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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 greater or less in the vicinity of a preferred host plant species than where the secondary host occurs alone. 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 patch. Neither the level of *R. conicus* oviposition on *C. undulatum,* nor the strength of the distance effect, were predicted by local musk thistle patch density or by local *C. undulatum* density ($\leq$ 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 co-evolved, 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 plant proximity and herbivore density strongly affected the herbivore-mediated indirect interaction, and that such interactions are important pathways by which invasive exotic weeds can indirectly impact native plants. (240 words)

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 (= 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" to draw herbivores away from associated crops (Hokkanen 1991). Alternatively, secondary host plants near more palatable 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 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 spillover 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. They predicted 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) hypothesize that associational susceptibility only occurs where insect herbivore populations are large 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, musk or nodding thistle (*Carduus nutans*). 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: 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, patch area or total patch 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 weedy 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,* is a widespread, native thistle of the central Great Plains of North America (Great Plains Flora Association 1986). In the northern 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, personal observation).

The Eurasian flower head weevil, *Rhinocyllus conicus*, was deliberately introduced from Europe into North America in 1968 and into Nebraska 1969-1972 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 et al. 2005). Larvae burrow into the flower head where they develop, consuming receptacle tissues, florets, ovules, and developing seeds (Zwölfer and Harris 1984, unpublished data). Development takes 53 - 76 d (Zwölfer and Harris 1984, unpublished data), allowing one generation per year in this region.

Site Selection

Data were collected at 20 sites in Custer, Dawson, Gosper, Lincoln and Keith Counties, with all sites centered around North Platte, NE (41.13°N, 100.76°W), in late June and early July 2001 - 2003. 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 *C. 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 sampled than to the focal musk patch. At each site, we sampled up to 10 wavyleaf thistles at each distance (mean = 9.1, range 3 – 10) within a 20 m x 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 m X 50 m plot, the number of slips in the bag was equal to the number of bolting wavyleaf thistles in the plot with 10 slips were marked "Y." In 2001, we 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 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 the egg covers fell 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 X area) in 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 the 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 NE, SE, SW and NW. 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 below 1 bolter per 20 m^2. We used an image analysis program (Scion Image) to calculate 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 of bolting musk thistles at 5 m intervals along north-south and east-west axes through each patch. In 2003 we also 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 loads in July 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 to both 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 (mean

number of egg cases/terminal head first subsidiary branch); N = 5 patches, p = 0.05, R^2 = 0.767)

and the mean number of adults per plant in patches (linear regression: mean adult *R. conicus* per

bolting musk thistle = -0.859 + 0.364 (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) x (density of bolting musk thistles) x (patch area).

Data Analyses

We used mixed model ANCOVA (SAS for Windows V8: PROC MIXED; SAS Institute

2003) to examine effects of variables for focal musk thistle patches (i.e., patch area, density and

total abundance of bolting musk thistles, *R. conicus* adult density and population size), as well as

distance to the focal musk patch and size of individual wavyleaf thistles on mean number of *R.

conicus* egg cases plus pinholes per wavyleaf thistle flower head (hereafter, "egg load"). Effects

of musk thistle patch area, density, total abundance of bolting musk thistles, *R. conicus* adult

density and population size each 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, *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 over-stating the precision of estimates that are based on sub-

sampling patches. Large fluctuations in musk thistle and *R. conicus* abundances between years

within sites meant that some sites that were sampled in multiple years had to be placed in

different categories ("high" vs. "low") in different years.

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 thistle musk 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 (ANCOVA tables included as electronic appendices). 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 X 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 zero. The dependent variable, *R. conicus* egg load, was natural log-transformed to meet the assumptions of ANCOVA.

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 X distance included as a random effect against the model that did not include the year X distance interaction. To identify year X distance combinations that might contribute to a significant interaction effect, we used t-tests to determine whether best linear unbiased predictors for each year X distance combination differed significantly from zero (Littell et al. 2002).

We included number of flower heads per wavyleaf plant as a covariate in ANCOVA models to control for differences in plant quality that might be correlated with distance to musk thistles or musk patch structure. Number of flower heads was our best measure of plant quality for wavyleaf thistles. 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 (SAS Proc Mixed) 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 provides 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 versus 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 palatable 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 conducted a second ANCOVA using only the local density of *C. undulatum* as the covariate. Finally, 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 m X 50 m plots at each distance on *R. conicus* egg load on wavyleaf thistles in the plot in 2001.

