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Echinacea angustifolia and its specialist ant-tended aphid: a multi-year study of manipulated and naturally-occurring aphid infestation

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Abstract.

1. The impact of herbivores on plant fitness depends on multiple ecological mechanisms, including interactions between herbivore guilds.
2. This study assessed the effects of a specialist aphid (*Aphis echinaceae*) on performance and foliar herbivore damage of a long-lived perennial plant (*Echinacea angustifolia*) native to the North American tallgrass prairie. A 2-year field experiment manipulating aphid infestation on 100 plants was compared with concurrent and past observations of unmanipulated plants in the same outdoor experimental plot. Because ants co-occur with aphids, the experiment tested the combined effects of aphids and ants.
3. Neither manipulated nor naturally-occurring aphid infestations led to measurable declines in plant performance. Results for foliar herbivore damage differed between experimental and observational studies: the occurrence of foliar herbivore damage decreased with aphid infestation in the first year of the experiment and increased with aphid infestation over 5 years in unmanipulated plants.
4. While the experimental results concur with other experiments of ant–hemipteran–herbivore relationships, the observational results suggest that ant–aphid interactions do not naturally play a major role in determining patterns of foliar herbivory in this system. This result demonstrates the value of using field observations to interpret the relevance of experimental results.

Key words. Ant–aphid interaction, habitat fragmentation, insect herbivory, leaf-chewers, long-lived perennial, plant–insect interactions, sap-feeders, tallgrass prairie.

Introduction

Despite many investigations into the potential benefits of herbivores for plants (see review by A. Belsky, 1986), most experimental evidence reveals that herbivory hinders plant growth and fitness (Massad, 2013). However, sap-feeding herbivores, such as aphids, may provide indirect benefits by attracting ants that deter more costly herbivores (Styrsky & Eubanks, 2007, 2010; Ando & Ohgushi, 2008; Neves *et al.*, 2011). For example, Styrsky and Eubanks (2010) found that cotton plants with abundant caterpillars experienced a net increase in seed production when aphids and ants were present. Because ants did not forage on plants when aphids were absent, the benefit ultimately came from the aphids. While this is the only experiment that has directly measured increase in plant fitness from aphids, field experiments in a wide variety of systems have found an increase in herbivore damage after excluding ants or ant-tended herbivores (see reviews by Styrsky & Eubanks, 2007; Zhang *et al.*, 2012).

Even if an experiment finds reduced herbivore damage or increased plant growth from aphids or other ant-tended herbivores, it is difficult to quantify its importance for plant fitness. The balance between costs and benefits of ant-tended herbivores depends on multiple biotic and abiotic factors that vary widely within the same study system (Styrsky & Eubanks, 2007,

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2010). For example, Pringle *et al.* (2011) found that the effectiveness of ant bodyguards on the tree *Cordia alliodora* varied at different times in season and between study sites. One strategy for interpreting the relevance of short-term experimental manipulations is to combine them with long-term observations of naturally-occurring patterns in the same system. Such observations require less labour than long-term experimental manipulations and provide a broader range of ecological variability to contextualise short-term experimental results.

This study examines costs and benefits of an ant-tended herbivore for *Echinacea angustifolia*, a long-lived herbaceous plant native to the North American tallgrass prairie. *Echinacea angustifolia* hosts a specialist aphid (*Aphis echinaceae* Lagos) that cannot survive on any other host species (Lauren Hobbs and Hillary Lyon, unpublished). The aphid is the most common and abundant herbivore observed on *E. angustifolia* and it is usually tended by ants (Muller, 2013). Aphids attached to leaves, stems, and flowering heads divert photosynthate from other functions such as growth and reproduction and can impose substantial costs to host fitness (Foster, 1984; Snow & Stanton, 1988). *Aphis echinaceae* can reach very high abundances on *E. angustifolia* – sometimes over 1000 aphids on one plant (Muller, pers. obs.) – and feeding may impose a high cost. At the same time, *A. echinaceae* may indirectly benefit host plants by attracting ant bodyguards to repel other herbivores.

Aphid infestation varies substantially among small isolated populations of *E. angustifolia* near our experimental plot (Muller, 2013), possibly as a result of limited dispersal in a fragmented habitat. If aphids influence host plant fitness – either owing to costs of feeding or benefits of ant attendance – differences in aphid infestation could contribute to differences in plant fitness among fragmented populations and consequently plant population growth. Therefore, studying the effects of *A. echinaceae* on *E. angustifolia* plants contributes to understanding the consequences of habitat fragmentation for this native prairie plant.

The goal of this study was to assess the consequences of repeated heavy aphid infestation versus local extirpation for the fitness of *E. angustifolia*. A field experiment quantified the extent to which aphids influence host plant performance and damage from other herbivores by adding and excluding aphids on the same plants over 2 years. Because the experiment took place outdoors without cages or other artificial means of boosting aphid numbers, the experimental treatments simulated levels of aphid infestation that may occur in fragmented populations. Because ants co-occur with aphids in this system, the experiment tested the combined effects of aphids and ants on plant performance and foliar herbivore damage. Owing to the potentially important role of plant apparency in herbivore movement (Endara & Coley, 2011), plant size and flowering status were considered as additional factors to explain aphid abundance and foliar herbivore damage. The experimental results were compared with naturally-occurring patterns of aphid infestation and foliar herbivore damage in the same experimental plot over 5 years. These long-term observations provided a broader context for interpreting the ecological relevance of the experimental results. Combining these approaches yielded insights that would have been missed with either experiments or observations alone.

