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Combined Effects of Folivory and Neighbor Plants on *Cirsium altissimum* (tall thistle) rosette performance

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Abstract

Predicting how herbivory and neighbor plant interactions combine to affect host plants is critical to explaining variation in herbivores' impact on plant population dynamics. In a field experiment, we asked whether the combined effects of neighbor plants and folivores upon performance of tall thistle (*Cirsium altissimum*), a monocarpic perennial, can be predicted as the product of their individual effects (i.e. effects of neighbor plants and folivores act independently in suppressing tall thistle performance). Alternately, the combined effects of neighbor plants and folivores might be greater, indicating a synergistic interaction, or less, indicating an antagonistic interaction, than the product of their individual effects. Our experiment involved a neighbor plant clipping treatment and a folivory reduction treatment in a factorial design with manipulations applied to naturally-occurring tall thistle rosettes in restored tallgrass prairie. Clipping neighbors at the soil surface within 40 cm of tall thistle rosettes increased light availability to rosettes, rosette growth and the transition rate of 2007 rosettes to reproductive adults in 2008. Folivores' and neighbor plants' effects acted independently upon rosette growth. By contrast, folivory reduced the rate at which 2007 rosettes transitioned to reproductive adults in 2008 only where neighbor plants were unclipped, indicating a possible synergistic interaction of neighbor plants and folivores in suppressing tall thistle performance. Our results suggest that 1) promoting neighbor plant aboveground biomass should suppress rosette-forming weeds and 2) folivory, which reduces light acquisition by rosettes, may generate synergistic herbivory X neighbor plant interaction effects on rosettes in grasslands, where light often limits rosettes. (251 words)

Keywords: folivory, plant competition, grasslands, *Cirsium*, plant compensatory ability

Introduction

Predicting the ecological conditions under which herbivores most strongly reduce host plant performance is central to the basic research objective of understanding herbivores' effects on plant evolution and population dynamics. Further, enhanced ability to predict and manipulate the magnitude of herbivores' effects is of applied significance in reducing the spread of weed populations. Accumulating empirical evidence suggests that the proximity and density of neighboring vegetation can drive spatial and temporal variation in herbivores' effects on their host plants (Maschinski and Whitham 1989, Louda and Rodman 1996, Sessions and Kelly 2002, Kuijper et al. 2004).

A current, primary goal of research that addresses the joint effects of neighbor vegetation and herbivory on host plants is to determine whether these two interactions combine independently, synergistically or antagonistically to reduce plant performance. Rees and Brown (1992) propose that when herbivory and neighbor vegetation act independently in reducing host plant performance, their joint effect will be the product of their individual effects. A synergistic interaction is defined as occurring if the joint effect of herbivory and neighbor plants on host plant performance exceeds the product of the individual effects and an antagonistic interaction is defined as occurring if the joint effect is less than the product of the individual effects. One set mechanisms by which synergistic or antagonistic interaction effects could occur is if neighbor plants either increase the amount of damage that host plants suffer, producing a synergistic interaction, or decrease the amount of damage that host plants suffer, producing an antagonistic interactions (Hamback and Beckerman 2003). At this point, studies have shown that independent effects of herbivory and neighbor plants (Rees and Brown 1992, Steets et al. 2006), synergistic interactions (Nötzold et al. 1998, Agrawal 2004) and antagonistic interactions (Haag

et al. 2004, Schädler et al. 2007) all can occur, but the small number of field experiments makes it difficult to conclude which outcome is most common or to predict the circumstances under which each outcome occurs in nature. Clearly, quantifying effects of neighbor plants on the amount of herbivore damage to host plants is essential to developing such predictive abilities.

In addition to altering herbivore behavior and density, neighbor plants can influence abiotic resource availability to host plants. Therefore, a mechanistic understanding of herbivory X neighbor plant interaction effects requires knowledge of how host plants' compensatory abilities vary with resource levels. Empirical studies provide examples in which host plants' ability to compensate for tissue loss to herbivores increased (Maschinski and Whitham 1989, Hersch 2006), decreased (Meyer and Root 1993) or has been unrelated to (Meyer 2000) the availability of abiotic resources, bolstering a long-standing and, as yet, unresolved debate concerning the relationship between compensatory ability and resource availability. Parmesan (2000) and Wise and Abrahamson (2005, 2007) suggest that ability to explain and predict variation in the relationship between plant compensation for herbivory and resource availability will be improved by considering which abiotic resource limits plant performance and whether the form of herbivory that the plant suffers inhibits acquisition of that resource. In their Limiting Resource Model, Wise and Abrahamson (2005, 2007) predict that plants will be less tolerant of herbivore damage with lower resource availability when 1) the resource that varies between the high- and low-resource environments limits plant performance in the low-resource environment and 2) herbivory hinders acquisition of this limiting resource. Therefore, synergistic interaction effects between herbivory and competition from neighbor plants should occur where herbivory reduces acquisition of the resource that limits host plants where neighbor plants are present.

