abstract. Theory predicts that damage by a shared herbivore to a secondary host plant species may either be higher or lower in the vicinity of a preferred host plant species. To evaluate the importance of ecological factors, such as host plant proximity and density, in determining the direction and strength of such herbivore-mediated indirect effects, we quantified oviposition by the exotic weevil *Rhinocyllus conicus* on the native wavyleaf thistle *Cirsium undulatum* in midgrass prairie on loam soils in the upper Great Plains, USA. Over three years (2001–2003), the number of eggs laid by *R. conicus* on *C. undulatum* always decreased significantly with distance (0–220 m) from a musk thistle (*Carduus nutans* L.) patch. Neither the level of *R. conicus* oviposition on *C. undulatum* nor the strength of the distance effect was predicted by local musk thistle patch density or by local *C. undulatum* density (≤ 5 m). The results suggest that high *R. conicus* egg loads on *C. undulatum* near musk thistle resulted from the native thistle's co-occurrence with the coevolved preferred exotic host plant and not from the weevil's response to local host plant density. Mean egg loads on *C. undulatum* also were greater at sites with higher *R. conicus* densities. We conclude that both preferred-plant proximity and shared herbivore density strongly affected the herbivore-mediated indirect interaction, suggesting that such interactions are important pathways by which invasive exotic weeds can indirectly impact native plants.

Key words: *apparent competition; associational defense; biological control; Carduus nutans L.; Cirsium undulatum Spreng.; insect herbivory; invasive plants; non-target effects; Rhinocyllus conicus; thistle; weeds; weevil.*

INTRODUCTION

Empirical results and emerging theory suggest that the presence of an alternative, preferred host species can strongly affect the intensity of herbivory on a less preferred (i.e., secondary) host plant species by a shared insect herbivore. Such indirect interactions may result in “associational defense” (Atsatt and O’Dowd 1976), in which occurrence with a preferred host reduces feeding on the secondary host. For example, in agroecosystems, palatable species may be used as “trap crops,” i.e., they draw herbivores away from associated crops (Hokkanen 1991). Alternatively, secondary host plants near more preferred neighbors may suffer greater damage (Hjalten et al. 1993, Wahl and Hay 1995, Rand 2003). This phenomenon has been called “associational susceptibility” (Brown and Ewel 1987) or “apparent competition” (Holt 1977). Theory predicts that apparent competition/associational susceptibility of less preferred hosts will occur if herbivore populations are limited by food availability or if herbivores aggregate in preferred host patches and spill over onto secondary hosts nearby

(Holt 1977, Holt and Kotler 1987, Abrams and Matsuda 1996). Few tests of these predictions exist.

Holt and Hochberg (2001) extended apparent competition theory for indirect effects of biological control insects. Their analysis predicts that biocontrol insects will mediate strong indirect effects of invasive weeds on native plants if the insect will attack native plants and its effects on the targeted weed are weak; weak effects allow the weed to remain abundant enough to support a large insect population. The possibility that biocontrol insects can mediate indirect effects of exotic weeds on native species rarely has been evaluated. In one of the first studies to document indirect effects of an invasive weed on a native plant mediated by a biocontrol insect, Rand and Louda (2004) found that nontarget damage by *Rhinocyllus conicus* Frölich, an exotic flower head weevil introduced against the exotic musk thistle (*Carduus nutans* L.), to native *Cirsium* spp. in midgrass prairies on loam soils was related to both site- and landscape-scale abundances of the targeted weed. That study provides the foundation for the more spatially explicit evaluation of the interaction presented here.

In theory, the strength of the population response by the shared herbivore is critical to determining the direction of the indirect effect (Holt 1977, Holt and Kotler 1987). White and Whitham (2000) and Blossey et al. (2001) hypothesized that associational susceptibility only occurs where insect herbivore populations are large

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enough to deplete their preferred host, forcing them to attack secondary hosts. This resource-dependent hypothesis suggests that the ratio of insect herbivores to preferred host plant resources drives associational susceptibility of secondary hosts. Further, because local population density of specialist insect herbivores often is related to the spatial structure of host plant patches (Root 1973, Bach 1984), patch structure of preferred hosts may affect the outcome of herbivore-mediated indirect effects on co-occurring, less preferred neighbors. In spite of the conceptual importance of herbivore abundance in such indirect interactions, we found no studies of herbivore-mediated indirect effects that quantified herbivore density across multiple sites or multiple years.

In this study, our first objective was to determine if the native North American wavyleaf thistle (*Cirsium undulatum* Spreng.) experienced associational susceptibility or associational defense to inflorescence damage by the introduced biological-control weevil, *Rhinocyllus conicus*, near patches of its exotic preferred host weed, *Carduus nutans* L. (musk or nodding thistle). Our second objective was to examine effects of the density of the preferred exotic host plant and the local abundance of the shared herbivore on the direction and strength of the herbivore-mediated indirect effect. To achieve these goals, we quantified *R. conicus* oviposition on *Cirsium undulatum* in relation to proximity of *Carduus nutans* at 20 sites across mid-grass prairie on loam soils in southwestern Nebraska, USA. Specifically, the questions we addressed were these: (1) Is *R. conicus* damage to *C. undulatum* plants higher or lower near patches of the weedy *C. nutans*? (2) Is *R. conicus* damage to *C. undulatum* higher at sites or in years with greater *R. conicus* abundances? (3) Does the structure of *C. nutans* patches, specifically the musk thistle patch density, area, or total abundance, explain spatial and temporal variation in *R. conicus* damage to the native *C. undulatum*? And (4), does *R. conicus* abundance or *C. nutans* patch structure affect the relationship between distance to the weed thistle's patches and weevil damage to the native *C. undulatum*?