Materials and methods

Study system

Echinacea angustifolia DC (Asteraceae) is a long-lived perennial native to the North American tallgrass prairie. While *E. angustifolia* hosts many herbivores in our study area – including beetles, slugs, grasshoppers, and various Hemiptera – one species of aphid is the most common (Ridley *et al.*, 2011, Muller pers. obs.). The aphid *A. echinaceae* Lagos is a specialist to *E. angustifolia* and was identified in 2009 from specimens collected in Douglas County, MN (Lagos & Voegtlin, 2009). Winged and wingless aphid morphs occur on the leaves, stems, and heads of plants, frequently exceeding 100 individuals per plant in late summer.

Aphis echinaceae is tended by multiple ant species in the genera *Formica*, *Myrmica*, and *Lasius* (Muller, 2013). In the field site for this study, *L. alienus* was by far the most common ant species tending aphids in 2011 and 2012, based on a small-scale collection of *E. angustifolia* plants and personal observations. In nearby prairie remnants, the dominant ant species were either *L. alienus* or *F. obscuripes* (Hallaman, 2014).

All field research took place in a common garden experiment located in Douglas County, MN in a landscape containing small patches of remnant and restored prairie interspersed among corn and soybean fields. The ($46 \times 123\,\mathrm{m}^2$) plot is a former agricultural field with multiple cohorts of *E. angustifolia* planted amongst existing native and non-native perennials. The experimental manipulation took place in a subplot planted in 2003 ($39 \times 60\,\mathrm{m}^2$). The long-term observations took place in a subplot planted in 1997 ($24 \times 23\,\mathrm{m}^2$). Both subplots contain *E. angustifolia* planted either 0.5 m apart (2003 subplot) or 1 m apart (1997 subplot) within rows spaced by 1 m. Management for the entire plot consists of biyearly spring burns, mowing between rows, and hand broadcasting native grass seed. All *E. angustifolia* plants were transplanted as seedlings from seed originating from natural populations within 5 km of the plot.

Measurements

Plants emerge each spring as either a non-flowering plant with one or more basal rosettes or as a flowering plant. Because *E. angustifolia* does not produce additional leaves or flowering stems after emerging each year, plant performance was classified based on changes in plant size and flowering status between years. We consider size as a proxy for fitness in non-flowering plants because previous investigations have shown a strong positive relationship between plant size and future fecundity (Shaw *et al.*, 2008). A transition from basal to flowering or an increase in the count of basal leaves constitutes an increase in performance. A decrease in performance occurs as a transition from flowering to basal, a decrease in the count of basal leaves, or death. Plants that had the same basal leaf count or remained flowering were considered to have no change in performance. Senescence was quantified by counts of yellow, purple, and crisp leaves in late summer. Foliar herbivore damage was defined as the presence of chew marks or holes in leaves recorded in mid-summer when aphid abundance was high (10 August 2011

Table 1. Co-occurrence of aphids and ants: the range of values represents the proportion of plants observed to harbour or not harbour aphids and/or ants during the experimental treatments throughout 2011 ($n = 100$ plants) and 2012 ($n = 97$ plants).

	Ants absent	*Ants present*
Aphids absent	2011: 0.53 to 0.91 2012: 0.31 to 0.86	2011: 0 to 0.03 2012: 0 to 0.04
Aphids present	2011: 0 in all observations 2012: 0.03 to 0.19	2011: 0.08 to 0.45 2012: 0.09 to 0.51

and 14 July 2012) and in late-summer after aphid infestation had declined (4 September 2011 and 25 August 2012). In the first year, the severity of foliar herbivore damage was quantified as the number of leaves with chew marks. In the second year, the count of damaged leaves was expanded to include both chew marks and holes.

Field experiment

Aphid infestation was manipulated on 100 non-flowering (basal) plants in the 2003 experimental subplot during the summers of 2011 and 2012 (Figure S1, Document S1). Ants roamed freely on plants; thus the experiment quantified combined effects of aphids and aphid-tending ants. Plants were randomly assigned to aphid addition and exclusion treatments ($n = 100$) and received the same treatment in both years. The addition treatment consisted of transferring adult aphids from plants in the experimental subplot onto leaf surfaces twice per week in early summer and once per week afterwards. Aphids were excluded by brushing them into a Petri dish after visually inspecting leaves. Exclusion took place once per week when aphid abundances were low in early- and late-summer and twice per week when aphid abundance were high in mid-summer. Before adding or removing aphids, a single observer recorded the abundance of aphids and ants present on the plant in categories of 0, 1–10, 11–80, and >80 aphids and 0, 1, 2–10, and >10 ants. Ants and aphids typically co-occurred on plants, and ants were seldom present when aphids were absent (Table 1).

The addition treatment differed slightly between years. In 2011, one adult aphid was added to every plant on the first visit and the number of aphids was adjusted on subsequent visits in accordance with the abundance present on the plant. Adding a large number of aphids did not cause a greater increase in aphid abundance than adding a small number of aphids (Figure S2, Document S1). In 2012, the addition treatment was simplified to adding two adult aphids to the underside of a single leaf during each visit. In each year, the addition and exclusion treatments effectively produced clear differences in aphid abundance between the two experimental groups (Figure S3, Document S1).

