OBSERVATIONAL AND EXPERIMENTAL APPROACHES TO UNDERSTANDING THE RELATIONSHIP BETWEEN A LONG-LIVED PERENNIAL AND ITS SPECIALIST APHID IN FRAGMENTED HABITAT

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☐ Pass with no revisions ☒ Pass with minor revisions

☐ Pass with major revisions ☐ Fail to pass

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In the case of revisions, specify below, on back, or on additional attached pages what they are and who on the committee (major adviser, or everyone) will review the revisions before the thesis is officially accepted as complete. After this approval has been met, the major adviser needs to sign again below and this form, along with a pdf file of the final approved thesis must be turned into the PBC program assistant. Students need to check the deadlines for June and December graduations with TGS to make sure these forms are completed on time.

Stuart Wagenius — 19 June 2013

Signature of major adviser confirming final approval — Date

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Abstract

Plant-aphid systems provide a valuable opportunity for studying the ecological consequences of land use change for interacting species. The North American tallgrass prairie has undergone severe reduction and fragmentation due to agricultural development. The prairie perennial *Echinacea angustifolia*, a model system for studying population-level effects of habitat fragmentation, hosts a specialist ant-tended aphid (*Aphis echinaceae*). My research explores various aspects of the relationship between aphids and *Echinacea* to evaluate consequences of habitat fragmentation for plant and aphid populations. I used a combination of experimental and observational approaches to investigate: 1) effects of aphid infestation on plant growth, senescence, and damage from other herbivores; 2) relationships between seasonal peaks of aphid infestation and host flowering over two years and across six populations, and 3) factors influencing aphid distribution in a plant population within and across seasons. Aphid infestation did not appear to harm or improve plant performance between 2011 and 2012 in the experiment or in unmanipulated plants. Experimentally manipulated aphid infestation accelerated leaf senescence and decreased foliar herbivore damage. The negative relationship between aphid infestation and foliar herbivore damage in the experiment did not appear in observational data, which showed a positive relationship between aphid abundance and foliar herbivore damage over five years. I found that the peak of winged aphids roughly coincided with peak flowering in multiple populations and years, shifting when a spring burn delayed peak flowering. Aphids dispersed short distances within a population and dispersed further to flowering plants than to non-flowering plants. Aphid distribution in 2012 corresponded to early-season clusters of aphid infestation from 2011, indicating across-year patterns in aphid dispersal. These results suggest that 1) aphid population dynamics are closely tied to host phenology and density, and 2) fragmentation of plant populations may limit dispersal of *A. echinaceae* across prairie remnants, leading to loss of aphids in some populations and heavy aphid infestation in others. Considering *Echinacea* is a long-lived perennial, multiple years of heavy aphid infestation may induce costs that were not apparent in this two-year experiment. In contrast, a loss of aphids within a population may heighten pressure from other herbivores. Opposing results on foliar herbivore damage between the experimental and observational studies suggest that manipulating aphid infestation may create patterns that do not occur when aphids disperse naturally.

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Introduction

Studies using model plant-aphid systems have addressed a variety of questions in ecology and evolutionary biology. These include molecular and physiological responses of plants to aphid feeding (Goggin 2007), tritrophic interactions involving parasitoids or mutualists (Styrsky and Eubanks 2007, Mooney and Singer 2013), and aphid population genetics across host populations (Loxdale et al. 2011). While most of our knowledge of plant-aphid interactions comes from agricultural systems, a growing number of studies are examining non-domesticated plant-aphid systems. One challenge in studying the role of aphids in natural systems is that their influence on plants is complex. Some studies have documented severe aphid-induced reductions in plant growth or fecundity (Foster 1984, Snow and Stanton 1988, Valdivia and Niemeyer 2005), while others have found little or no negative consequences of aphid infestation for host plants (Siquera-Neves et al. 2011, Zhang et al. 2012). The effects of aphids on host plants may vary due to abiotic factors in the environment, such as nutrient availability (Haase et al. 2008), or through biotic interactions with mutualists or other herbivores (Styrsky and Eubanks 2007, Ando and Ohgushi 2008). Aphids may deter other herbivores from host plants by altering plant nutrient quality (Ando and Ohgushi 2008) or by attracting ants that harvest aphid honeydew and aggressively defend aphid colonies (Styrsky and Eubanks 2007; Zhang et al. 2012). Aphids and aphid-tending ants play central role in shaping the community of arthropods that visit host plants, leading some to refer to their relationship as a "keystone interaction" for plant and insect communities (Styrsky and Eubanks 2007).