METHODS

Natural history of study system and study sites

Musk (or nodding) thistle, *Carduus nutans*, was introduced into the United States over 100 years ago from Eurasia (Rees 1982). It spread to become a noxious weed in grasslands, including those of the upper Great Plains. Musk thistle occurs primarily on loam and loamy-clay soils (Dunn 1976). It can grow as a biennial, winter annual, or annual. In Nebraska, most musk thistles bolt (initiate a reproductive stem) in May, begin flowering in June, and begin releasing seeds in July (McCarty 1982).

Wavyleaf thistle, *Cirsium undulatum* (see Plate 1), is a widespread native thistle of the central Great Plains of North America (Great Plains Flora Association 1986).

In the central plains, it is a tap-rooted, short-lived, iterocarpic perennial (Great Plains Flora Association 1986). In Nebraska, *C. undulatum* bolts in mid- to late May, begins flowering in early June, and disperses most seeds in late July (McCarty 1982, Louda 1998; S. Louda, *personal observation*).

The Eurasian flower head weevil, *Rhinocyllus conicus* (see Plate 1), was deliberately introduced from Europe into North America in 1968 and into Nebraska from 1969 to 1972 against weedy exotic thistles, especially musk thistle, *Carduus nutans* (Zwölfer and Harris 1984, Gassmann and Louda 2001). In Nebraska, overwintering adult weevils emerge in early May, and the females lay eggs on thistle flower heads under externally obvious egg covers of masticated tissues between mid May and late June (Louda 1998, Louda et al. 2005). Larvae burrow into the flower head where they develop, consuming receptacle tissues, florets, ovules, and developing seeds (Zwölfer and Harris 1984; S. Louda, *unpublished data*). Development takes 53–76 d (Zwölfer and Harris 1984; S. Louda, *unpublished data*), allowing one generation per year in this region. *Rhinocyllus conicus* often is only marginally effective in reducing musk thistle seed production (Milbrath and Nechols 2004; for review see Gassmann and Louda 2001), causing 30–45% reductions in seed production by terminal flower heads and having little effect on most lateral flower heads that often develop after the oviposition period (Hodgson and Rees 1976).

Site selection

Data were collected at 20 sites in Custer, Dawson, Gosper, Lincoln, and Keith Counties, with all sites centered around North Platte, Nebraska, USA (41.13° N, 100.76° W), in late June and early July 2001–2003 (Appendix A). At each site in each year, a musk thistle (*C. nutans*) patch and the associated naturally occurring wavyleaf thistles (*C. undulatum*) were sampled. We refer to the sampled musk patches as “focal musk patches.” Criteria for selecting sites were (1) presence of a musk thistle patch, (2) naturally occurring wavyleaf thistles in and near the musk patch, and (3) naturally occurring wavyleaf thistles from 80 to 200 m from the focal musk patch and at least 100–200 m from any other musk thistles. We found and sampled 11, 8, and 14 sites that met these criteria in 2001, 2002, and 2003, respectively.

Rhinocyllus conicus oviposition use of the native Cirsium undulatum

At each site in each year we quantified *R. conicus* egg load on naturally occurring wavyleaf thistles (*C. undulatum*) within a focal musk thistle patch and at 30–50 m, 80–100 m, and if available, 200–220 m from the focal musk patch along a transect that began at the patch edge. Transect direction varied among sites and was determined by the occurrence of musk thistle; transect direction was chosen to ensure that no musk thistles were closer to wavyleaf thistles at all distances



PLATE 1. Clockwise from left panel: *Cirsium undulatum* in flower, *Rhinocyllus conicus* adult on *C. undulatum* flower head, *R. conicus* larvae inside receptacle base of *C. undulatum* flower head, and *R. conicus* egg cases on phyllaries of *C. undulatum* flower head. Photo credits: S. M. Louda.

sampled than to the focal musk patch. At each site, we sampled up to 10 wavyleaf thistles at each distance (mean = 9.1 thistles, range: 3–10 thistles) within a 20×50 m plot, with the 50-m axis perpendicular to and centered on the transect. If more than 10 individuals occurred, the wavyleaf thistles sampled were selected randomly by blindly drawing paper slips marked “Y” or “N” from a bag as we approached each bolting wavyleaf thistle. For each 20×50 m plot, the number of slips in the bag was equal to the number of bolting wavyleaf thistles in the plot with 10 of those slips marked “Y.” In 2001, we also recorded our counts of the total number of bolting wavyleaf thistles in each plot.

We quantified wavyleaf thistle size by counting flower heads. We counted *R. conicus* egg cases (one egg per case) and “pinholes” on each flower head on each sampled plant. Pinholes are made by first instar *R. conicus* larvae as they burrow into the flower head, allowing us to detect successful oviposition even after egg covers fall off. In 2002 and 2003, we estimated local thistle density around each sampled wavyleaf plant by counting all thistles by species within 5 m of each sampled plant; we also measured their heights. We sampled within a 5-m radius because, in the uncommon case when wavyleaf thistles form a dense aggregation, the radii of the patches average <5 m. In addition to wavyleaf and musk thistles, three later flowering thistles sometimes occurred: two native species, *Cirsium ochrocentrum* A. Gray (yellowspine thistle) and *Cirsium*

flodmanii [Rydb.] Arthur (Flodman’s thistle) and one exotic species, *Cirsium vulgare* (Savi) Tenore (bull thistle).

Characteristics of focal musk thistle patches

Since host patch density (Root 1973, Turchin 1987), area (Bach 1984, Kareiva 1985), and total plant resource (Bach 1984, 1986) can affect local density of specialist insect herbivores, we estimated the density of bolting musk thistles, patch area, and the total abundance of bolting musk thistles (density \times area) for each focal musk thistle patch. The aim was to test the hypothesis that variation in the patch structure of the preferred, exotic musk thistle among sites would explain variation in the outcome of *R. conicus*-mediated indirect effects on the secondary, native host plant, *C. undulatum*.