Observational studies

Naturally-occurring patterns of aphid infestation, plant growth, and foliar herbivory were observed concurrently with the experiment in the 2003 subplot. The concurrent observational study took place within a $20 \times 20\,\text{m}^2$ area of the subplot that was 11 m from any edge. Plants were observed every 2 weeks between 1 July and 10 September 2011 and once a month between 15 June and 18 August 2012 and insect abundances were recorded in categories of 0, 1–10, 11–80, and >80 aphids and 1, 2–10, and >10 ants. Foliar herbivory was recorded only in 2012 as the presence of chew marks or holes. A total of 548 plants were observed in 2011 (64 flowering and 484 basal) and 520 plants in 2012 (44 flowering and 476 basal).

Long-term patterns of aphid infestation and foliar herbivore damage were assessed from observations annually from 2004 to 2008 in the 1997 cohort. During this time period, aphid infestation in the 2003 cohort was infrequent (<2%) and varied too little to assess a relationship with foliar herbivore damage. Because these annual observations were completed within a few days each year, the long-term dataset accurately reflects quantitative variation in aphid abundance among individual plants within years. However, the dataset may not accurately convey differences in total aphid abundance between years because the observation periods may have occurred at different times relative to peak seasonal aphid abundance (Muller, 2013). Nevertheless, the long-term dataset presents a great opportunity to assess relationships between aphid abundance and foliar herbivore damage across multiple years.

Statistical analysis

The statistical software R version 2.15.2 was used for all data analysis (R Core Team, 2013). The relationship between aphid infestation and changes in plant performance was examined in contingency tables using χ^2 tests or Fisher's exact tests (for tables with counts less than five). Aphid infestation was categorised by experimental treatment (addition or exclusion) or by the highest recorded aphid abundance (0, 1–10, 11–80, and >80). If aphids harmed plants, then plants with high aphid abundance would be expected to die or decrease in size the following year at a greater rate than plants with few or no aphids. We also used contingency tables to investigate relationships between plant size and aphid abundance and plant performance. Accounting for the role of plant size in aphid attraction helps interpret the effects of aphid infestation on plant performance.

The effects of aphid infestation on senescence and foliar herbivore damage were assessed using generalised linear models (GLMs) with a binomial response. Because the residuals indicated overdispersion, the quasibinomial family was used throughout the analysis. Responses were defined as the proportion of senesced leaves per plant, the proportion of damaged leaves per plant, or the presence of foliar herbivore damage. Maximal models for the manipulative experiment contained predictors of experimental treatment and plant size. Because aphid abundance varied within treatments, we conducted an additional analysis of senescence and herbivory based on maximum observed aphid abundance, instead of treatment. Plants that became crisp before the herbivory observations in late-summer were included in the analysis of senescence and excluded from the analysis of herbivory. As an alternative approach, we also combined both years into a mixed-effects GLM, using the package lme4 and the R-function glmer (Bates & Maechler, 2009), to account for pseudo-replication owing to repeated measurements. Experimental treatment and plant size were treated as fixed effects and plant identity was treated as a random effect. Results for the mixed-effects models were qualitatively similar to the single-year GLM models; for simplicity, we report only the single-year GLM models.

For the long-term observational dataset, the relationship between aphid infestation and foliar herbivore damage was modelled with a binomial mixed-effects GLM combining observations over 5 years (Bates *et al.*, 2015). Maximal models for the observational studies contained fixed effects of highest observed aphid abundance, plant size, and spatial coordinates within the experimental plot (spatial coordinates were not included for the experiment because the locations of plants in the aphid addition and exclusion treatment were randomly assigned). Because the mixed-effects model did not converge when the interaction between year and aphid abundance was included, a separate analysis was conducted to assess the relationship between aphid abundance and foliar herbivore damage in each year, using separate binomial GLMs. Results for this analysis were qualitatively similar to the mixed-effects GLM and are reported in the supplement (Tables S4 and S5, Document S1).

For all data analysis, minimal adequate models were selected using backwards model selection (Crawley, 2008). This method begins with a maximal model containing all predictor terms, removes one term at a time, and compares the simpler model to the more complex model using a likelihood ratio test. The minimal adequate model omits all predictors that yield a *P*-value of > 0.05 when removed from the model. For models comparing categories of aphid abundance, post-hoc Tukey's tests were used to identify significant differences among categories, using the package multicomp and the R-function glht (Hothorn *et al.*, 2008).

Flowering plants were excluded from analyses of foliar herbivore damage and senescence for several reasons: (i) flowering plants produce relatively few leaves and their size cannot be approximated by leaf count, (ii) rates and abundances of aphid infestation were markedly higher among flowering plants than basal plants (Muller, 2013), and (iii) only nine out of 100 plants in the experiment flowered in 2012. Flowering plants were excluded from models of observational data to provide a meaningful comparison with experimental results.

Results

Effects of aphid infestation on plant performance and senescence

Aphid addition and exclusion treatments did not affect plant performance after the first year of the experiment (Table 2). However, in the second year, plants in the aphid addition treatment were more likely to decline in performance (0.44, *n* = 48) than plants in the exclusion group (0.24, *n* = 49) and plants in the exclusion group were more likely to improve in performance (0.59) than plants in the addition group (0.38). While this association was not statistically significant ($P = 0.079$, $\chi^2 = 5.08$, d.f. = 2), the greater decline among plants in the addition group may suggest a negative effect of aphids in the second year. In the concurrent observational study, no associations were present between maximum aphid abundance in the first year and changes in plant performance in the second year ($P = 0.33$, $\chi^2 = 6.87$, d.f. = 6, Table 2).