Aphid population dynamics depend on both individual and population-level characteristics of host plants. Individual plants vary in their resistance to aphid infestation based on their genotype (Johnson 2008, Utsumi et al. 2011, Genung et al. 2012, Moreira and Mooney 2013). At the population level, plants influence aphid population dynamics through phenology

(Mopper and Simberloff 1995), spatial distribution (Elzinga et al. 2005), and genotypic diversity (Utsumi et al. 2011; Moreira and Mooney 2013). In order to adequately address the ecological complexity of plant-herbivore interactions, it is necessary to consider both top-down effects of herbivores on plants and bottom-up effects of plants on herbivores in variety of ecological contexts (Hunter and Price 1992).

Due to their complex web of direct and indirect interactions, plant-aphid systems provide a useful opportunity to study the ecological consequences of land use change for terrestrial communities. Changes in human land use, such as agricultural development, have led to the loss and fragmentation of native habitat throughout the world. A commonly observed consequence of habitat fragmentation is a loss of herbivore species in small or isolated populations—especially among specialist herbivores (Valladares et al. 2012a; Cagnolo 2009). For plants, the loss of specialist herbivores may benefit performance (Elzinga et al. 2005). Considering the protective influence of aphid-tending ants against other herbivores (Styrsky and Eubanks 2007), local extinction of specialist aphid species may augment pressure from generalist herbivores, which are considered to be less susceptible to negative effects of habitat fragmentation (Jonsen and Fahrig 1997, Holt et al. 1999).

This study explores the relationship between a specialist aphid (*Aphis echinaceae*) and a prairie perennial (*Echinacea angustifolia*) that has experienced dramatic reduction and fragmentation in its habitat. Since European settlement, the North American tallgrass prairie has dwindled to less than 1% of its original range (Samson and Knopf 1994) and the remaining prairie persists in small patches varying in size and quality. Habitat fragmentation has altered the population dynamics of *Echinacea* in ways that reduce individual fitness and threaten the persistence of populations (Wagenius 2006, Wagenius and Lyon 2010, Wagenius et al. 2011). In

particular, plants experience declined reproduction due to spatial isolation and divergent flowering phenology within and among populations (Wagenius and Lyon 2010, Ison and Wagenius *in press*). We do not know how changes in plant spatial distribution or phenology influence the population dynamics of *A. echinaceae.* We also do not know to what extent aphid infestation influences host plant fitness. Considering the aphid is a species specialist and is tended by ants, it is unclear whether *A. echinaceae* is harmful to *E. angustifolia*.

The goals of this study are to quantify top-down and bottom-up interactions between *E. angustifolia* and its specialist aphid and to evaluate their importance in natural populations. For top-down interactions, I manipulated aphid infestation and measured effects on plant performance, leaf senescence, and foliar herbivory in an experimental population. In order to evaluate the importance of top-down effects in nature, I compared the experimental results on plant performance and foliar herbivore damage with observational results from nearby plants in the same experimental plot. I also assessed the relationship between naturally-occurring aphid infestation and foliar herbivore damage over five years in the experimental plot and over one year in six remnant populations. For bottom-up effects, I examined the relationship between plant phenology, spatial distribution, and annual flowering status and aphid population dynamics using observational data from the experimental plot and six remnant populations. First, I compared the timing of peak flowering, peak aphid infestation, and the peaks of winged and wingless morphs within an experimental population over two years and across six remnant populations within a single season. Because a spring burn delayed flowering phenology at one site, I was able to observe populations on different flowering schedules within the same year. If aphid phenology depends on flowering phenology, I would expect that the relative position of these peaks would remain consistent over time and across populations. Second, I examined

mechanisms explaining the spatial distribution of aphid infestation within a plant population by quantifying the extent to which a plant's susceptibility to aphid infestation is influenced by isolation from previously-infested plants and by its size and flowering status (Table 1). Lastly, I examined across-year patterns of aphid distribution by investigating the relationship between a plant's likelihood of hosting aphids within a season and its proximity to plants with early-season or late-season infestation in the previous year.

Materials and Methods

Study organisms

The narrow-leaved purple coneflower *Echinacea angustifolia* is native to the North American Great Plains. Due to the large-scale conversion of prairie to agricultural fields, *Echinacea's* habitat is limited to small patches of remnant prairie. While *E. angustifolia* is not classified as threatened or endangered, the severity of its habitat alteration and the popularity of wild-harvested Echinacea make *E. angustifolia* a species of conservation concern.