Unfortunately, studies that address joint effects of neighbor plants and herbivory on host plants rarely quantify how neighbor manipulations affect resource availabilities.

In this study, we examined the individual and joint effects of neighbor plants and folivory on growth in the current growing season and on the probability of reproducing in the next growing season for tall thistle (*Cirsium altissimum*) rosettes in a Kansas prairie. Tall thistle is native to Kansas, but because it shares its rosette growth form and short-lived monocarpic perennial life-history with numerous invasive exotic weeds in North America, including *Cirsium vulgare* and *Carduus nutans*, understanding tall thistle's interactions with neighbor plants and folivores may provide broad insights for limiting weed performance. Specifically, we asked 1. Do neighbor plants, insect and rodent folivory individually affect rosette growth and probability of reproduction? 2. How do neighbor plants affect availability of photosynthetically active radiation and soil moisture to rosettes? 3. Does the amount of leaf tissue removed from rosettes by folivores depend upon the presence of neighbor plants? and 4. Do effects of neighbor plants and folivores act independently or do they combine synergistically or antagonistically to reduce rosette performance? By quantifying folivore damage and availability of two abiotic resources that often are important in prairie ecosystems, light and soil water, our goal is to illuminate mechanisms by which interaction effects on plant performance may occur. Our study draws upon a key insight of the Limiting Resource Model: variation in compensatory ability may be explained by considering the identity of resources limiting plant performance and the form of herbivory that a plant suffers. Our study, however, is not a test of the Limiting Resource Model because we examine fitness correlates, and not fitness itself.

Methods

Study Species

Tall thistle (*Cirsium altissimum* (L.) Spreng.) (Asteraceae) is a rosette-forming, short-lived, monocarpic perennial plant that is native to the eastern United States (Great Plains Flora Association 1986). Central Kansas is at the western limit of this species' range. Tall thistle occurs on roadsides, in pastures and on mildly disturbed non-cultivated land. It does not reproduce clonally. In south-central Kansas adult plants begin stem elongation ("bolt") in early May, flower late July - October and disperse seeds September - early November.

Insect folivores on tall thistle rosettes at our study site remove leaf tissue by chewing leaves from the edges, chewing or scraping holes through the leaf, scraping away the upper epidermis and mesophyll leaving only the lower epidermis and by mining or chewing the mid-rib. We observed *Vanessa cardui* L. (Lepidoptera: Nymphalidae) larvae, adult *Systena hudsonius* Forster (Coleoptera: Chrysomelidae) and grasshoppers consuming leaf tissue on tall thistle rosettes. During research at our study site in 2006, we noticed many leaves were removed from tall thistle rosettes leaving a neat 45º cut on the petiole, suggesting either rodent or rabbit folivory.

Study Site

This study was conducted in a restored prairie on sandy-loam and silty-loam soils at Wichita State University's Ninnescah Reserve (37.53ºN, 97.7ºW). Mean annual precipitation at Wichita, Kansas (USA), 32 km northeast of the study site, for 1993-2007 was 84 cm per year (National Oceanic and Atmospheric Administration). Restoration to tallgrass prairie, after decades of crop agriculture, began 20 years before our study. The vegetation is dominated by *Andropogon gerardii* Vitman, *Sorghastrum nutans* (L.) Nash, *Panicum virgatum* L. and *Schizachyrium scoparium* (Michx.) Nash. Mid-successional forbs, including *Conyza canadensis*

(L.) Cronq., *Lespedeza capitata* Michx. and *Helianthus maximilianii* Schrad., are a much smaller fraction of total plant cover. Therefore, tall thistle rosettes occur in a matrix that is primarily grasses.

Experimental Design

In May 2007 we selected 104 non-seedling tall thistle rosettes by choosing the nearest rosette at 5 m intervals along six east-west transects across the study site. Adjacent transects were separated by 5 m. Transect lengths differed as dictated by the irregular shape of the site. The longest transect was 120 m (24 intervals) and the shortest was 20 m (4 intervals). Plants were tagged and measured May 17-26 2007. From these initial measurements, we ordered rosettes from smallest to largest and used a stratified, random assignment of individual rosettes to treatment combinations based on rosette diameter.

Folivory reduction and the neighbor plant clipping treatments were applied to individual rosettes in a factorial design. The five levels of the folivory reduction treatment were; control, insect folivory reduction, water application control, rodent folivory reduction and rodent pseudo-barrier control. The neighbor plant clipping treatment included two levels; neighbors unclipped vs. clipped. The six treatment combinations that included control, insect folivory reduction and rodent folivory reduction were applied to 12 rosettes each. The four treatment combinations that included water control and pseudo-barrier were applied to 8 rosettes each.