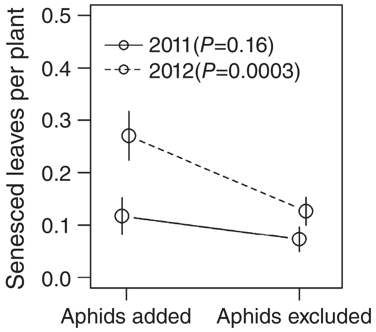

Fig. 1. Effect of aphid addition and exclusion on leaf senescence: senescence was quantified as the proportion of leaves that were yellow, purple, or crisp in late-summer (9 September 2011, 25 August 2012). Values are fitted means and SEs for a plant with the modal leaf count (>5 leaves) from a quasibinomial generalised linear model (GLM). Minimal adequate models were chosen by backwards elimination from maximal models that included aphid treatment, plant size (basal leaf count in categories of 1–3, 4–5, and >5 leaves), and an interaction term. Plant size significantly contributed to senescence in 2012 ($P = 0.018$), but not in 2011. The aphid addition treatment contained $n = 50$ plants in 2011 and $n = 42$ plants in 2012. The aphid exclusion treatment contained $n = 50$ plants in 2011 and $n = 45$ plants in 2012.

Adding aphids accelerated leaf senescence in 2012. The proportion of senesced leaves per plant in the aphid addition group exceeded that in the exclusion group by 5% in 2011 and 14% in 2012 (2011, $P = 0.16$, $n = 100$ Table S1, Document S1; 2012, $P = 0.0003$, $n = 87$; GLM quasibinomial, Table 3, Fig. 1). Six plants were completely crisp by the end of the study period in 2012 and all of them were in the addition group. All but one of these plants re-emerged in 2013, indicating that their early senescence did not foreshadow death.

Effects of aphid infestation on foliar herbivore damage

The two types of foliar herbivore damage observed (chew marks and holes) showed qualitatively similar patterns across experimental treatments and aphid abundance categories (Figure S5, Document S1). For simplicity, all results for foliar herbivore damage will refer to plants with either chew marks or holes.

Aphid infestation and foliar herbivore damage were negatively associated in the experiment. The exclusion group contained a greater proportion of basal plants with chew marks or holes in late-summer (0.66, *n* = 49, in 2011 and 0.78, *n* = 45 in 2012) relative to the addition group (0.43, *n* = 50 in 2011 and 0.68, *n* = 38 in 2012) – although the difference was not statistically significant in the second year (2011, $P = 0.04$, $n = 99$; 2012, $P = 0.13$, $n = 83$; GLM quasibinomial, Fig. 2a, Table 4). The exclusion group also had a greater proportion of chewed leaves per plant (0.22, *n* = 49 in 2011 and 0.51, *n* = 45 in 2012) than the addition group in both years (0.12, *n* = 50 in 2011 and 0.35,

Table 2. Effect of aphid addition and exclusion on plant performance: values are the proportion of plants within each treatment or abundance category whose performance improved (increase in basal leaf count or transition from basal to flowering), declined (decrease in basal leaf count, transition from flowering to basal, or mortality), or remained the same (no change in basal leaf count or flowering status). Independence among categories was assessed with χ^2 tests for each year.

(a) Aphid addition-exclusion experiment

Year	***Plant performance***				
	Treatment	**Declined**	**No change**	**Improved**	***n***
2011–2012	Addition	0.28	0.12	0.60	50
$P = 0.63$, $\chi^2 = 0.92$, d.f. = 2	Exclusion	0.30	0.18	0.52	50
2012–2013	Addition	0.44	0.19	0.38	48
$P = 0.079$, $\chi^2 = 5.08$, d.f. = 2	Exclusion	0.24	0.16	0.59	49

(b) Concurrent observational study

Max. aphid abundance 2011	***Plant performance***			
	Declined	**No change**	**Improved**	**n**
0	0.32	0.25	0.43	310
1–10	0.41	0.17	0.41	58
11–80	0.34	0.23	0.43	91
>80	0.45	0.22	0.33	89
$\chi^2 = 6.87$, d.f. = 6, $P = 0.33$				

Table 3. Analysis of deviance table for the relationship between aphid infestation and senescence (experiment): backwards model selection results for a generalised linear model (GLM) with a quasibinomial error analysing the proportion of senesced leaves (basal plants only) as a function of aphid addition or exclusion treatment and basal leaf count (in categories of 1–3, 4–5, and >5 leaves).

Term	Resid. d.f.	Resid. deviance	Test d.f.	Deviance	*F*	*P*
Late summer, 2012 (n = 87)						
Null	86	212.43	–	–	–	–
Treatment	85	187.36	1	25.07	14.15	0.00032
Basal leaf count	83	172.56	2	14.80	4.18	0.019
Treatment × basal leaf count	81	170.13	2	2.43	0.68	0.51

Grey highlight indicates statistical significance at $P < 0.05$.

P-values are for the *F*-test of the null hypothesis that a model excluding each term is not different from a model including each term. The minimal adequate model included main effects of treatment and basal leaf count and excluded their interaction.

$n = 38$ in 2012) – although the difference was not statistically significant (2011: $P = 0.05$, $n = 99$; 2012: $P = 0.16$, $n = 83$; GLM quasibinomial, Fig. 2b; Table S2, Document S1). In the first year, differences between the addition and exclusion group were less pronounced in mid-summer when aphid infestation was high ($P = 0.075$, $n = 100$, GLM quasibinomial, Table S2, Document S1).