Results

Oviposition on Wavyleaf Thistle with Distance from Focal Musk Thistle Patch

The ANCOVA model that included density of *R. conicus* adults in the focal musk thistle patch, wavyleaf distance to the focal musk patch, number of flower heads per wavyleaf plant and a year X distance random effect fit the data best, as measured by the AIC (Table 1). Oviposition on native wavyleaf thistles by *R. conicus* decreased significantly with increasing distance from musk thistle patches (distance: $F_{3,10.5} = 15.07$, $p < 0.001$; Fig. 1). Mean *R. conicus* egg load on wavyleaf thistles within the focal musk patches averaged across all three years was 2.86 (s.e. 0.18) eggs per flower head; this was 2.0X, 3.4X, and 3.6X the mean egg cases per flower head observed on wavyleaf thistles at $30 - 50$ m (1.42, s.e. 0.14), at $80 - 100$ m (0.83, s.e. 0.09), and at $200 - 220$ m (0.79, s.e. 0.12) from musk thistle patches, respectively. There was a trend that did not reach statistical significance toward differences in the effect of distance among years (likelihood ratio test for models with and without year X distance as a random effect: $\chi^2_1 = 2.5$, $p = 0.114$; see electronic appendices for -2 log likelihood values). The largest best linear unbiased estimator was for egg load on wavyleaf thistles in 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 X distance combination may have contributed to the trend toward a year X distance interaction effect.

Oviposition on Wavyleaf Thistle in Relation to <u>R. conicus</u> Density Within Musk Focal Patches

Egg loads on the native wavyleaf thistles at sites with high densities of *R. conicus* adults in 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). However, variation in the density or population size of *R. conicus* adults within focal musk thistle patches did not predict either the

presence of associational susceptibility or the extent to which *R. conicus* egg load on *C. undulatum* decreased with distance from the patch (*R. conicus* adult density x distance: $F_{3,78} = 1.24$, $p = 0.299$; *R. conicus* adult population size x distance: $F_{3,90.4} = 0.33$, $p = 0.806$). Also, egg load on wavyleaf thistles was not related to the estimated population size of *R. conicus* adults within the focal musk patch (*R. conicus* adult population size: $F_{1,34.8} = 0.02$, $p = 0.89$). The relationship between *R. conicus* adult density and egg load on wavyleaf thistles at a site likely did not result from differences in over-all quality of plant growing conditions between sites. Heights of 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 focal patches.

Oviposition on Wavyleaf Thistle in Relation to Wavyleaf Plant Flower Head Resources

For individual wavyleaf thistles, 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 (size X 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 ($t = 0.29$, $p = 0.775$). However, egg load increased significantly with wavyleaf plant size at 30 – 50 m ($t = 3.47$, $p = 0.001$), and marginally so at 80 – 100 m ($t = 1.84$, $p = 0.066$), and 200 –220 m ($t = 1.84$, $p = 0.066$) from focal musk patches. Slopes for these relationships, which were calculated for ln(egg load), correspond 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 amid musk thistles, at 30-50 m, 80-100 m and 200-220 m,

respectively. Smaller sample sizes for wavyleaf thistles at the greater distances likely

contributed to the lack of a highly significant relationship between plant size and egg load there.

Oviposition in Relation to Exotic and Native Thistle Densities

We found no significant effect of musk thistle focal patch area, density, or total

abundance of musk thistles on *R. conicus* egg load on wavyleaf thistles at a site. Further, we

found no significant interactions between patch density, area or total musk thistle abundance and

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

bolting ($F_{1,411}$=0.98, P = 0.323) or to the number of wavyleaf thistles bolting within 5 m. Also,

density of bolting wavyleaf thistles in the 20 m X 50 m sampling plots did not explain variation

in *R. conicus* egg load on wavyleaf thistles in those plots in 2001 ($F_{1,5}$ = 1.36, P = 0.297).

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*) in 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 host plant, musk thistle.

High egg loads on wavyleaf thistles within musk thistle patches likely did not result from a positive response by weevils to overall density of flower head resources on all thistle species. Oviposition on wavyleaf thistle by *R. conicus* was not related to local densities (within 5 m) of bolting native thistles outside musk thistle patches, nor was it related to bolting wavyleaf density at the larger plot spatial scale. Although *R. conicus* oviposition on wavyleaf thistle can be high in areas without musk thistle (Russell and Louda 2005), the results here 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 shared insect herbivores near versus far from the preferred host (White and Whitham 2000, Blossey et al. 2001).

Insect Herbivore Abundance, Behavior, and Associational Susceptibility

Theory predicts that secondary hosts 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. Densities of *R. conicus* in this study averaged higher in focal musk patches than in local areas around sampled wavyleaf plants or in sampled larger plots at all distances outside the focal patches. Also, during the peak of adult weevil activity in early June 2003, the average number of *R. conicus* per musk thistle flower head in musk patches was 29X higher than the average number of *R. conicus* per wavyleaf thistle flower head in relatively dense wavyleaf patches (5 m diameter) at three intensive study sites (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, *R. conicus* use of *C. undulatum*, 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 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. 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%. However, because of a lack of other studies of insect herbivore-mediated indirect effects that quantified herbivore abundance, it was impossible to compare herbivore population sizes and densities in our survey with those in other cases that reported associational susceptibility. 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 have greater egg loads on wavyleaf thistles (*C. undulatum*), averaged across all distances sampled, than do sites with low *R. conicus* densities in musk patches may be explained if sites with high weevil densities in musk patches 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. At the three sites with the lowest densities of *R. conicus* adults in focal musk thistle patches, egg loads on wavyleaf flower heads still were 25% of the egg loads observed on the first subsidiary terminal flower head of musk thistles in the focal patches. Therefore, attack on the secondary host remained high even when resources on the preferred, exotic host were available.