For the neighbor plant clipping treatment, we either clipped at the soil surface all aboveground vegetation within a 40 cm radius of the focal tall thistle rosette ("clipped") or neighbor plants were not clipped ("unclipped"). Clipping was first imposed May 26–June 1 2007 and was repeated at 2-3 week intervals until early October 2007. We reduced insect folivory on tall thistle rosettes by applying the non-systemic, synthetic pyrethroid insecticide

Bifin I/T at 2-3 week intervals June 10–October 7 2007. The dilution rate was 16 ml Bifin I/T in 1 liter of water. In greenhouse trials, no phytostimulatory or phytotoxic effects of Bifin I/T were found (T. Suwa, J. Eckberg and S. Louda, unpublished data). 'Water control' rosettes were sprayed with a volume of water that was equal to the volume of insecticide mixture that we sprayed on insecticide-treated rosettes. Water and insecticide mixture were applied on the same dates. To reduce rodent folivory on tall thistle rosettes, we constructed aluminum flashing barriers. Each barrier enclosed a 40 cm radius circle around a rosette. Barriers were 36 cm tall aboveground and 10 cm deep belowground. Pseudo-barriers were identical to real barriers except that eight 10 cm X 10 cm holes were cut in the pseudo-barriers at the soil surface. Pseudo-barriers were used to identify potential confounding effects of surrounding plants with aluminum flashing, such as altered light regimes or lower soil moisture perhaps due to greater evaporation from the soil surface inside the barriers. Barriers and pseudo-barriers were installed June 3–10 2007. In August 2008 we evaluated the rodent barriers' efficacy by installing six aluminum track plates with graphite powder inside barriers and six track plates beside rosettes that were not surrounded by barriers or pseudo-barriers and did not receive insecticide. We exposed track plates for 24 hrs. Track plates were baited with peanut butter rolled in oats.

We censused tall thistles May 17-26 2007, August 10-17 2007, October 12-21 2007 and June 2-4 2008. Although our research questions did not explicitly address seasonality in folivore and neighbor plant effects, the August census provided insight into the mechanisms of these effects because it partitioned the growing season into a moist early season during which grasshoppers were not major folivores and a dry late season during which grasshoppers were the predominant insect folivore. At each census, we recorded rosette presence / absence and three measures of rosette size: root crown and rosette diameters and number of leaves. Folivore

damage was quantified as the number of leaves with any folivore damage and the number of leaves damaged by folivores over > 50% of their surface. Only folivore damage that was visible with the naked eye was recorded. In June 2008, we noted whether each plant was bolting. Although we did not impose neighbor clipping and insect folivory reduction treatments after October 2007, we decided that evaluating the carry-over effects of these treatments to the following growing season was biologically meaningful because it provides insight into effects of processes that temporarily reduce neighbor plant aboveground biomass, such as haying or grazing, and insect populations, which are often highly variable from year-to-year.

To evaluate effects of the experimental treatments on resource availability to rosettes in 2007, we measured soil moisture and light penetration around each rosette August 19-26 2007. We measured volumetric soil water content at 20 cm west and east of each rosette's center using a TDR 100/200 soil moisture meter (Spectrum Technologies, Inc.) with a 12 cm probe. We measured photosynthetically active radiation at the rosette surface and above the canopy of the surrounding vegetation using an AccuPAR model LP-80 PAR/LAI ceptopmeter (Decagon Devices, Inc.). We expressed percent light penetration to rosettes as the proportion of PAR above the surrounding canopy that was available at the rosette surface.

Statistical Analysis

Light penetration and soil water availability

We evaluated effects of folivory reduction and neighbor clipping treatments on light penetration and soil water availability using 2-way ANOVA. Proportion of light penetrating to rosettes and volumetric soil water content were arcsine (square root) transformed, as were all dependent variables that were proportions in our statistical analyses.

Folivore damage

To evaluate the effect of neighbor plants on leaf damage to tall thistle rosettes and the efficacy of our folivory reduction treatment, we analyzed proportion of leaves damaged per rosette and proportion of leaves damaged over >50% of their surface per rosette using repeated-measures 2-way ANCOVAs. Initial rosette size, quantified as number of leaves per rosette in May, was the covariate. We used the Huynh-Feldt adjustment for degrees of freedom to address slight violations of the sphericity assumption. After finding significant interactions between treatments and time, we used 2-way ANCOVA to examine treatment effects on each 2007 census date. We used number of leaves per rosette as our measure of initial size because R^2 values for ANCOVAs conducted separately on each census date were greater for models that included number of leaves per rosette than for models with either initial root crown diameter or initial rosette diameter. Single degree-of-freedom contrasts among successive dates were used to evaluate seasonal trends in folivory. After finding a significant effect of the folivory reduction treatment on leaf damage, we compared each level of the folivory reduction treatment with the control using a series of repeated-measures 2-way ANCOVAs.