We estimated the densities of focal musk patches by counting bolting musk thistles in 2 m wide belt transects along north–south and east–west axes through the patch. We estimated patch areas from field measurements of distances from the center to the edge of each patch along eight transects that radiated from the center of the patch in the four cardinal directions plus northeast, southeast, southwest, and northwest. Edges were defined by an abrupt decrease in musk thistle density and were identified by consensus among members of the data collection team. As a result of using these criteria, patch edges corresponded to a decrease in musk thistle density to <1 bolter/20 m². We

used an image analysis program (Scion Image, Scion, Frederick, Maryland, USA) to calculate the area of each musk patch from the eight radial measurements.

To estimate *R. conicus* adult density and activity in focal musk patches we counted egg cases on the terminal head of the first subsidiary branch, the terminal head of the third subsidiary branch, and the first lateral head of the second subsidiary branch of bolting musk thistles at 10-m intervals along north-south and east-west axes through each patch. The timing of development of these heads coincides with the duration of the *R. conicus* oviposition period. Because egg load on the terminal head of the first subsidiary branch significantly predicted the sum of egg loads on the terminal head of the third subsidiary branch and on the first lateral head of the second subsidiary branch (ANCOVA [site was included as an independent variable]: egg load on terminal head first subsidiary branch, $F_{1,127} = 37.92$, $P < 0.001$, $R^2 = 0.46$), and because the latter two heads were more frequently missing, we used egg load on the terminal head of the first subsidiary branch as our index of *R. conicus* use of musk thistle plants.

In 2003, we sampled five musk patches in early June, the time of peak *R. conicus* adult activity, as well as in early July, after oviposition ended, to determine the reliability of egg load estimates made in July: specifically, estimates made on the terminal head of the first subsidiary branch, as an index of the number of *R. conicus* adults per musk thistle flower head and per musk thistle plant in the musk patch during peak activity. Mean egg load on the terminal flower head of the first subsidiary branch of musk thistles in patches in July was significantly, positively related both to the mean number of *R. conicus* adults per flower head in patches in early June (linear regression: mean adult *R. conicus* per head = $-0.231 + 0.095 \times$ [mean number of egg cases/terminal head first subsidiary branch]; $N = 5$ patches, $P = 0.05$, $R^2 = 0.767$) and to the mean number of adults per plant in patches (linear regression: mean adult *R. conicus* per bolting musk thistle = $-0.859 + 0.364 \times$ [mean number of egg cases per terminal head of first subsidiary branch]; $N = 5$ patches, $P = 0.003$, $R^2 = 0.963$). Thus, to estimate *R. conicus* adult population size in each musk patch, we multiplied "estimated *R. conicus* density per bolting musk thistle" by "density of bolting musk thistles" by "patch area."

Data analyses

We used mixed-model ANCOVA (SAS version 8: PROC MIXED; SAS Institute 2003) to examine effects of variables describing the focal musk thistle patch structure (i.e., patch area, density, and total abundance of bolting musk thistles), as well as *R. conicus* adult density and population size for the patch, plus distance to the focal musk patch and size of individual wavyleaf thistles on egg load; egg load was defined as mean number of *R. conicus* egg cases plus pinholes per wavyleaf thistle flower head. Effects of musk thistle

patch area, density, total abundance of bolting musk thistles, *R. conicus* adult density, and population size were examined in separate ANCOVAs. We compared explanatory abilities of models that included the different musk thistle patch variables by using the Akaike Information Criterion (AIC; Burnham and Anderson 2002).

To analyze effects of focal musk thistle patch density, area, total bolting musk thistles in the patch, as well as *R. conicus* adult density and population size on the weevil egg load on the native wavyleaf thistles for each of these independent variables, we divided sites in each year into high and low categories. We treated these potential explanatory variables as categorical, rather than continuous, to avoid overstating the precision of estimates that are based on subsampling patches. Large fluctuations in musk thistle and *R. conicus* abundances between years within sites meant that (1) some sites that were sampled in multiple years were placed in different categories (high vs. low) in different years and (2) few sites could be sampled in all three years because musk thistles were absent at some sites in some years (Appendix A).

To ensure that results did not depend upon a particular method of classifying sites, we repeated each analysis using three different classification approaches. First, we equally divided sites in each year between high and low categories if there were an even number of sites. If the number of sites was odd, the median site was assigned to either the high or low category, according to the degree of separation from the most similar patch in each category. Second, we divided sites between high and low categories using the largest gap in values for the explanatory variable as the breakpoint, with the constraint that each category had to contain at least 35% of sites in each year. Third, we used three categories: high, medium, and low. Results obtained using the three different methods of classification did not differ. We present results from analyses in which sites were classified as high vs. low, with category boundaries chosen to achieve near-equal numbers of sites in each category.

In mixed-model ANCOVAs, variables related to properties of focal musk thistle patches and distance to the focal musk patch were treated as fixed effects; wavyleaf thistle size (number of flower heads) was used as the covariate; and year and site were treated as random effects (for statistical details see Appendices B–F). Variance components were estimated using maximum likelihood. We used the Kenward-Rogers method to calculate degrees of freedom associated with tests of each independent variable (Littell et al. 2002). Denominator degrees of freedom in tests of musk thistle patch variables were larger than the number of sites sampled because the year \times site variance was relatively large and sites that changed categories between years allowed comparisons within sites. We used protected least significant differences to make a posteriori comparisons

among means and to determine whether relationships between plant size and *R. conicus* egg load differed significantly from 0. The dependent variable, *R. conicus* egg load, was natural-log transformed to meet the assumptions of ANCOVA.