Echinacea's breeding system is typical of plants adapted to a large, continuous range—i.e. it is strictly self-incompatible and pollinated by native solitary bees (Wagenius 2004). Individual *E. angustifolia* re-sprout annually as rosettes of basal leaves from a single taproot. Flowering occurs initially at least 3 years after germination and then intermittently throughout the individual's lifetime. Plant growth and flowering are determinate within a season and plants rarely produce additional stems, basal leaves, or flowering heads after they emerge in early spring. The aboveground parts of the plant senesce in the fall, with heads becoming crisp and leaves turning yellow or purple before becoming crisp.

The aphid *Aphis echinaceae* Lagos was identified in 2009 from specimens collected in Douglas County, MN (Lagos and Voegtlin 2009). *Aphis echinaceae* is a specialist to *Echinacea*

angustifolia, as confirmed by its inability to survive or reproduce on other *Echinacea* species during a field experiment (Hobbs and Lyon, *unpub. data*). While a variety of arthropods visit *E. angustifolia*—including other sap-feeders—*A. echinaceae* is the most common herbivore observed in the Douglas County area (Ridley et al. 2011, *pers. obs*.). We have observed colonies of aphids, including winged and unwinged morphs, on the leaves, stems, and heads of plants—with aphid abundances frequently exceeding 100 individuals per plant in late summer. *Aphis echinaceae* is tended by a variety of ant species (Table 2), including *Formica obscuripes* and *Lasius alienus.* While ants occasionally tend other sap-feeding insects on *Echinacea*, their presence on plants most closely associates with the presence of *Aphis echianceae* (Ridley et al. 2011, Table 3). Ants sometimes build structures of soil and thatch on the leaves, stems, or heads of aphid-infested plants. These structures occur more frequently when the soil is moist and may function in the cultivation of aphids.

Study Site

All field research took place within Douglas County, MN, an area containing a patchwork of remnant and restored prairie interspersed among corn and soybean fields (Fig. 1). The primary study area is an experimental plot of restored prairie on a former agricultural field (CG). The plot contains *E. angustifolia* planted on an x-y coordinate system of rows spaced by 1m (east-west axis) and positions spaced by 0.5m (north-south axis). Management consists of biyearly fall burns, mowing between rows, and hand broadcasting native grass seed. The study also includes six remnant *Echinacea* populations located within 6 km of the experimental plot. These populations vary in size, the density of flowering plants, and other characteristics (Table 4). The largest is a 40 hectare Nature Conservancy prairie preserve (SPP) that is divided into two management units that are each burned every 5 years. When this research took place, the

east unit (SPPE) had been last burned in 2010 and the west unit (SPPW) had been burned in May 2012. The three smallest sites (NWLF, NNWLF, and NESS) are adjacent to gravel roads and experience frequent disturbance through mowing, road maintenance, and herbicide application. The sites EELR and ALF are medium-sized remnants that each includes a ditch and a hill. Only SPPE and SPPW are under active management.

Field research

Aphid addition and exclusion experiment

I manipulated aphid infestation on plants from a larger common garden experiment within CG (Fig. 1), examining the effects of inter-population breeding on offspring fitness. All plants were derived from populations within Douglas County, though their precise parental identity is unknown. Plants were germinated in controlled conditions and transplanted as seedlings to the study site in 2003. In 2011, I randomly selected 100 non-flowering (basal) plants from this group and divided them into aphid addition and exclusion treatments. I visited each plant once or twice a week to add or exclude aphids between July 21st and September 9th 2011 (12 visits) and June 23rd and September 1st 2012 (12 visits to the addition group, 20 visits to the exclusion group). At the onset of this experiment, I placed wire and mesh cages over all plants to exclude natural enemies. However, these were removed after the first two weeks of the experiment due to a severe wind storm and plants were not covered for the remainder of the study period. To minimize spatial effects on aphid abundance, I randomized the order of rows I visited during the addition treatment. For the exclusion treatment, I visited each plant twice a week to inspect leaves visually and remove aphids by hand into a petri dish. Before adding or removing aphids, I 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. I minimized variation due to observer by conducting all visual estimations of aphid and ant abundance myself.

The addition treatment differed slightly between 2011 and 2012. In 2011, I added 1 adult aphid to every plant on the first visit and subsequently varied the number of aphids I added according to the abundance of aphids present on the plant, adding fewer aphids to plants with higher levels of aphid infestation. I did not add aphids to any plant with >50 aphids after the initial visit. The greatest variation in the number of aphids added occurred on Aug. 17th, when I added between 0 and14 aphids on each plant. Adding a large number of aphids did not cause in a greater increase of aphid abundance than adding a small number of aphids (Fig. S1). In 2012, I simplified the addition treatment by adding 2 adult aphids to the underside of a single leaf during each visit.