Rosette growth

We used 2-way ANCOVAs to evaluate treatment effects on tall thistle rosette growth. The three size-related dependent variables that were analyzed in separate 2-way ANCOVAs were the absolute changes in root crown diameter, rosette diameter and number of leaves per rosette between May and October 2007. These absolute changes were calculated as the difference between the size variable (e.g. root crown diameter or rosette diameter or number of

leaves per rosette) in October 2007 minus the value for the same size variable in May 2007. All

dependent variables were natural-log transformed in accordance with a multiplicative model of

the combined effects of herbivory and neighbor plants (Rees and Brown 1992, Hamback and

Beckerman 2003). The independent variables were the folivory reduction and clipping

treatments, the folivory reduction X clipping interaction effect and natural log transformed initial

size. The measurement of initial size that was used (initial root crown diameter, initial rosette

diameter, initial number of leaves) corresponded to the size measurement that was being

analyzed as the dependent variable (e.g. if we were analyzing treatment effects on change in

rosette diameter then we included initial rosette diameter as the covariate in the 2-way ANCOVA

model). To complement the separate univariate analyses and to determine whether the

treatments resulted in an over-all plant growth response, we used MANCOVA that included all

three growth-related dependent variables. The independent variables were the folivory reduction

and clipping treatments and the folivory reduction X clipping interaction effect. Initial root

crown diameter was the covariate because it provided the best fit of the MANCOVA model as

indicated by the largest R^2 value.

To allow comparisons of the intensity of neighbor plant – tall thistle interactions in our

study with plant-plant interactions in other studies that have manipulated the presence of

neighbor plants' aboveground parts, we calculated relative competitive indices, one of the most

widely used indices of competitive intensity (Weigelt and Joliffe 2003). We calculated two

relative competitive indices, the first used change in root crown diameter as a measure of plant

performance and the second used change in rosette diameter as a measure of plant performance.

In all analyses of treatment effects on rosette performance, we pooled plants in the water

control and rodent pseudo-barrier treatment levels with controls after we found no differences

between control, water control and pseudo-barrier treatment levels in effects on growth (Pillai's Trace = 0.123; $F_{6,80} = 0.83$; p = 0.552) or rate of bolting in the next growing season (Fishers exact test: p =0.262). We also pooled rosettes from the rodent exclusion level of the folivory reduction treatment with control, water control and pseudo-barrier treatment levels in analyses of rosette performance. Metal flashing barriers impeded rodent access to only a limited extent. Four of six track plates inside barriers had rodent tracks, whereas all plates near rosettes that were not inside barriers had rodent tracks. This moderate reduction in rodent access had no effect on damage to rosettes (proportion of leaves damaged: $F_{1,33} = 0.01$, p = 0.939; proportion of leaves damaged > 50%: $F_{1,33} = 0.03$, p = 0.873). Further, the barriers did not alter light penetration ($F_{1,44} = 0.17$, p = 0.678) or soil moisture availability ($F_{1,44} = 0.46$, p = 0.503).

Rosette survival and bolting

We used Fisher's exact tests to examine effects of clipping neighbor plants and, separately, effects of folivory reduction on 1) survival of 2007 rosettes until June 2008, 2) bolting of rosettes that survived until June 2008 and 3) transition of 2007 rosettes to adulthood in 2008. For example, to examine the effect of neighbor plants on 2007 rosette survival until June 2008 we first constructed a 2 X 2 contingency table with the rows representing levels of the neighbor plant clipping treatment and the columns representing rosette survival categories (survived vs. died). In this table for examining neighbor plant effects, we pooled across levels of the folivory reduction treatment. We then used Fisher's exact test to evaluate whether the observed counts of rosettes in the cells of this 2 X 2 contingency table deviated significantly from the expected counts if rosette survival were independent of neighbor clipping. We used the same procedure to evaluate effects of neighbor plant clipping on the other two categorical rosette

performance variables (e.g. bolting among survivors and transition to adulthood) and to evaluate effects of folivory reduction on categorical rosette performance variables. "Transition of 2007 rosettes to adulthood in 2008" combines rosette survival and probability of bolting for survivors. "Adulthood" refers to the reproductive life-stage. The rate at which 2007 rosettes transition to adulthood in 2008 is meaningful for tall thistle population dynamics because both greater juvenile mortality rates and longer generation times (Crawley 1997) could reduce population growth rates.