The repeated-measures structure of our ANCOVA was complicated by our inability to sample all sites in all three years. To deal with the problem of sites that were sampled more than one year apart (i.e., in 2001 and 2003, but not 2002), we assumed that correlation between observations two years apart were the same as observations one year apart. We made this assumption because sites with observations more than one year apart were relatively few. This assumption is conservative, likely overestimating the correlation between observations two years apart and so likely resulting in an overestimation of the standard errors for between-site comparisons. To account for the repeated-measures structure of the data, the model included random effects for year (Y), site (S), year \times site, and plot \times year \times site (P), in addition to the usual residual. The associated variances are σ_Y^2 , σ_S^2 , σ_{YS}^2 , σ_P^2 , and σ_e^2 . The resulting within-site covariance structure would have a covariance between years within a site of σ_S^2 , between plots within a site of $\sigma_S^2 + \sigma_{YS}^2$, and between plants within a plot of $\sigma_Y^2 + \sigma_{YS}^2 + \sigma_P^2$.

For the ANCOVA model that best fit the data as indicated by the AIC, we evaluated whether the effect of distance to the musk thistle patch varied significantly among years by using a likelihood-ratio test, comparing the model with year \times distance included as a random effect against the model that did not include the year \times distance interaction. To identify year \times distance combinations that might contribute to a significant interaction effect, we used t tests to determine whether best linear unbiased predictors for each year \times distance combination differed significantly from 0 (Littell et al. 2002).

We included number of flower heads per wavyleaf plant as a covariate in ANCOVA models to control for potential differences in plant quality that might be correlated with distance to musk thistles or musk patch structure. Based on results from previous studies of native prairie thistles (Rand and Louda 2004, 2006, Russell and Louda 2004, 2005), we used number of flower heads as the best measure of wavyleaf thistle plant quality for this flower head feeding weevil. We also analyzed variation in musk thistle heights across sites, as an index of variation in plant growing conditions, in relation to each of the focal musk-patch structure variables considered. Using separate ANCOVAs for each focal musk patch structure variable (i.e., patch density, area, total musk abundance, *R. conicus* density, population size), we tested whether musk thistle height differed between sites in different levels of the patch structure variable. Patch structure variables were fixed effects, whereas site and year were random effects. Musk thistle height provided an independent measure of site

quality for plant growth that would not have been accounted for already by including wavyleaf plant reproductive size in models as a covariate.

Differences in *R. conicus* egg loads on native wavyleaf thistles within vs. outside the focal musk patches could occur either because wavyleaf thistles within the focal musk thistle patches were closer to the preferred host (musk thistle) or because they had higher densities of acceptable neighbors (regardless of the species composition of those neighbors) than did wavyleaf thistles outside of the focal musk thistle patches (White and Whitham 2000). To evaluate the possibility that local density of all acceptable host plant neighbors was driving variation in *R. conicus* egg load and damage, we used ANCOVA to examine the relationship between the number of bolting native thistles within 5 m and *R. conicus* egg load for wavyleaf thistles sampled outside musk patches. Distance to the musk thistle patch was treated as a fixed effect, and number of all bolting native thistles within 5 m was the covariate. Year and site were random effects. However, because *R. conicus* uses *Cirsium ochrocentrum* and *C. flodmanii* very little due to their later reproductive phenology, we also conducted a second ANCOVA using only the local density of *C. undulatum* as the covariate. To examine whether wavyleaf densities at larger spatial scales affected *R. conicus* oviposition and damage, we used ANCOVA to analyze effects of bolting wavyleaf thistle density in 20 \times 50 m plots at each distance on *R. conicus* egg load on wavyleaf thistles in the plot in 2001.

To provide a preliminary examination of the possibility that musk thistle negatively affects wavyleaf thistle through interspecific competition, we used linear regression to examine whether the density of bolting wavyleaf thistles in focal musk thistle patches was negatively related to the density of musk thistles in the patch in 2001, the only year in which counts of all wavyleaf thistles in focal musk patches were recorded.

RESULTS

Oviposition by R. conicus on wavyleaf thistle with distance from focal musk thistle patch

The ANCOVA model that fit the data best, as measured by the AIC, included density of *R. conicus* adults in the focal musk thistle patch, wavyleaf thistle distance to the focal musk patch, number of flower heads per wavyleaf plant, and the year \times distance random effect (Table 1). Oviposition by *R. conicus* on native wavyleaf thistles decreased significantly with increasing distance from musk thistle patches (distance: $F_{3,10.5} = 15.07$, $P < 0.001$; Fig. 1, Appendix B). Mean *R. conicus* egg load on wavyleaf thistles within the focal musk patches averaged across all three years was 2.86 eggs (SE = 0.18) per flower head; this was 2.0-, 3.4-, and 3.6 times the mean number of egg cases per flower head observed on wavyleaf thistles at 30–50 m (1.42 egg cases/flower head, SE = 0.14), at 80–100 m (0.83 egg cases/flower head, SE = 0.09), and at 200–220 m (0.79

TABLE 1. Values for the Akaike Information Criterion (AIC) to evaluate fit of ANCOVA models that differed in the musk thistle patch structure variable (a fixed effect) and whether distance to the musk thistle patch \times year was included as a random effect.

Musk thistle patch structure variable	Distance \times year included (random effect)?	AIC values
<i>R. conicus</i> density	yes	1931.6
<i>R. conicus</i> density	no	1932.1
<i>R. conicus</i> population size	no	1938.9
Musk thistle density	no	1939.8
Musk patch area	no	1934.5
Total musk thistle abundance	no	1935.8

Note: AIC values were calculated from variance components estimated by maximum likelihood; the lower the AIC value, the better is the model fit.

egg cases/flower head, SE = 0.12) from musk thistle patches, respectively. There was a trend toward differences in the effect of distance among years but it did not reach statistical significance (likelihood-ratio test for models with and without year \times distance as a random effect: $\chi^2_1 = 2.5$, $P = 0.114$). The largest best linear unbiased estimator was for egg load on wavyleaf thistles within musk thistle patches in 2002 (best linear unbiased estimator = 0.122, $t_{4,02} = 1.26$, $P = 0.275$). High egg loads in this year \times distance combination could contribute to the trend toward a year \times distance interaction effect.