In order to account for variation in aphid abundance within aphid addition and exclusion treatments (Figure S3, Document S1), we also considered the effects of aphids on foliar herbivore damage based on maximum observed aphid abundance. Similar to the results for aphid addition and exclusion, herbivore damage and aphid abundance were negatively related in the first year, but not the second year (Table S2, Document S1; Fig. 3a). In the first year, the proportion of basal plants with foliar herbivore damage decreased with increasing aphid abundance ($P = 0.02$, GLM quasibinomial) and was lower on plants with >80 aphids (0.31, $n = 13$) than on plants with 11–80 aphids (0.39, $n = 33$), 1–10 aphids (0.63, $n = 16$), or no aphids (0.72, $n = 37$). According to a post-hoc Tukey's test, the only two categories that differed significantly were plants with 0 aphids versus 11–80 aphids ($P = 0.049$, Table 5a).

In the concurrent observational study, no significant relationship existed between aphid infestation and foliar herbivore damage among basal plants ($P = 0.85$, n = 472, GLM binomial, Table S3, Document S1).

The relationship between aphid abundance and foliar herbivore damage in the long-term observational study was the opposite of that in the experiment. Between 2004 and 2008, foliar herbivore damage increased with increasing aphid abundance ($P < 0.0001$, $n = 248–345$, mixed-effects GLM, Table 6, Fig. 3). When years were modelled separately, the positive relationship between aphid abundance and foliar herbivore damage was statistically significant ($P < 0.05$) only in 2004 and 2007 (Table S4, Document S1). The positive relationship between aphid abundance and the occurrence of foliar herbivore damage was apparent within all plant size groups and among flowering plants (Figure S4, Document S1). Post-hoc tests of the multi-year mixed-effects GLM showed significant differences between plants with 0 aphids versus 11–80 or >80 aphids and between plants with 1–10 aphids versus > 80 aphids. However, post-hoc tests of the single-year models found that differences in the occurrence of herbivore damage among aphid abundance categories were not consistent between years. Plants with 0 aphids versus 11–80 aphids differed significantly in 2004 and 2007, whereas plants with 0 aphids versus > 80 aphids differed significantly only in 2004. No other categories differed significantly in the single-year models. Therefore, it is likely that the

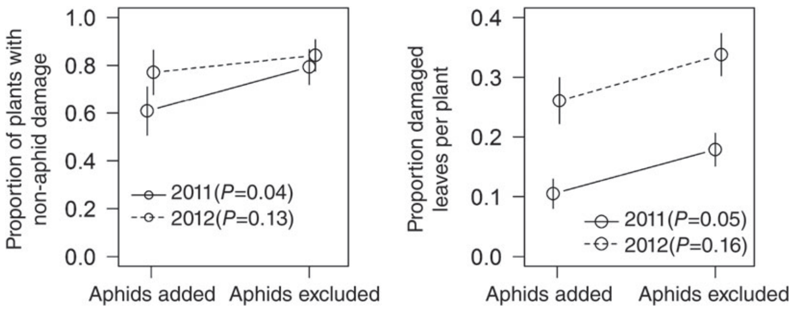

Fig. 2. Effect of aphid addition and exclusion on the occurrence (a) and extent (b) of foliar herbivore damage: herbivore damage was recorded after the seasonal decline of aphid infestation. The occurrence and extent of foliar herbivore damage were quantified, respectively, as the presence and proportion of leaves with chew marks or holes (extent only included chew marks in 2011). Sample sizes, excluding plants that senesced before herbivory was recorded, were $n = 49$ in 2011 and $n = 38$ in 2012 for the addition treatment and n = 50 in 2011 and n = 45 in 2012 for the exclusion treatment. Values are fitted means and SEs for a plant with modal leaf count (>5 leaves) from a quasibinomial generalised linear model (GLM). The minimal adequate models, chosen by backwards elimination, had predictors of treatment (aphids added or excluded) and plant size (basal leaf count in categories of 1–3, 4–5, and >5 leaves). Plant size contributed significantly to only one model, the occurrence of foliar herbivore damage in 2011($P = 0.032$, Table 3). Plant size did not contribute significantly to the extent of herbivore damage and was not included in either model.

Table 4. Analysis of deviance table for the relationship between aphid treatment and the occurrence and extent of foliar herbivore damage (experiment): backwards model selection results for a generalised linear model (GLM) with quasibinomial error analysing the proportion of plants with chew marks or holes (occurrence) or the proportion of leaves with chew marks or holes (extent) as a function of treatment and basal leaf count (categories of 1–3, 4–5, and >5 leaves).