The behavioral responses that determine the magnitude of *R. conicus* use of wavyleaf thistle where it co-occurs with musk thistle appear to be complex. Our finding that egg load on *C. undulatum* is better explained by *R. conicus* density per musk thistle plant than by *R. conicus* population size might indicate that interference among adults on the preferred host or depletion of the preferred resource increases use of the less-preferred host within a site, as suggested by White and Whitham (2000) and Blossey et al. (2001). However, interference among *R. conicus* adults cannot explain the relatively high level of use of the secondary host, wavyleaf thistle, observed even when weevil densities in the focal musk patches were low. Alternatively, use of wavyleaf thistles where *R. conicus* densities on musk thistles are low may occur if in early season *R. conicus* adults use wavyleaf thistle primarily when local musk thistle resources are

saturating, whereas later in the season *R. conicus* use of wavyleaf thistle may instead be determined by frequency of encounters between herbivore and secondary host. *Rhinocyllus conicus* preference for musk thistle over wavyleaf thistle appears to decrease as absolute oviposition levels decline with time (M. Manning and S. M. Louda, unpublished data).

Individual Plant Properties Affected Level of Floral Herbivory

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). We found that the number of *R. conicus* egg cases per flower head on wavyleaf thistle plants occurring at sites with musk thistle increased as the number of its flower heads increased per plant. However, the relationship between *R. conicus* egg load and number of flower heads per plant was modified by ecological context, specifically 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 plant size did help explain the variation in oviposition and damage to wavyleaf thistle outside of musk thistle patches where these species co-occur in the loam midgrass prairie region of the upper Great Plains.

Studies that have explored the effects of ecological context on the relationship between floral display size and insect pre-dispersal seed predation have focused on effects of unpalatable vegetation in hiding host plant inflorescences (Brody and Mitchell 1997, Leimu et al. 2002), not on the effects of co-occurring alternate hosts. Our results suggest that the influence of floral display size on host selection within unpalatable vegetation decreases with increasing proximity to alternative, preferred host plants. An herbivore on an isolated host plant must survey a larger area to find a second suitable host and, therefore, may be influenced to a greater extent by floral display, compared to an herbivore within a patch of many host plants. Further studies that

integrate the effects of individual plant traits and ecological context on levels of pre-dispersal seed predation are needed (e.g., Louda 1982, 1983; Leimu et al. 2002) to improve our ability to predict the extensive spatial and temporal variation in seed loss and its demographic consequences.

Indirect Effects Imposed by Invasive Species

Invasive exotic species, such as musk thistle (*Carduus nutans*) and the flower head weevil (*R. conicus*), represent a significant threat to biodiversity; yet, the mechanisms by which invaders affect native species and communities remain poorly understood (Parker et al. 1999, Levin et al. 2002). A mechanistic understanding of invasive species effects on native species is essential to predicting their impacts 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 and do not strongly suppress the targeted weed can mediate indirect effects of invasive weeds on acquired native hosts, as shown in this study. Further, empirical evidence suggests that less-preferred host plants, in most cases, suffer associational susceptibility to damage by shared insect herbivores where they co-occur with a preferred host (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 may exemplify the type of synergistic interactions among exotic species that could 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 associational susceptibility near the preferred, exotic weed.

Acknowledgments

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Table 1. Values for the Akaike Information Criterion (AIC) to evaluate fit of ANCOVA models
that differed in 1) the musk thistle patch structure variable that we included as a fixed effect in
the model and 2) whether distance to the musk thistle patch X year was included as a random
effect. AIC values were calculated from variance components estimated by maximum
likelihood.

Musk thistle patch structure variable included in model	Distance X year included as a random effect?	Akaike Information Criterion Values
R. conicus density	Yes	1931.6
R. conicus density	No	1932.1
R. conicus population size	No	1938.9
Musk thistle density	No	1939.8
Musk patch area	No	1934.5
Total musk thistle abundance	No	1935.8

Figure Legends

Fig. 1. Mean number of *Rhinocyllus conicus* egg cases per wavyleaf thistle (*Cirsium undulatum*) flower head as a function of distance to the nearest musk thistle (*Carduus nutans*) patch. Bars are untransformed means ± 1 SE.

Fig. 2. Mean number of *Rhinocyllus conicus* egg cases per wavyleaf thistle (*Cirsium undulatum*) flower head for sites with low vs. high densities of *R. conicus* adults in focal musk thistle patches. Estimated densities of *R. conicus* averaged 0.55 (range: 0.07 – 1.19) and 1.8 (range: 1.26 – 3.15) adults per musk flower head (*Carduus nutans*) during peak oviposition in low and high density sites, respectively. Bars are untransformed means ± 1 SE.

Figure showing *R. conicus* eggs / wavyleaf head versus Density of *R. conicus* adults in musk thistle patch