Oviposition on wavyleaf thistle in relation to R. conicus density within musk focal patches

Egg loads on the native wavyleaf thistles at sites with high densities of *R. conicus* adults within the focal musk thistle patch were significantly greater than those at sites with low *R. conicus* densities (*R. conicus* density: $F_{1,33.5} =$

5.08, $P = 0.031$; Fig. 2, Appendix B). However, the observed variation in *R. conicus* adult density or population size within focal musk thistle patches was not sufficient to predict the magnitude of decrease in weevil egg load on *C. undulatum* with distance from the patch (*R. conicus* adult density \times distance: $F_{3,78} = 1.24$, $P = 0.299$; *R. conicus* adult population size \times distance: $F_{3,90.4} = 0.33$, $P = 0.806$). Also, egg load on wavyleaf thistle was not related to the estimated population size of *R. conicus* adults within the focal musk patch (*R. conicus* adult population size: $F_{1,34.8} = 0.02$, $P = 0.89$, Appendix C). The relationship between *R. conicus* adult density and egg load on wavyleaf thistle at a site likely did not result from differences in overall quality of plant growing conditions between sites. For example, plant size (height) of the musk thistles in focal patches did not differ between high vs. low *R. conicus* density sites in 2002 or 2003 ($F_{1,16.3} = 1.27$, $P = 0.276$), the two years in which we measured heights of the musk thistles in the focal patches.

Oviposition on wavyleaf thistle in relation to wavyleaf plant flower-head resources

For individual wavyleaf thistles, *R. conicus* egg load was positively related to the number of flower heads per plant (size: $F_{1,1033} = 12.3$, $P < 0.001$). There was some indication that effects of wavyleaf plant size on *R. conicus* oviposition may interact with plant proximity to musk thistle (Fig. 3; size \times distance: $F_{3,1029} = 2.17$, $P = 0.09$). Egg loads on wavyleaf thistles within musk patches were not related to the number of flower heads per plant ($t = 0.31$, $P = 0.758$). However, egg load increased significantly with wavyleaf plant size at 30–50 m ($t = 3.45$, $P < 0.001$) and marginally at both 80–100 m

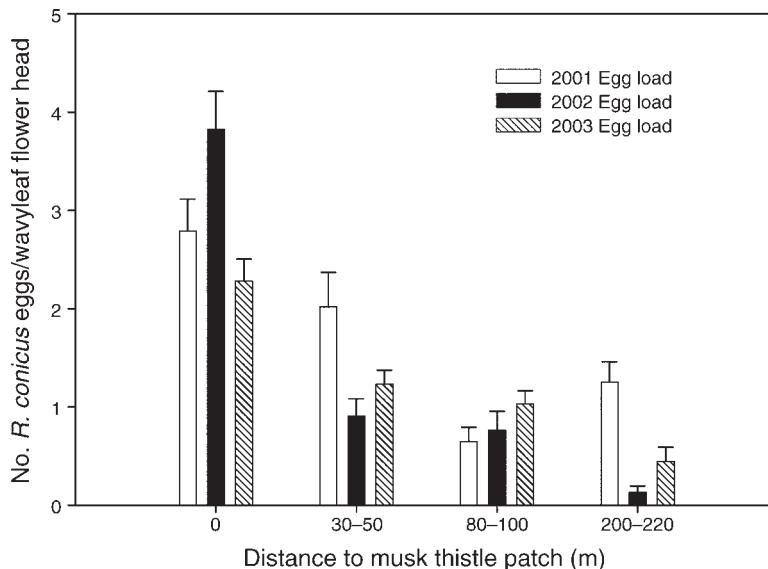


FIG. 1. Number (mean + SE) of *Rhinocyllus conicus* egg cases (each egg case contains one egg) per wavyleaf thistle (*Cirsium undulatum*) flower head as a function of distance to the nearest musk thistle (*Carduus nutans*) patch. Egg loads (mean number of eggs plus pinholes per wavyleaf thistle flower head) were measured for 2001–2003.

($t = 1.9$, $P = 0.058$) and 200–220 m ($t = 1.84$, $P = 0.066$) from focal musk patches. Slopes for these relationships, which were calculated for $\ln(\text{egg load})$, corresponded to 0.2%, 4.7%, 2.7%, and 4.6% increases in number of eggs per flower head for each additional head on wavyleaf plants within the musk thistle patch, and at 30–50 m, 80–100 m, and 200–220 m, respectively. Smaller sample sizes for wavyleaf thistles at the greater distances reduced our ability to detect a significant relationship between plant size and egg load there.

Oviposition by R. conicus in relation to densities of exotic and native thistles

We found no significant effect of musk thistle patch structure traits—focal patch density, area, or total abundance of musk thistles—on *R. conicus* egg load on wavyleaf thistles at a site (Appendices D–F). Further, we found no significant interactions between patch density, patch area, or total musk thistle abundance with distance in predicting *R. conicus* egg load on wavyleaf thistles.