Term	Resid. d.f.	Resid. deviance	Test d.f.	Deviance	*F*	*P*
(a) Occurrence: late summer, 2011 ($n = 99$)						
Null	98	136.42	–	–	–	–
Basal leaf count	96	128.79	2	7.63	3.85	0.032
Treatment	95	124.34	1	4.45	4.18	0.043
Treatment × basal leaf count	93	123.95	2	0.39	0.18	0.83
(b) Occurrence: late-summer, 2012 ($n = 83$)						
Null	82	95.99	–	–	–	–
Basal leaf count	80	91.38	2	4.61	2.14	0.12
Treatment	79	90.61	1	0.77	0.71	0.40
Treatment × basal leaf count	77	89.96	2	0.65	0.30	0.74
(c) Extent: late-summer, 2011 ($n = 99$) – includes only chew marks						
Null	98	166.62	–	–	–	–
Treatment	97	159.98	1	6.64	3.95	0.05
Basal leaf count	95	159.61	2	0.37	0.11	0.89
Treatment × basal leaf count	93	159.01	2	0.59	0.18	0.84
(d) Extent: late-summer, 2012 ($n = 83$) – chew marks and holes						
Null	82	161.21	–	–	–	–
Treatment	81	157.72	1	3.49	2.03	0.16
Basal leaf count	79	157.37	2	0.34	0.1	0.91
Treatment × basal leaf count	77	156.60	2	0.77	0.22	0.80

Grey highlight indicates statistical significance at $P < 0.05$.
P-values are for the *F*-test of the null hypothesis that a model excluding each term is not different from a model including each term.

results from the mixed-effects GLM primarily reflect patterns from 2004 and 2007.

Role of plant size and flowering status

Plant size played an important role in both aphid infestation and foliar herbivore damage. Compared with small plants, large basal plants (i.e. leaf count >5) had a higher incidence of foliar herbivore damage in the first year of the experiment ($P = 0.032$ in 2011, Table 4), the second year of the concurrent observational study ($P < 0.0001$ in 2012, Table S3, Document S1), and the long-term observational study ($P < 0.0001$, Table 6). In the first year of the experiment, large plants more frequently hosted > 80 aphids in the concurrent observational study ($P < 0.001$, χ^2 test), but not in the experiment ($P = 0.45$, Fisher's test, Table 7).

Flowering plants (excluded from herbivory models) had a substantially higher occurrence of foliar herbivore damage and aphid infestation than basal plants in the experiment and in the concurrent observational study. In the experiment, all of the plants that flowered in the second year had foliar herbivore damage and medium or heavy aphid infestation ($n = 9$). In the concurrent observational study, 91% of flowering plants had

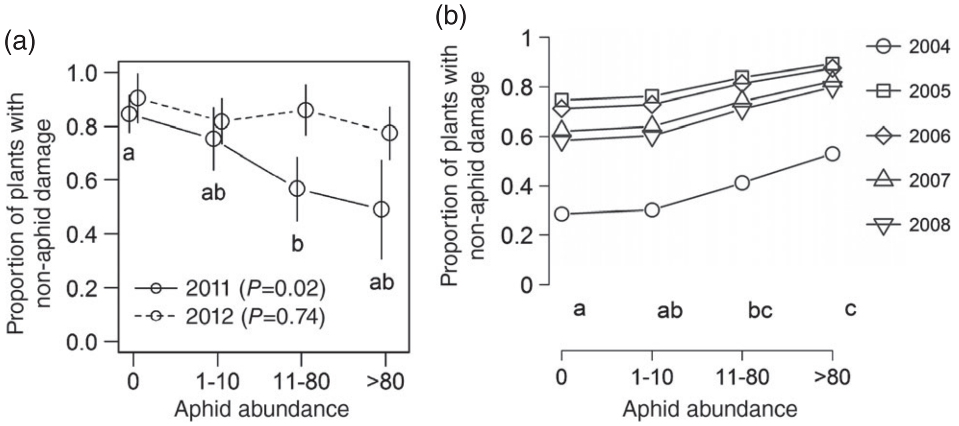

Fig. 3. Relationship between aphid abundance and foliar herbivore damage: (a) experiment (first year): the proportion of plants with non-aphid herbivory (chew marks or holes) in four aphid abundance categories. Values are fitted means and SEs from a quasibinomial generalised linear model (GLM) with predictors of highest observed aphid abundance and plant size (basal leaf count in categories of 1–3, 4–5, and >5), selected by backwards elimination. The occurrence of foliar herbivore damage decreased with increasing aphid abundance ($P = 0.02$, GLM quasibinomial). Letters refer to pairwise differences with $P < 0.05$, according to a Tukey's post-hoc test (Table 5a). Sample sizes in each category were as follows: 0 aphids ($n = 37$ in 2011, $n = 8$ in 2012), 1–10 aphids: ($n = 16$ in 2011, $n = 28$ in 2012), 11–80 aphids ($n = 33$ in 2011, $n = 14$ in 2012), >80 aphids ($n = 13$ in 2011, $n = 33$ in 2012). (b) Long-term observational study: aphid abundances were recorded once annually on each plant between mid-July and mid-August. Values are fitted means from a mixed-effects GLM for plants with modal leaf count (>5), excluding flowering plants. The minimal adequate model, selected by backwards elimination, had fixed effects of aphid abundance, year, and leaf count and random effects of individual plant (to account for repeated measures). Letters refer to pairwise differences according to a post-hoc Tukey's test (Table 5b). The number of plants observed was $n = 272$ in 2004, $n = 345$ in 2005, $n = 272$ in 2006, $n = 308$ in 2007, and $n = 248$ in 2008.

Table 5. Tukey's post-hoc comparison of herbivore damage among aphid abundance categories presented in Fig. 3.