Fisher's exact test is designed for two-way contingency tables, so we could not directly test neighbor clipping X folivory reduction interaction effects upon rosette survival and bolting. Instead, we asked whether the effects of insect folivory on rosette performance differed between rosettes with unclipped neighbor plants vs. with neighbors clipped. Specifically, for each categorical rosette performance variable we constructed two 2 X 2 contingency tables; one table contained counts for rosettes that had neighbor plants present and the second table contained counts for rosettes with clipped neighbors. In each table, rows represented the two folivory reduction treatment levels and columns represented the two categories for the rosette performance variable. For each table, we then used Fisher's exact test to ask whether the observed counts of rosettes in cells differed from the expected counts if the state of the rosette performance variable were independent of folivory. If the outcome for whether rosette performance was independent of the folivory treatment differed between rosettes with clipped neighbors and rosettes with unclipped neighbors then we interpreted this as evidence that insect folivores' impacts were influenced by neighbor plants.

Results

Resource availability to tall thistle rosettes

Clipping neighbor plants within 40 cm of tall thistle rosettes increased light penetration to rosettes from 59.9% to 96.2% ($F_{1,94} = 156.8$, $p < 0.001$). The folivory reduction treatment did not affect light penetration ($p > 0.91$). Neither neighbor plant clipping nor the folivory reduction treatment affected soil water availability (p-values > 0.73).

Folivore damage to tall thistle rosettes

There was a marginally significant effect of time on the proportion of leaves damaged per rosette (Fig 1; $F_{2,154} = 2.49$, $p = 0.086$) reflecting a trend toward a greater proportion of leaves per rosette having folivore damage in August than in May ($F_{1,77} = 2.61$, $p = 0.11$).

Both the proportion of leaves damaged per rosette ($F_{1,77} = 12.18$, $p < 0.001$) and the proportion of leaves damaged over >50% of their surface area ($F_{1,77} = 7.03$, $p = 0.01$) were significantly, positively related to the number of leaves on the rosette in May.

As the growing season progressed, tall thistle rosettes with clipped neighbors suffered damage on a marginally significantly smaller proportion of their leaves than rosettes with unclipped neighbors (Fig. 1; time X clip: $F_{2,154} = 2.59$, $p = 0.078$). In May before clipping, rosettes assigned to "neighbors clipped" had 70.0% ($\pm$3.5) of leaves damaged whereas rosettes assigned to "neighbors unclipped" had 71.8% ($\pm$2.8) of leaves damaged ($F_{1,77} = 0.00$, $p = 0.955$). By October, rosettes with clipped neighbors had damage on 73.6% ($\pm$2.5) of leaves whereas rosettes with unclipped neighbor plants had damage on 82.4% ($\pm$3.0) of leaves ($F_{1,77} = 13.63$, $p < 0.001$). Clipping neighbors did not affect the proportion of leaves that were damaged over >50% of their surface area.

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The folivory reduction treatment significantly affected the amount of leaf damage

suffered by rosettes ($F_{4,77}$ = 3.62, p = 0.009). In October, rosettes sprayed with insecticide

averaged 57.4% (±4.6) of leaves damaged vs. 83.7% (±3.4) of leaves damaged in controls ($F_{1,34}$

= 17.76, p < 0.001). Proportion of leaves damaged also was lower on insecticide rosettes in

August (insecticide: 73.5% (±4.0) vs. control: 81.3% (±3.9)), but not significantly so ($F_{1,34}$ = 1.5,

p = 0.23). No other levels of the folivory reduction treatment, including rosettes surrounded by

rodent barriers, differed significantly from controls in proportion of leaves damaged. There were

no significant differences among levels of the folivory reduction treatment in proportion of

leaves per rosette that were damaged on >50% of their surface area.

Effects of neighbor plants and folivory on tall thistle rosette growth

Clipping neighbor plants increased tall thistle rosette growth (Pillai's Trace = 0.345; $F_{3,81}$

= 14.42; p < 0.001) May - October 2007. The absolute increases in root crown diameter (Fig 2A;

$F_{1,83}$ = 12.04; p = 0.0008), rosette diameter (Fig 2B; $F_{1,83}$ = 10.9; p = 0.001) and number of

leaves per plant (Fig 2C; $F_{1,83}$ = 40.55; p < 0.001) were all greater where neighbor vegetation

was clipped. The relative competitive intensity of neighbor plants on tall thistle rosettes was

0.436 when rosette performance was quantified using the change in root crown diameter and

0.541 when rosette performance was quantified using change in rosette diameter.

The folivory reduction treatment did not affect rosette growth. Further, the interaction of

the neighbor clipping and folivory reduction treatments was not significant.

Tall thistle rosette growth was negatively and marginally significantly related to initial

root crown diameter (Pillai's Trace = 0.0864; $F_{3,81}$ = 2.55; p = 0.061). This relationship was

driven by a negative relationship between initial root crown diameter and the increase in rosette

diameter ($F_{1,83}$ = 4.75, p = 0.032). For other measures of growth, the increase in size was independent of initial root crown diameter.

Effects of neighbor plants and folivory on bolting

91.7% of tall thistle rosettes that began the experiment in May 2007 survived until June 2008. Among these survivors, 94.4% bolted.