For wavyleaf thistles outside of focal musk thistle patches (e.g., 30–50 m, 80–100 m, and 200–220 m), *R. conicus* egg load was not related either to the total number of native thistles flowering ($F_{1,411} = 0.98$, $P = 0.323$; Appendix G) or to the number of wavyleaf thistles flowering within 5 m ($F_{1,442} = 0.38$, $P = 0.377$; Appendix H). Also, density of bolting (i.e., flowering) wavyleaf thistles in the 20×50 m sampling plots did not explain variation in *R. conicus* egg load on wavyleaf thistles in those plots (2001; $F_{1,5} = 3.05$, $P = 0.141$; Appendix I). The density of bolting wavyleaf thistles within focal musk thistle patches was not significantly related to the density of the bolting musk thistles there (2001; $F_{1,11} = 0.25$, $P = 0.626$).

DISCUSSION

Ecological context affects R. conicus oviposition and damage to C. undulatum

Associational susceptibility is defined as increased predation on a prey species that results from close proximity to another prey species (Brown and Ewel 1987). We found that native wavyleaf thistles (*Cirsium undulatum*) within patches of the exotic musk thistle (*Carduus nutans*) suffered greater damage by the exotic flower head weevil, *R. conicus*, than did wavyleaf thistles that were even short distances (30–50 m) from those musk patches. The decrease in herbivory with increasing distance from musk thistles suggests that *C. undulatum* experiences associational susceptibility where it is near the weevil's preferred, targeted host plant, musk thistle. Although *R. conicus* oviposition on wavyleaf thistle can be high in areas without musk thistle, such as sand prairie (Russell and Louda 2005), the results here in richer prairies are consistent with other recent studies that showed associational susceptibility with host plant co-occurrence in an area; in these studies, as in ours, the secondary host species suffered more damage from

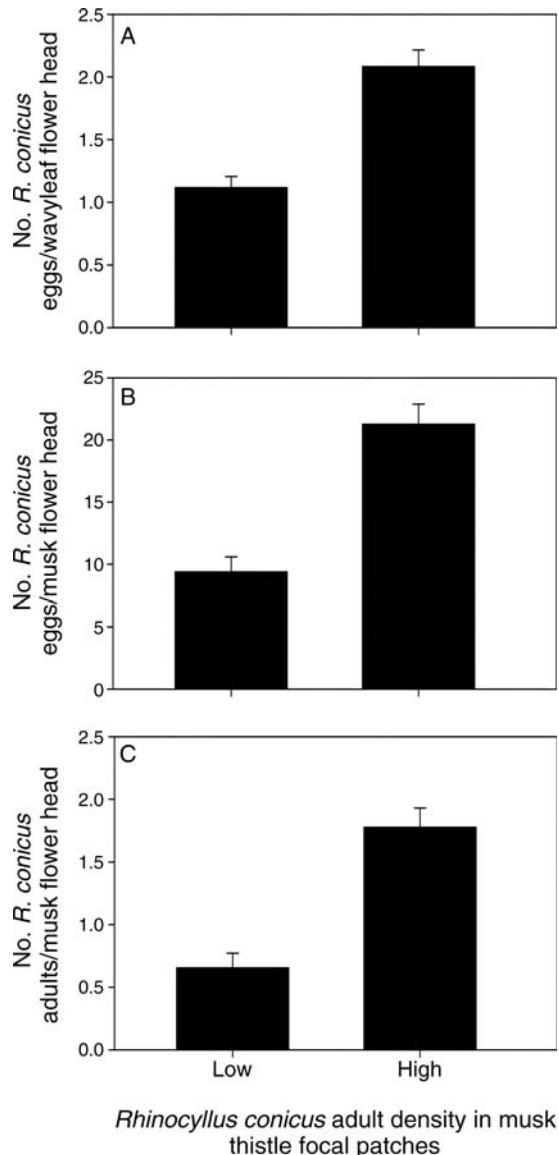


FIG. 2. (A) Number (mean + SE) of *Rhinocyllus conicus* eggs per wavyleaf thistle (*Cirsium undulatum*) flower head for sites with low vs. high densities of *R. conicus* adults in focal musk thistle patches. (B) Number (mean + SE) of *R. conicus* eggs on the terminal flower head of the first subsidiary branch of musk thistles in focal patches for sites with low vs. high densities of *R. conicus* adults in focal patches. (C) Predicted number (mean + SE) of *R. conicus* adults per musk thistle flower head in focal patches for sites with low vs. high densities of *R. conicus* adults in focal patches.

shared insect herbivores near vs. far from the preferred host (White and Whitham 2000, Blossey et al. 2001).

High *R. conicus* egg loads on wavyleaf thistles within musk thistle patches likely did not result from a positive response by the weevils to overall density of flower head resources on all thistle species. We found that oviposition on wavyleaf thistle by *R. conicus* was not related to local densities (within 5 m) of bolting native thistles