I recorded foliar herbivore damage twice annually during the experiment: once while seasonal aphid infestation was relatively high (August 10th, 2011 and July 14th, 2012) and again after aphid abundance had declined for the season (September 4th, 2011 and August 25th, 2012). In 2012, I also recorded foliar herbivore damage on June 24th, before manipulating aphid infestation. I defined foliar herbivore damage as the presence of chew marks or round holes in leaves. While these are not the only types of herbivore damage that occur on *Echinacea*, they are the most unambiguous. In 2012 I assessed the severity of herbivore damage on each plant by recording the number of leaves with chew marks or holes. In 2011, this analysis included the count of leaves with chew marks, but not holes. I assessed the timing of leaf senescence by recording the number of yellow, purple, or crisp leaves present after aphid infestation declined for the season (September 9th, 2011 and August 25th, 2012).

I measured traits of plant performance in July 2011 and 2012. For basal plants, I recorded 3 traits that have been measured annually for each plant since 2003: the number of rosettes, the number of basal leaves, and the length of the longest basal leaf. Because growth is determinate within a year, I measured plant performance based on changes in rosette count, leaf count, and status (flowering and mortality) between 2011 and 2012. For flowering plants, I measured those three traits along with the number of flowering heads, the stem height, and the leaf count on flowering rosettes. Because only 6 out of 100 plants flowered in 2012, my analysis did not include traits specific to flowering plants.

Observational study in experimental plot

In addition to manipulating aphids on plants, I observed patterns of naturally-occurring aphid infestation on different plants within the same 2003 common garden experiment. In the summers of 2011 and 2012, I surveyed a 20 x 20m section of the study area that was 11m from any edge of the experimental plot and did not include any plants in the field experiment. With assistance from a team of observers and recorders, I visited plants every 2 weeks between July 1st and September 10th in 2011 and once a month between June 15th and August 18th, 2012. In order to minimize observer effects, each recorder/observer pair completed a randomized assignment of rows on each survey date. We recorded the abundance of aphids and ants in categories of 0, 1-10, 11-80, and >80 aphids and 1, 2-10, and >10 ants. We also recorded the presence of winged and non-winged morphs and nymphs, the parts of the plant occupied by aphids (leaves, stem, or head), and the presence of ant domatia and non-aphid herbivores. In 2012 we also recorded the presence of chew marks and holes indicative of foliar herbivore damage. The plant traits we measured were identical to those measured in the field experiment.

There were a total of 548 plants measured in 2011 (64 flowering and 484 basal) and 520 plants measured in 2012 (44 flowering and 476 basal).

Observational study in remnant populations

In 2012 I surveyed aphid and ant abundance in six remnant *Echinacea* populations located within 6 km of the experimental plot (Table 4). In order to maximize my ability to compare aphid infestation across sites, I decided to limit my survey to plants that flowered during the current and previous year. This decision was based on a previous study that found lower rates of aphid infestation on basal plants (0.05, n = 212) than flowering plants (0.32, n = 10) in 10 remnant populations (Rath and Dykstra 2010, *unpub. data*). I included flowering plants from the previous year because few plants flowered in 2012. My survey included all flowering plants from 2011 and 2012 in the three smallest populations and a random sample of up to 30 plants from each year in larger populations. I visited each site three times during the summer of 2012: once in early July (Jul. 5th- 7th), once in late July (Jul. 23rd-25th), and once in mid-August (Aug. 16th-18th). Similar to the previous observational study, I recorded the abundance of aphids and ants; the presence of chew marks and holes; the presence of ant domatia and other herbivores; and the presence of winged, non-winged, and juvenile aphids. Although I received assistance in recording data, I conducted all observations myself to minimize observer variation. I measured the count of rosettes and flowering heads to assess plant performance. When multiple ants were present, I collected one or two specimens for identification.

Long-term data—relationship between aphid infestation and foliar herbivore damage

I expanded the results on aphid infestation and foliar herbivore damage from the experiment and observational studies with 5 years of observational data from older plants in the experimental plot. In 1997 Wagenius collected achenes from 14 prairie remnants within 6 km of

the plot, germinated them in controlled conditions, and planted seedlings on a 1x1m grid. Between 2004 and 2008, he collected annual measurements of plant traits, aphid and ant abundances, and foliar herbivore damage (chew marks and holes). The reason I examined foliar herbivore damage in the 1997 cohort instead of the 2003 cohort is that the plants were mature in 2004. Aphid infestation was infrequent among juvenile plants in the 2003 cohort in 2004 (0.01, n = 4060) compared to the 1997 cohort (0.76, n = 398) and varied too little to assess a relationship with foliar herbivore damage.