Neither clipping neighbor plants in 2007 nor reducing insect folivory in 2007 affected tall thistle rosette survival until June 2008. Among rosettes that did survive until June 2008, clipping neighbor plants in 2007 increased the probability of bolting in 2008 (p = 0.02; 87.8% ± 3.5% of survivors bolt with neighbors unclipped vs. 100% bolt with neighbors clipped). Reduced insect folivory did not affect the probability of bolting among surviving rosettes.   There were no significant interaction effects of clipping neighbor plants and reduced insect folivory on either rosette survival or bolting of surviving rosettes.

For the annual transition rate of tall thistle rosettes to adulthood (the proportion of rosettes that began 2007 that bolted in 2008), clipping neighbor plants in 2007 (Fig. 3; p = 0.007) and the folivory reduction treatment in 2007 (folivory reduction: p = 0.036) significantly increased the number of rosettes that bolted in 2008. However, effects of folivory reduction on the transition rate of rosettes to adulthood differed depended upon whether neighbor plants were clipped. Folivory reduction did not affect bolting where neighbors were clipped in 2007 (94.9% ± 3.5% survived and bolted without insecticide vs. 100% with insecticide), but where neighbors were not clipped folivory reduction increased the number of rosettes that bolted in 2008 (Fig. 3; p = 0.046; 69.4% ± 7.7% survived and bolted without insecticide vs. 100% with insecticide).

Discussion

Tissue loss to herbivores (Crawley 1983) and competition with neighbors (Wilson 1993, Cahill 2002) are challenges that confront plants in many ecosystems. Therefore, understanding neighbor plants' and herbivores' individual and interaction effects on host plant performance is broadly important for predicting spatial and temporal variation in factors limiting plant populations. Further, greater ability to predict individual and combined outcomes of these interactions may improve strategies for slowing the spread of weed populations (Shea and Kelly 1998).

Effects of Neighbor Vegetation on Rosette Performance

At our study site at the western edge of tallgrass prairie, the presence of aboveground parts of neighbor plants, primarily native grasses, strongly limited tall thistle rosette performance. This outcome is consistent with predictions by Jongejans et al. (2006) that suppressive effects of aboveground parts of neighbor plants would be especially strong for short-lived, rosette-forming species. Relative competitive intensities of aboveground neighbor vegetation on tall thistle rosette growth that we calculated using two common measures of thistle size, root crown diameter (Guretzky and Louda 1997, Rose et al. 2005) and rosette diameter (Bullock et al. 1994, Tenhumberg 2008), were comparable to those observed for other rosette-forming plants in grasslands. For example, using plant biomass to calculate relative competitive intensities, Wilson (1993) found relative aboveground competitive intensity in alpine grassland for the rosette-forming composite, *Celmesia longifolia*, to be 0.26. Cahill (2002) found much stronger relative aboveground competitive intensities in productive old-fields on the rosette-forming biennials *Rumex crispus* and *Plantago lanceolata* of 0.65 and 0.92. Therefore, our

results support previous studies that suggest that aboveground parts of neighbor plants often strongly reduce growth of rosette-forming species. This conclusion can inform management of several invasive exotic weeds in North America, including *Cirsium vulgare* and *Carduus nutans*, which share a growth form and life-history with tall thistle.

Reduced rosette growth where aboveground biomass of neighbor plants was present likely drove the significant reduction in the transition rate of 2007 rosettes to adulthood in 2008. Neighbor plants reduced the probability that tall thistles that survived until 2008 would bolt. Reduced bolting frequency among survivors is not surprising because, for many monocarpic plants, attempting reproduction is closely related to size (Metcalf 2003) and aboveground parts of neighbor plants strongly reduced growth. For example, Bullock et al. (1994) found that the proportion of large (> 20 cm rosette diameter) *Cirsium vulgare* rosettes that bolted depended upon the size distribution of rosettes within their "large rosette" size category. Where grazing practices allowed many small and medium rosettes to survive into the "large" size category, the size distribution of large rosettes shifted toward the lower limit of the size class and the bolting rate declined.

Relative to effects of neighbor plants on the probability that surviving tall thistle rosettes would bolt, neighbor plants had much weaker effects on rosette survival. Similarly, large *C. vulgare* rosettes had very high survival rates and their survival rates were unaffected by removal of neighbor aboveground biomass and litter by grazing in any season (Bullock et al. 1994). In fact, all but four tall thistle rosettes in our study had initial rosette diameters >20 cm and, therefore, would fall into Bullock et al. (1994)'s "large" rosette category. Silvertown et al. (1993) showed that transitions related to progression among life-stages had high elasticity values in matrix models for short-lived, monocarpic herbs. Hence, lower rates of progression to

adulthood where aboveground biomass of neighbor vegetation is large are likely to strongly affect tall thistle population growth.