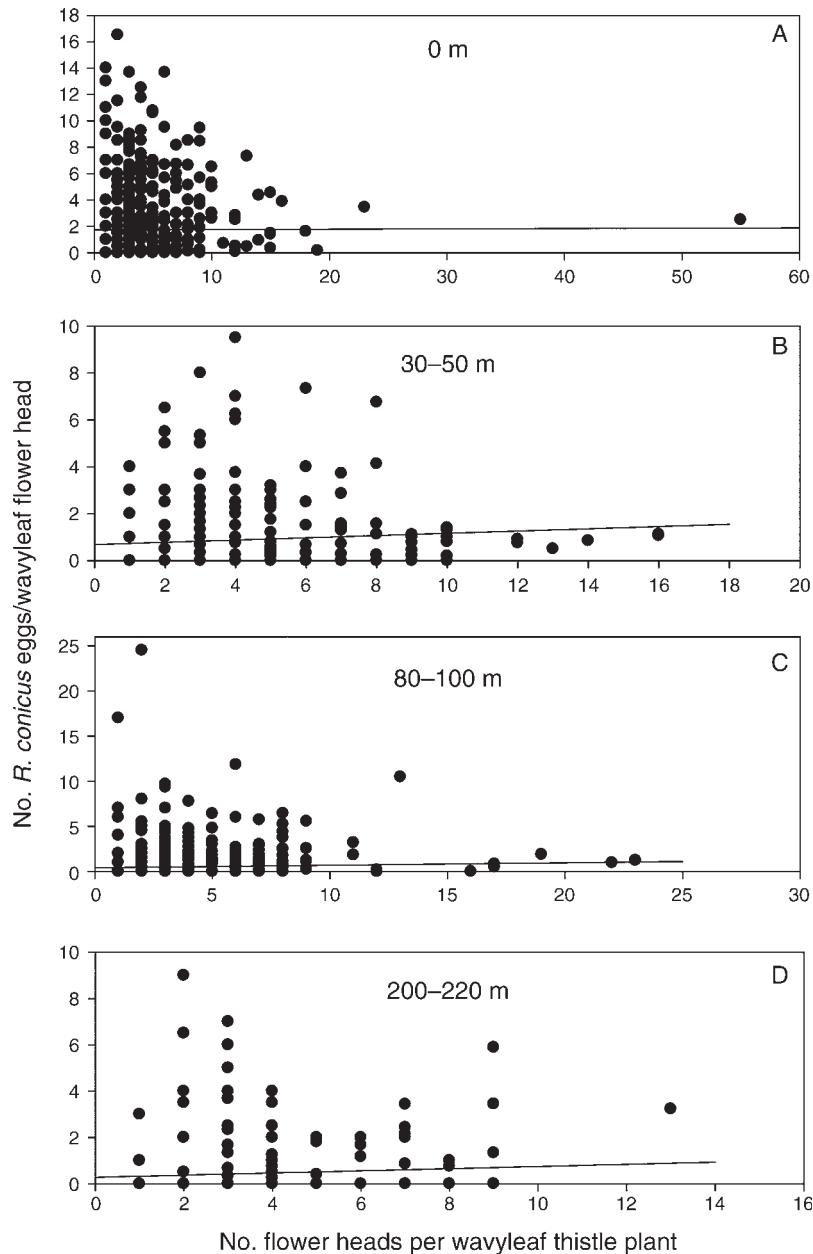


FIG. 3. Mean number of *Rhinocyllus conicus* eggs per wavyleaf thistle (*Cirsium undulatum*) flower head as a function of the number of flower heads produced by the plant for each distance from the focal musk thistle (*Carduus nutans*) patch at which plants were sampled. Regression equations are: (A) in focal musk thistle patches, egg load per head = $1.741 + 0.0025 \times (\text{number of flower heads})$; (B) at 30–50 m, egg load per head = $0.687 + 0.048 \times (\text{number of flower heads})$; (C) at 80–100 m, egg load per head = $0.408 + 0.028 \times (\text{number of flower heads})$; (D) at 200–220 m, egg load = $0.322 + 0.0467 \times (\text{number of flower heads})$.

outside musk thistle patches, nor was it related to bolting wavyleaf density at the larger plot spatial scale.

In sum, the results strongly suggest a relationship between proximity to the preferred host plant and high *R. conicus* egg loads on wavyleaf thistles. The strength of this correlative pattern suggests that the next step is an experimental test of the relationship between distance and egg load on wavyleaf thistle to verify that no unmeasured variable, in addition to the key variables

assessed here (e.g., local thistle density, site quality, individual plant size), is confounded with distance to preferred host.

*Insect herbivore abundance, behavior,
and associational susceptibility*

Theory predicts that secondary host plant species are likely to suffer associational susceptibility where they co-occur with a preferred host, if populations of the shared

herbivore respond numerically to the preferred host species (Holt 1977, Holt and Kotler 1987). Our data suggest that adult *R. conicus* occurred more densely and were more abundant in patches containing their coevolved exotic host, musk thistle, than in patches containing only the native wavyleaf thistle. For example, during the peak of adult weevil activity in early June 2003, the mean number of *R. conicus* per musk thistle flower head in musk patches was 29 times higher than the mean number of *R. conicus* per wavyleaf thistle flower head in relatively dense wavyleaf patches (5 m diameter) at three intensive study sites (L. Russell and S. Louda, unpublished data). Given the large annual variation in musk thistle density observed at our sites, active dispersal by *R. conicus* adults that allows aggregation in dense musk patches, may explain why wavyleaf thistle experiences associational susceptibility, rather than associational defense, amid its preferred musk thistle hosts.

White and Whitham (2000) and Blossey et al. (2001) hypothesized that variation in the density of the shared insect herbivore drives variation in the strength of associational susceptibility. In fact, they predicted that associational susceptibility would occur only where the shared herbivore is sufficiently abundant to deplete its preferred host. In our study, use of *C. undulatum* by *R. conicus*, averaged across all distances to the focal musk thistle patch, was higher at sites with greater densities of adult *R. conicus* in the focal musk patch. This result may provide partial support for the resource limitation hypothesis of White and Whitham (2000) and Blossey et al. (2001). However, contrary to this resource limitation hypothesis, we also found that variation in *R. conicus* adult density in patches of the preferred musk thistle did not predict either the presence of associational susceptibility or the magnitude of decrease in *R. conicus* egg load on the acquired secondary host plant, *C. undulatum*, with increasing distance from the patch.

Wavyleaf thistles within musk thistle patches suffered greater use than wavyleaf thistles away from musk patches over a wide range of *R. conicus* population sizes and densities within musk patches. Estimated population sizes of adult *R. conicus* observed in musk patches varied 2200%, and the estimated number of *R. conicus* adults per musk thistle flower head varied 360%. To our knowledge, our study is the first empirical test of the role of herbivore density and population size in determining the outcome and strength of herbivore-mediated indirect effects of a preferred host on a secondary host.