Analyses

Effects of aphid infestation on plant performance

I examined the relationship between aphid infestation and plant performance in the experiment using a contingency table analysis with categorical predictors of aphid infestation and responses of plant performance. In order to compare experimental results with patterns of naturally-occurring aphid infestation, I repeated this analysis with all basal plants from the observational study in the experimental plot. The contingency table analysis tested the independence of categorical variables of aphid infestation and plant response using χ^2 tests and Fisher's exact tests for tables with any cell values of less than 5. I characterized plant response as the direction of change in basal leaf count, rosette count, and plant status (flowering, basal, dead) between 2011 and 2012. Because flowering represents a gain in plant performance, I characterized plants that transitioned from basal to flowering as having an increase in basal leaf count, even though they may have fewer basal leaves. Similarly, I characterized plants that died as having a decrease in basal leaf count and rosette count. I also examined the relationship between aphid infestation and change in the length of the longest basal leaf using a linear model. In this model, I excluded plants that flowered or died in 2012 because they had no basal leaves. I

characterized aphid infestation in four ways: 1. based on treatment, 2. based on their highest observed aphid abundance in 2011 (in categories of 0, 1-10, 11-80, and >80), 3. based on the presence or absence of aphids in 2011, and 4) based on and the presence or absence of aphids on the first survey in 2011 (observational study only). A gain in leaf count, rosette count, leaf length, or flowering associated with aphid infestation would reflect a potential positive influence of aphids. A significant association between aphid infestation and an increase in mortality or decrease in growth would suggest that aphids harm plant performance. Conversely, an association with an increase in flowering and an increase in growth would suggest a beneficial influence with aphids. However, if large plants are more likely to host aphids, any patterns associated with aphid infestation could be due to plant size. I addressed this possibility by examining changes in plant performance based on a categorical predictor of plant size (1-3, 4-5, and >5 basal leaves). I also included plant size as a covariate in the model of basal leaf length.

Effects of aphid infestation on foliar herbivore damage and senescence

To quantify the association between aphid infestation and damage from other herbivores, I modeled the presence chew marks and holes as a function of aphid infestation, using generalized linear models (GLMs) with a binomial response. I specified a quasibinomial error distribution for all models in which the degrees of freedom substantially exceeded the residual deviance. Due to differing rates of herbivore damage between basal and flowering plants, I excluded flowering plants from the analysis of herbivory and senescence. I also excluded one plant that died in 2011 and four plants that were completely crisp in late-summer 2012. For all datasets, I measured the response of foliar herbivore damage as the presence of chew marks or holes on the plant. In the experiment, I also measured foliar herbivore damage as the proportion of leaves with chew marks and holes (this measurement did not include holes in 2011). The

maximal model in the experiment included treatment, plant size (basal leaf count), and the interaction between treatment and plant size. Because aphid abundance varied within treatments, I created a separate model with highest observed aphid abundance, plant size, and the interaction between aphid abundance and plant size.

In the observational study in the experimental plot, the maximal model included highest observed aphid abundance, plant size (basal leaf count), row, position, and the interaction between aphid abundance and plant size. In the observational study in remnant populations, the maximal model included highest observed aphid abundance, rosette count, site, and interactions between aphid abundance and rosette count and aphid abundance and site. I excluded NNWLF from this model because aboveground parts from the majority of plants were removed during a road maintenance project before I was able to record rosette count.

For the long-term data, I used two statistical approaches to assess the relationship between aphid infestation and the presence of foliar herbivore damage between 2004 and 2008: separate binomial GLMs for each year and a generalized linear mixed effects model (GLMM) for all years (lme4 package in R). The maximal model for each year included aphid abundance, row, position, plant size, and measure date. The maximal model for all years combined included fixed effects of aphid abundance, row, position, plant size, year, and the interaction between plant size and aphid abundance and random effects of individual plants. A negative relationship between aphid infestation and foliar herbivore damage would suggest that aphids deter other herbivores. Conversely, a positive relationship would suggest that similar factors influence a plant's susceptibility to aphids and to foliar herbivores.

I analyzed the relationship between aphid infestation and senescence in the experiment using a binomial GLM to assess the proportion of senesced (yellow, purple, or crisp) vs. green

leaves at the end of the study period. Similar to the analysis of herbivory, this model excluded flowering plants. A greater proportion of senesced leaves among plants in the addition group would suggest that aphids accelerate leaf senescence.