Comparison	Estimate	Std. error	z value	p
(a) Aphid addition-exclusion experiment, 2011 (quasibinomial GLM)				
0 vs. 1–10 aphids	−0.5814	0.6876	−0.846	0.8297
0 vs. 11–80 aphids	−1.4291	0.5564	−2.569	0.0487
0 vs. >80 aphids	−1.7379	0.7527	−2.309	0.0938
1–10 vs. 11–80 aphids	−0.8477	0.6762	−1.254	0.5873
1–10 vs. >80 aphids	−1.1565	0.8569	−1.35	0.5257
11–80 vs. >80 aphids	−0.3087	0.756	−0.408	0.9765
(b) Long-term observational study, 2004–2008 (mixed-effects binomial GLM)				
0 vs. 1–10 aphids	0.08146	0.20345	0.4	0.97712
0 vs. 11–80 aphids	0.56224	0.15336	3.666	0.00122
0 vs. >80 aphids	1.03802	0.26437	3.926	<0.001
1–10 vs. 11–80 aphids	0.48078	0.21179	2.27	0.0992
1–10 vs. >80 aphids	0.95656	0.29771	3.213	0.00675
11–80 vs. > 80 aphids	0.47578	0.24557	1.937	0.2025

Pairwise significant differences ($P < 0.05$) are highlighted in grey.
Post-hoc comparisons for single-year GLMs for the long-term observational data are included in the supplement (Table S5, Document S1).

foliar herbivore damage and 68% hosted aphids ($n = 44$). In contrast, 64% of basal plants had foliar herbivore damage and 43% hosted aphids ($n = 440$). The rates of aphid infestation and foliar herbivore damage for basal and flowering plants in the long-term observational study are included in the supplement (Table S6, Document S1).

Discussion

Overall, our results showed that the specialist aphid *A. echinaceae* did not strongly affect host plant performance after 2 years of infestation. The most dramatic effect of aphid infestation – the greater rate of leaf senescence in the aphid addition group – did not lead to mortality. This suggests that *A. echinaceae* does not impose a strong fitness cost on *E. angustifolia,* at least over the short-term, indicating that any benefit of ant–aphid interactions would likely outweigh fitness costs of aphid feeding. However, because *E. angustifolia* is a long-lived perennial, it is possible that fitness costs of aphid infestation accumulate over multiple years. This interpretation is supported by the slight decline in performance among plants in the aphid addition treatment after the second year.

Table 6. Analysis of deviance table for the relationship between aphid abundance and the occurrence of foliar herbivore damage in the long-term observational study (basal plants only): backwards model selection results for a mixed-effects generalised linear model (GLM) that accounts for a random effect using individual plants as a grouping variable and fixed effects of plant size (basal leaf count in categories of 1–3, 4–5, and >5), aphid abundance (0, 1–10, 11–80, and >80), and observation year.

Term	d.f.	AIC	BIC	logLik	χ^2	χ^2 d.f.	P
Plant	2	1986	1996.6	−991	–	–	–
Year	6	1865	1896.7	−926.52	128.98	4	<0.0001
Basal leaf count	8	1743.8	1786	−863.88	125.26	2	<0.0001
Aphid abundance	11	1727.5	1785.5	−852.73	22.30	3	<0.0001
Basal leaf count × aphid abundance	17	1732.1	1821.8	−849.07	7.32	6	0.29

Grey highlight indicates statistical significance at $P < 0.05$.
P-values are from χ^2 tests of the null hypothesis that a model simplified by excluding each term is not different from a model including each term.

Table 7. Contingency tables of the association between plant size and aphid abundance in 2011: cell values are the proportion of basal plants in each size category (1–3 leaves, 4–5 leaves, and >5 leaves) with a maximum aphid abundance of 0, 1–10, 11–80, or >80 aphids. Plant size had a positive relationship with aphid abundance in the concurrent observational study (a) and in the second year of the experiment (c), but not in first year of the experiment (b).

Plant size	0 aphids	1–10 aphids	11–80 aphids	>80 aphids	n
(a) Concurrent observational study (2011)*					
1–3 lvs	0.69	0.11	0.16	0.04	198
4–5 lvs	0.65	0.11	0.13	0.10	173
>5 lvs	0.45	0.12	0.90	0.25	113
(b) Experiment (2011)†					
1–3 lvs	0.29	0.15	0.44	0.12	34
4–5 lvs	0.43	0.11	0.25	0.21	28
>5 lvs	0.39	0.21	0.32	0.08	38
(c) Experiment (2012)‡					
1–3 lvs	0.25	0.25	0.21	0.29	24
4–5 lvs	0.04	0.33	0.04	0.58	24
>5 lvs	0.03	0.38	0.23	0.36	39

*$P < 0.001$, χ^2 test.
†$P = 0.45$, Fisher's test.
‡$P = 0.02$, Fisher's test.

The negative relationship between foliar herbivore damage and aphid abundance in the first year of the experiment supports the possibility that ant–aphid interactions deter other herbivores in *E. angustifolia*. However, the association between aphids and foliar herbivore damage was weak and only apparent in the first year of the experiment. Without experimental manipulation of aphid infestation, the relationship between aphids and foliar herbivore damage was positive over 5 years.