Effects of Insect Folivory on Rosette Performance.

Insect folivory often has been shown to reduce rosette growth for other *Cirsium* species (Bevill et al. 2000, Suwa et al. *in press*). Interestingly, Guretzky and Louda (1997) showed that insect folivores strongly reduced growth of tall thistle rosettes in an eastern Nebraska grassland, 500 km northeast of our study site. By contrast, we found no effects of insect folivory upon tall thistle rosette growth. These differing outcomes do not appear to result from differences in the magnitude of insect folivore damage in these two geographic regions of tall thistle's range. In fact, levels of damage to leaves of rosettes in our study appear similar to those found by Guretzky and Louda (1997). In eastern Nebraska, over the course of the growing season the average percentage of leaves per rosette that were damaged over >10% of their surface area varied between 67% and 75% for large rosettes (>4.0 mm root crown diameter) and for small rosettes (2.0 – 4.0 mm root crown diameter) the average percentage of leaves damaged over >10% of their surface area varied between 50% and 67%. Applying the same designations for "large" and "small" rosettes to our study, we found that over the course of the growing season large rosettes averaged 77% to 92% of leaves suffering any damage by folivores and small rosettes averaged between 67% and 84% of leaves damaged. Instead, it seems likely that we found less effect of reducing insect folivory upon tall thistle growth because our insecticide application was less effective. In particular, Guretzky and Louda (1997) achieved a significant reduction in folivore damage throughout the growing season whereas we succeeded in reducing late-season folivore damage only.

In spite of weak effects of insect folivory on tall thistle rosette growth, we did find that reducing insect folivory late in the growing season increased the transition rate of 2007 rosettes to adulthood in 2008. Similarly, Bevill et al. 2000 showed that insect herbivory on rosettes of the rare, monocarpic perennial thistle, *Cirsium pitcheri* reduced the transition rate to adulthood. Whereas the effects of aboveground parts of neighbor plants on the transition rate of rosettes to adulthood were strongly influenced by a reduction in the bolting rate of surviving rosettes, the effects of insect folivory on the transition rate to adulthood arise from moderate reductions that did not reach statistical significance, in both rosette survival and bolting rate of survivors.

Combined effects of neighbor plants and insect folivory

Effects of neighbor plants and herbivores may act independently in reducing some fitness correlates, such as plant growth, yet combine non-independently to affect others, such as reproductive traits (Nötzold et al. 1998, Steets et al 2006). Our finding that effects of aboveground parts of neighbor plants and insect folivory acted independently in reducing growth of tall thistle rosettes adds to a short but growing list of field experiments in natural or agro-ecosystems that demonstrate that independent effects of neighbor plants and insect folivory on host plant growth may be more common than either synergistic or antagonistic interactions (independent effects on growth: Rees and Brown 1992, McEvoy et al. 1993, Erneberg 1999, Newingham and Callaway 2006, Suwa et al. *in press*; synergistic effects on growth: Nötzold et al. 1998, Agrawal 2004; antagonistic effects on growth: Haag et al. 2004). In spite of the independence of their effects on plant growth, we did find evidence that the presence of neighbor plants magnified the impact of insect folivory on the transition rate of rosettes to reproductive life-stages.

Hamback and Beckerman (2003) identified two categories of mechanisms that could generate interaction effects of neighbor plants and herbivory on plant performance, 1) neighbor plants either indirectly, through effects on predator or parasitoid abundance or foraging behavior, or directly, though altering herbivores' abilities to locate host plants, change the amount of damage that herbivores' impose on their host plant and 2) neighbor plants affect abiotic resource availability and, thereby, alter the host plant's ability to compensate for tissue loss to herbivores. The amount of folivory suffered by tall thistle rosettes late in the growing season was greater where neighbor plants were unclipped suggesting the possibility that a synergistic interaction effect on transition rate to adulthood could have arisen through modification of damage levels. However, the magnitude of change in damage as a function of neighbor plant clipping, only a 10.8% reduction in the proportion of leaves damaged as compared to where neighbors were unclipped, was sufficiently small to suggest that it likely plays a minor role in generating any interaction effect. To put this 10.8% reduction in folivore damage into perspective, the 42% reduction in folivore damage relative to ambient levels of folivory that was caused by applying insecticide where neighbor plants were clipped had no effect upon tall thistle rosette growth or transition to reproductive life-stages.