For the experiment, I also assessed the relationship between aphid infestation and foliar herbivore damage and senescence with binomial GLMMs combining responses in 2011 and 2012. All models included fixed effects of treatment (or maximum aphid abundance), plant size (in categories of 1-3, 4-5, and >5 basal leaves), and year and random effects of individual plant. The results for foliar herbivore damage were similar between two-year and single-year models. The two-year model for senescence did not adequately fit the data. The results section presents only the single-year GLMs. Analysis of deviance tables for two-year models are included in the appendix (Table S3A.1).

Model selection

For all statistical models involving multiple predictors (GLM, GLMM, linear models, and POLR), I selected the simplest adequate model using a stepwise backwards selection approach described by Crawley (2008). I began with maximal model containing all predictors and relevant interaction terms and tested importance of each term by removing it and comparing the simpler model to the more complex model. I compared models using analysis of variance for linear models, analysis of deviances for GLMMs and POLRs, and GLMs. I removed predictors in order of their complexity: 3-way interactions, followed by 2-way interactions, followed by single terms. Within each level of complexity, I removed predictors in the order in which they contributed to model variance—i.e. beginning with the predictor that generated the largest ANOVA p-value when removed from the model. The simplest adequate model did not include terms that resulted in p-values >0.1 when removed from the model.

For any model in which experimental treatment or aphid abundance influenced variance foliar herbivore damage or senescence, I tested asymptotic assumptions using bootstrap resampling with 100,000 bootstraps. In general, the bootstrap p-values were similar to those produced by the model, confirming the suitability of the models for explaining variance in the data. I used the statistical software R version 2.1.5.2 for all data analysis.

Phenology of aphid infestation and plant flowering (experimental plot, remnant populations)

My observations of aphid population phenology were limited to June-September 2011 and 2012. Though the fall and winter phases of the aphid life cycle are unknown, I have observed aphids descending below the surface of the soil at the base of plants in late-summer, leading me to believe that aphids lay eggs and over-winter on *Echinacea*'s taproot. I evaluated summer aphid phenology based on seasonal peaks of aphid infestation and incidence of winged and wingless morphs. I defined these peaks as the dates on which I observed the highest proportion of plants with any aphids, winged aphids, or wingless aphids. I defined peak flowering as the day on which the highest proportion of plants showed emerging styles. In 2012, I was not able to observe the peak of aphid infestation due to widespread aphid mortality before my final survey on August 10th. Because of this, I estimated peak levels of aphid infestation as the proportion of plants with live or dead aphid colonies on August 10th. I estimated the peak date of aphid infestation for 2012 as the date on which I observed the highest levels of aphid abundance in the experiment (Jul. 21st, Fig. S2).

I compared the timing of peak flowering and peak aphid infestation by creating graphs showing the timing of each peak and comparing their relative positions over two years in the experimental plot (2011 and 2012) and over one year in six remnant populations (2012). For the remnant populations, I only presented peak flowering dates for the two largest populations: East

Elk Lake Road (EELR) and east and west burn units of the Staffanson Prairie Preserve (SPPE and SPPW). A spring burn in SPPW delayed peak flowering relative to other populations.

Aphid dispersal (experimental plot)

In order to explore mechanisms explaining variation in aphid infestation within a population, I tested factors contributing to patterns of aphid dispersal within and across seasons in the 20x20m survey area in the experimental plot (Table 1). I used binomial GLMs to model a plant's likelihood of hosting aphids based on its status (***S***) and isolation (***I***) from plants infested earlier in the season (2011 and 2012) or in the previous year (2012 only). I modeled aphid infestation at four time periods: any time during the summers of 2011 and 2012, during peak aphid infestation in 2011, and during the peak of winged morphs in 2011. I did not include within-year peaks for 2012 because the timing of my survey missed peak aphid infestation. I defined plant status (***S***) a category of plant size and flowering status: flowering, or basal with 1-3, 4-5, or >5 leaves. For within-season dispersal I quantified ***I*** as the distance (m) to the k^{th} nearest plant ($k < 4$) that hosted aphids earlier in the season (Jul. 1^{st}, 2011; Jul. 12^{th}, 2012). For across-season dispersal, I quantified ***I*** as the distance (m) to the k^{th} nearest neighbor ($k < 4$) with early or late-season aphid infestation in 2011 (Jul. 1^{st} or Sep. 8^{th}). All models included ***I, S,*** and the interaction between ***I*** and ***S.*** For within-season dispersal, I also included a term for plant clustering (***P***) and the interaction between ***S*** and ***P*** to account for variation in plant density throughout the experimental plot. I quantified ***P*** as the distance (m) to the k^{th} nearest plant—including plants outside the 20x20m survey area ($k < 40$). Setting isolation as $1 < k < 4$ for ***I*** and $6 < k < 40$ for ***P*** did not qualitatively change the results. I chose to present results based on the 3^{rd} nearest infested neighbor (***I***) and the 14^{th} nearest plant (***P***). One caveat to the results on