Our observation that sites with high adult *R. conicus* densities within musk thistle patches averaged greater egg loads on wavyleaf thistles (*C. undulatum*) across all distances sampled than did sites with low *R. conicus* densities in musk patches may be explained if sites with high weevil densities within musk patches also have high weevil densities throughout the site, leading to greater egg loads even for plants that are relatively isolated from musk thistles. The egg load on individual wavyleaf

thistles appears to reflect the effects of proximity to musk patches overlaid on a background level of weevil floral herbivory that is determined by site- and landscape-level factors (Rand and Louda 2004). However, it is important to note that *R. conicus* damage to *C. undulatum* was not restricted only to sites with high weevil densities (Fig. 2). Attack on the secondary host remained high even when resources on the preferred, exotic host were available.

In addition to effects of ecological context, specifically proximity to musk thistle patches, the magnitude of use of flower heads on individual wavyleaf thistle plants was influenced by the properties of individual plants. We found that the number of *R. conicus* egg cases per flower head on wavyleaf thistle plants at sites with musk thistle increased as the number of its flower heads increased per plant. Damage to individual plants by insects feeding as floral herbivores and predispersal seed predators often increases with the size of the plant's floral display (Leimu et al. 2002). However, the relationship between egg load of the exotic *R. conicus* and number of flower heads per native thistle plant was modified by distance to a patch of the weevil's preferred host, musk thistle. Number of flower heads per plant did not explain variation in *R. conicus* damage for wavyleaf thistle within the musk patches, but it did help explain the variation in oviposition and damage to wavyleaf thistles outside of musk thistle patches where these two host plant species co-occurred, in the loam midgrass prairie region of the upper Great Plains.

Indirect effects imposed by invasive species

Invasive exotic species, such as musk thistle (*Carduus nutans*) and the flower head weevil (*R. conicus*), can represent a significant threat to biodiversity; yet, the mechanisms by which such invaders affect native species and communities remain poorly understood (Parker et al. 1999, Levin et al. 2002). An understanding of these mechanisms is essential to predicting impacts of exotic species if they invade new communities (Louda et al. 2003a, b, 2005). Holt and Hochberg (2001) suggested that exotic biological control insects that attack native plants as secondary hosts and do not strongly suppress the targeted weed can mediate indirect effects of invasive weeds on acquired native hosts, as shown in this study. In some cases, it is possible that the negative effects of damage by a shared herbivore on native plants may be balanced by reduced competition from the exotic host plant if the herbivore reduces the density of the exotic species. However, for the interaction between musk thistle and wavyleaf thistle we have found no evidence of interspecific competition between the two thistle species nor did our test for negative correlations in densities of these two species reveal evidence of interspecific competition. We conclude that it is likely that herbivore-mediated negative indirect effects dominate the interaction between this native thistle and this exotic weedy thistle.

Accumulating empirical evidence suggests that less preferred host plant species frequently suffer associa-

tional susceptibility to damage by shared insect herbivores where they co-occur with preferred host species (White and Whitham 2000, Rand and Louda 2004). Therefore, indirect interactions between exotic weeds and native plants that share an invasive, marginally effective insect herbivore likely exemplify the type of synergistic interactions among exotic species that can promote "invasional meltdown" of invaded communities (Simberloff and Von Holle 1999). Our results document such a case of an indirect interaction mediated by an invasive biocontrol insect, in which the less preferred, native host plant suffers increased associational susceptibility near the preferred, exotic weed.

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APPENDIX A

Characteristics of focal musk thistle patches sampled (*Ecological Archives* E088-026-A1).

APPENDIX B

ANCOVA results for effects of *Rhinocyllus conicus* density in the focal musk thistle patch and distance to the focal patch on *R. conicus* egg load on wavyleaf thistles (*Ecological Archives* E088-026-A2).

APPENDIX C

ANCOVA results for effects of *Rhinocyllus conicus* population size in the focal musk thistle patch and distance to the focal patch on *R. conicus* egg load on wavyleaf thistles (*Ecological Archives* E088-026-A3).

APPENDIX D

ANCOVA results for effects of focal musk thistle patch density and distance to the focal patch on *Rhinocyllus conicus* egg load on wavyleaf thistles and a bar graph of mean *R. conicus* egg load on wavyleaf thistles at sites with low vs. high musk thistle densities in the focal patch (*Ecological Archives* E088-026-A4).

APPENDIX E

ANCOVA results for effects of focal musk thistle patch area and distance to the focal patch and on *Rhinocyllus conicus* egg load on wavyleaf thistles and a bar graph of mean *R. conicus* egg load on wavyleaf thistle at sites with small vs. large total area of the focal patch (*Ecological Archives* E088-026-A5).

APPENDIX F

ANCOVA results for effects of musk thistle abundance in the focal patch and distance to the focal patch on *Rhinocyllus conicus* egg load on wavyleaf thistles. Bar graph of mean *R. conicus* egg load on wavyleaf thistle at sites with low vs. high total abundance of musk thistles in the focal patch (*Ecological Archives* E088-026-A6).

APPENDIX G

Regression of mean number of *Rhinocyllus conicus* egg cases per wavyleaf thistle flower head as a function of the number of bolting native thistles within a 5-m radius of the sampled wavyleaf plant (*Ecological Archives* E088-026-A7).

APPENDIX H

Regressions of mean number of *Rhinocyllus conicus* egg cases per wavyleaf thistle flower head as a function of the number of bolting wavyleaf thistles within a 5-m radius of the sampled wavyleaf plant (*Ecological Archives* E088-026-A8).

APPENDIX I

Mean number of *Rhinocyllus conicus* egg cases per wavyleaf thistle (*Cirsium undulatum*) flower head in 20 × 50 m sampling plots as a function of the number of bolting wavyleaf thistles within the sampling plot (*Ecological Archives* E088-026-A9).