Lower compensatory ability by juvenile tall thistles for folivory when aboveground parts of neighbor plants are present likely contributes to the greater effect of insect folivory on the transition to adulthood where neighbor plants were unclipped. Parmesan (2000) and, more formally, Wise and Abrahamson's (2005, 2007) Limiting Resource Model pointed out that larger effects of herbivory on plant performance under low resource conditions should be expected if the resource that is in short supply limits plant performance and the herbivory further impedes acquisition of this resource. Our experiment is not a test of the Limiting Resource Model

because we did not quantify tall thistle fitness and levels of folivore damage did differ between levels of the neighbor plant clipping treatment late in the growing season. However, consideration of the identity of limiting abiotic resources and of the tissue type damaged by the herbivore may help explain the apparent synergism between aboveground parts of neighbor plants and insect folivory in reducing the transition rate to adulthood in tall thistle. Bolting rate in 2008 among surviving rosettes and growth between May-October 2007 were both greater when light availability was increased by clipping neighboring plants, providing correlational evidence that light limits plant performance. Folivory, by reducing photosynthetic surface area, is likely to reduce a rosette's ability to acquire light. Therefore, our outcome provides support for the idea that prediction of synergistic interaction effects may be enhanced by identifying which resource availabilities are altered by competitors and by considering whether or not the herbivore damage is likely to affect acquisition of the limiting resource.

Developing a predictive framework for the conditions under which effects of neighbor plants and herbivores combine non-independently to affect host plant performance will depend upon identifying the mechanisms that underlie synergistic or antagonistic interaction effects. Our results illustrate that herbivory and neighbor plant effects may act independently upon some fitness correlates, particularly growth, and non-independently upon others, emphasizing the value of quantifying impacts upon lifetime seed production, instead of fitness correlates when possible. Nevertheless, when folivory and neighbor plants' effects combine non-independently to affect performance of rosette-forming, grassland plants it seems likely that the interaction will be synergistic. This prediction is based upon accumulating evidence that light competition often limits rosette performance in grasslands and folivores are likely to further impede acquisition of light A comprehensive understanding of the joint effects of folivores and neighbor plants on

rosette-forming species, however, requires integrating predictions based on theories of how plant compensatory ability varies in relation to resource availability, with more detailed information on neighbor plants' effects on herbivore behavior and densities.

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Table 1. Effects (P-value; direction of effect) of clipping neighbor plants, insecticide, initial rosette size and the neighbor clipping X insecticide interaction on resource availability to tall thistle (*Cirsium altissimum*) rosettes, folivory and performance. Statistical tests are indicated by superscript numbers: [1] 2-way ANOVA, [2] 2-way repeated measures ANCOVA, [3] 2-way MANCOVA, [4] 2-way ANCOVA and [5] Fisher's exact test. Empty cells indicate that initial size was not included in the analysis. For dependent variables analyzed with Fisher's exact test, we asked whether insecticide had differing effects with neighbor plants unclipped vs. clipped.

Dependent variable	Initial size	Neighbor clipping	Insecticide	Neighbor clipping X insecticide
Resource availability				
Light penetration[1]		$P < 0.001$; increase	NS	NS
Soil moisture availability[1]		NS	NS	NS
Folivore damage				
Leaves damaged per rosette[2]	$P < 0.001$; increase	$P < 0.001$; decrease Oct.	$P < 0.001$; decrease Oct.	NS
Leaves damaged over >50% per rosette[2]	$P = 0.01$; increase	NS	NS	NS
Rosette Performance				
Growth[3]	$P = 0.061$; decrease	$P < 0.001$	NS	NS
Change root crown diam[4]	NS	$P < 0.001$; increase	NS	NS
Change rosette diam[4]	$P = 0.032$; decrease	$P < 0.001$; increase	NS	NS
Change no. of leaves[4]	NS	$P < 0.001$; increase	NS	NS
Transition to adulthood[5]		$P = 0.007$; increase	$P = 0.036$; increase	Insecticide effect with neighbors
Rosette survival[5]		NS	NS	No insecticide effect with or without neighbors
Bolting rate of survivors[5]		$P = 0.02$; increase	NS	No insecticide effect with or without neighbors

Figure Legends

Fig. 1. Seasonal trends in folivory to tall thistle rosettes with neighbor plants clipped to the ground within 40 cm (open circles) vs. neighbor plants unclipped (filled circles). Mean ± standard error for proportions of leaves per rosette with folivore damage are shown.

Fig. 2. Effects of neighbor plant clipping on tall thistle rosette growth May-October 2007. Absolute changes in rosette size May-October 2007 are shown on the Y-axis. These absolute changes were calculated as (rosette size in October 2007 – rosette size in May 2007). Rosette size is quantified as root crown diameter in panel A, as rosette diameter in panel B and as number of leaves per rosette in panel C. Bars are means ± standard errors.

Fig. 3. Transition rates of 2007 rosettes to adulthood in 2008 for combinations of the neighbor plant clipping and folivory reduction treatments. 2007 rosettes that did not transition to adulthood in 2008 may have died before June 2008 or may have survived but remained rosettes. Standard errors were calculated as S.E. $\hat{p} = (p(1-p)/n)^{0.5}$. Sample sizes are in parentheses above bars.

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