The insight gained from combining experimental and observational approaches was that bottom-up factors such as plant apparency play a more important role than aphid–ant interactions in shaping natural patterns of herbivory on *E. angustifolia.* One likely reason for our opposing experimental and observational results is that the addition and exclusion treatments created patterns of infestation that would not occur with natural dispersal. Without experimental manipulation, *E. angustifolia* plants are more likely to be infested by aphids if they are flowering, have many basal leaves, or are located near other infested plants (Muller, 2013). All of these factors probably contribute to the apparency of plants to herbivores (Endara & Coley, 2011). An experiment with oak saplings found that trees with taller neighbours had lower levels of herbivory than trees with shorter neighbors, suggesting that apparency influences susceptibility to herbivores (Castagneyrol *et al.*, 2013). Flowering *E. angustifolia* produce vertical stems often 2–3 times taller than basal leaves. Aphids and other herbivores may be more likely to land on flowering plants or large basal plants than on small basal plants. The experiment controlled for plant size, flowering status, and location by randomly assigning plants to the aphid addition and exclusion group and limiting the experimental manipulation to non-flowering plants. Thus, even although the experiment was conducted outdoors in an ecologically realistic setting, it created patterns of aphid infestation that would not occur under natural dispersal. The experimental treatments added aphids to plants with low natural susceptibility (i.e. small basal plants far away from other infested plants) and removed them from plants with high susceptibility (i.e. large basal plants nearby other infested plants). Thus, even if aphid–ant interactions deter other herbivores, their effect is only measurable when controlling for plant size and location. Based on these results, it is not likely that ant–aphid interactions provide a substantial defence benefit for *E. angustifolia* in nature.

Although the effect was minor, the negative association between aphid infestation and foliar herbivore damage in the experiment indicates that costs of aphid infestation may be balanced by reduced damage from other herbivores. This is consistent with results highlighted in a meta-analysis of 49 manipulative experiments in a wide variety of systems (Zhang *et al.*, 2012). Many of these studies found that ant–hemipteran interactions deterred other herbivores from host plants and did not strongly influence plant growth or reproduction (Zhang *et al.*, 2012). One experiment that manipulated both ants and aphids found that aphids reduced plant growth when ants were excluded, but not when ants were present, suggesting that the benefits of ants balanced the costs of aphid infestation (Neves *et al.*, 2011). Because our experiment did not attempt to separate the effects of aphids and ants, it cannot distinguish whether aphids or ants were responsible for the observed reduction in foliar herbivore damage in the aphid addition group.

While our results do not suggest substantial defence benefits from ants or fitness impacts from aphids, the effects of ant–aphid interactions on plants may depend on which ant species are present. The predominant ant tender in this study was *L. alienus*. A survey of nearby prairie remnants found that most sites were dominated by either *L. alienus* or *F. obscuripes*, both of which have been observed on *E. angustifolia* (Hallaman, 2014). Studies comparing native and invasive ants reveal that the species of ant affects aphid abundance and honeydew production, and thus plant fitness (Paris & Espadaler, 2009; Powell & Silverman, 2010). Differences in aggressiveness between ant species could influence their effectiveness as bodyguards (Rosumek *et al.*, 2009). Thus, the effects of aphids on *E. angustifolia* may differ between sites dominated by *Formica* or *Lasius* species.

Limited dispersal among fragmented *E. angustifolia* populations probably leads to consistently heavy infestation in some populations and the absence of aphids in others. Although there has been no direct study of aphid dispersal on *E. angustifolia*, an annual census of plant populations in Minnesota found that some populations exhibited consistently high or low levels of aphid infestation relative to other populations across multiple years (Muller, 2013). Another study in a similar system found a high degree of genetic heterogeneity in specialist aphids among subpopulations of another grassland perennial, *Tanacetum vulgare* (Asteraceae), separated by a few kilometres (Loxdale *et al.*, 2011). This is similar to the spatial scale of isolation among *E. angustifolia* populations in Minnesota. In light of our field observations and these results, it is likely that aphid dispersal is limited between populations.

Host genetics may also contribute to the unequal distribution of aphids among populations. Habitat fragmentation has the potential to increase or decrease the genotypic diversity of plant populations through inbreeding (Wagenius *et al.*, 2007; Wagenius & Lyon, 2010) or outcrossing between distant populations (Schulke & Waser, 2001). A study of three genotypic classes of *Echinacea* that are expected to be prevalent in remnant prairie populations found substantial differences in tolerance of aphid infestation. Fitness of inbred plants decreased much more with peak annual aphid infestation than did fitness of the other genotypes (Shaw *et al.*, 2015). This study was based in the same plot as the present experiment and found no difference in infestation among inbred and outbred genotypes, whether accounting for host size or not. An earlier investigation of the same experiment population found that inbred and outcrossed genotypes had greater aphid infestation early in the summer – although the genotypic effects disappeared by late summer and were not persistent across years (Ridley *et al.*, 2011).

Increased genotypic diversity (such as that due to outcrossing between fragmented populations) may increase herbivore abundance in a plant population (Cook-Patton *et al.*, 2011; Utsumi *et al.*, 2011; Castagneyrol *et al.*, 2012). For example, Utsumi *et al.* (2011) found higher aphid abundance in plots with multiple genotypes of *Solidago altissima* compared with monoclonal plots –independent of the genetic susceptibility of the component genotypes. Genotypic variation may create source-sink dynamics with herbivores spreading from more susceptible hosts to less susceptible hosts (Utsumi *et al.*, 2011).

Whatever the cause, it is likely that habitat fragmentation alters the costs and benefits of aphid infestation for *E. angustifolia*. Additional research is needed to determine whether the costs of aphid infestation accumulate over multiple years and whether different ant species alter effects of aphids on plants. Both of these questions are important for understanding the role of aphids in the long-term persistence of *E. angusifolia* populations.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference:
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Document S1. Provides additional detail on experimental methods and statistical analysis along with results and observations mentioned, but not included, in the main text.

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