dispersal is that they do not include distance to previously infested plants outside the 20x20m survey area, since I did not observe early or late season aphid infestation outside this area.

Results

Effects of aphid infestation on plant performance

Overall, aphid infestation was not associated with declines in plant performance between 2011 and 2012. However, one out of 12 tests in the experiment and three out of 12 tests in the observational study found an association between aphid infestation and gains in plant performance (Table 5a,b). The aphid addition and exclusion treatments were effective in creating differences in aphid abundance between treatment groups in both years (Fig. 2). In the experiment, there was a slight association between aphid infestation and change in basal leaf count (Table 5a). Of the plants that hosted aphids in 2011, 41 out of 63 (65%) increased in basal leaf count, compared to 15 out of 37 (41%) plants with no aphids ($p = 0.055$, $\chi^2 = 5.8$, $df = 2$). However, there was no significant association between treatment, highest observed aphid abundance, or aphid presence and changes in rosette count, plant status, or length of the longest basal leaf (Table 5a).

In the observational study in the experimental plot, naturally-occurring aphid infestation and change in plant status were generally positively associated. Plants that hosted aphids during the study period were more likely to flower in 2012 (19 out of 189, 10%) than plants that did not host aphids (9 out of 295, 3%) ($p = 0.0003$, $\chi^2 = 11.9$, $df = 2$). Of these, plants that hosted a medium abundance of aphids (11-80) were the most likely to flower (12 out of 75, 16%), followed by plants with >80 aphids (5 out of 54, 9%), followed by plants with 1-10 aphids (2 out of 54, 4%) ($p = 0.004$, Fisher Test, Table 5b). Plant size was positively associated with the transition to flowering (Table 5b) and with aphid abundance (Table 6). Large plants (> 5 basal

leaves) were more likely to flower in 2012 (21 out of 113, 19%) than medium (5 out of 173, 3%) or small basal plants (2 out of 198, 1%) ($p < 0.0001$, Fisher test). Large plants were also more likely to host a high abundance of aphids (> 80 aphids) (28 out of 113, 25%) than medium plants (18 out of 173, 10%) or small plants (8 out of 198, 4%) plants ($p < 0.001$, $\chi^2 = 36.2$, df = 6). Contingency tables and model selection results for all tests of the relationship between aphid infestation and plant performance, along with results tables of the relationship between plant size and plant performance, are included in the Appendix (Tables S1 and S2).

Effects of aphid infestation on foliar herbivore damage and senescence

Aphid infestation and foliar herbivore damage were negatively associated in the experiment. The proportion of plants with chew marks or holes in late-summer was higher in the exclusion group than the addition group by 30% in 2011 and 9% in 2012 (2011, $p = 0.04$, $n = 99$; 2012, $p = 0.13$, $n = 83$; GLM quasibinomial). The proportion of chewed leaves per plant was higher in the exclusion group than the addition group by 80% in 2011 and 21% 2012 (2011, $p = 0.05$, $n = 99$; 2012, $p = 0.16$, $n = 83$; GLM quasibinomial) (Fig. 3). The relationship between aphid infestation and foliar herbivore damage in 2011 was weaker during mid-season when aphid abundances were high ($p = 0.075$, $n = 99$, GLM quasibinomial). Aphid abundance also had a negative relationship with foliar herbivore damage in 2011 ($p = 0.03$, GLM quasibinomial): the rate of foliar herbivore damage was 42% lower on plants with >80 aphids than on plants with no aphids during the summer of 2011 (Fig. 4). The relationship between aphid infestation and the presence of chew marks and holes was not significant in the observational study in the experimental plot ($p = 0.85$, $n= 472$, GLM binomial, Table S3B) or in remnant populations ($p = 0.09$, $n = 209$, GLM binomial, Table S3C) in 2012.

The relationship between aphid abundance and foliar herbivore damage in the long-term data was the opposite of that in the experiment. During 2004 and 2008 the rate of foliar herbivore damage was 19-38% higher on plants with >80 aphids than on plants with no aphids ($p < 0.0001$, $n = 248$-345, GLMM). While the strength of this relationship varied in individual year models, the positive relationship was present in 3 out of 5 years (Fig. 5; Table S3D).

Plant size had a positive relationship with the rate of foliar herbivore damage in the experiment ($p = 0.032$ in 2011), both observational studies ($p < 0.0001$ in the experimental plot in 2012, $p = 0.067$ in remnants), and the long-term data ($p < 0.0001$) (Table S3). Generally large plants were more likely to have foliar herbivore damage, and were also more likely to host aphids (Table 6). The relationship between plant size and aphid abundance was not present in the experiment (Table 6).

Adding aphids accelerated leaf senescence in 2012. While the addition group had four plants that were completely crisp by late summer, the exclusion group had no crisp plants in 2012. The proportion of senesced leaves per plant was greater in the addition group than the exclusion group by 5 percentage points in 2011 and 14 percentage points in 2012 (2011, $p = 0.16$, $n = 100$; 2012, $p = 0.0003$, $n = 87$; GLM quasibinomial, Table S4) (Fig. 6).

Aphid population phenology vs. plant flowering phenology

Comparing dates of peak flowering, peak aphid infestation, and the peaks of winged and wingless morphs in multiple years and sites yielded the following observations:

1. Winged morphs peaked occurred earlier than the overall peak of aphid infestation.
2. The peak of winged morphs matched the overall peak of aphid infestation.
3. Aphid infestation peaked later than peak flowering.
4. The peak of winged morphs roughly coincided with peak flowering.

These patterns were consistent across both years in the experimental plot (Fig. 7) and across all remnant populations except NNWLF, which had no aphids in 2012 (Fig. 8). The site SPPW, which experienced delayed flowering (Jul. 14th) relative to other sites (Jul. 1st in EELR, Jul. 2nd in SPPE), also experienced a delay in the peak of winged morphs (Fig. 8). The timing of peak aphid infestation varied across years in the experimental plot, occurring approximately 3 weeks earlier in 2012 (Jul. 21st, Fig. S1) than in 2011 (Aug. 12th) (Fig. 8).

Dispersal of aphid infestation in the experimental plot

The models of within-season aphid dispersal support the scenarios that 1) aphids disperse short distances, 2) plant status influences susceptibility to aphid infestation, and 3) aphid dispersal distance varies by plant status (scenarios 4 and 5 from Table 1). A plant's likelihood of hosting aphids during the summers of 2011 and 2012 was influenced by its size and flowering status (2011 and 2012, $p < 0.0001$), the distance to its 3rd nearest previously infested neighbor (2011 and 2012, $p < 0.0001$), and the interaction between these two terms (2011, $p = 0.02$, $n = 499$; 2012, $p = 0.05$, $n = 419$; binomial GLM; Table S5.A,B). A plant's likelihood of hosting aphids was not influenced by plant clustering (2011, $p = 0.17$; 2012, $p = 0.41$) or the interaction between plant clustering and plant size and flowering status (2011, $p = 0.39$, 2012, $p = 0.84$). The observed rate of aphid infestation was highest among flowering plants (0.88 in 2011; 0.75 in 2012), followed by large basal plants (>5 leaves, 0.57 in 2011; 0.55 in 2012), medium basal plants (4-5 leaves, 0.35 in 2011; 0.32 in 2012), and small basal plants (1-3 leaves, 0.32 in 2011; 0.19 in 2012). The predicted rate of aphid infestation during the summer of 2011 dropped by 50% for flowering plants, 98% for large basal plants, 100% for medium and small basal plants between 1m and 9m from the 3rd nearest previously infested neighbor (Fig. 9a). During the summer of 2012, this drop was 92% for flowering plants, 93% for large basal plants, 40% for

medium basal plants, and 100 % for small basal plants (Fig. 9b). In 2011, the predicted difference in aphid infestation between basal and flowering plants was more pronounced at intermediate distances from previously infested plants (18 percentage points at 5m) than in clusters near previously infested hosts (8 percentage points at 1m). While these results reflect combined aphid infestation for the entire summer, I found similar patterns during peak aphid infestation and the peak of winged morphs in 2011 (Table S5.E,F).

A plant's likelihood of hosting aphids during the summer of 2012 was influenced by the distance to its 3rd nearest infested neighbor with early-or late-season aphid infestation in 2011 (early-season, $p < 0.0001$, $n = 477$; late-season, $p = 0.009$, $n = 459$; GLM binomial). Rates of aphid infestation declined between 1m and 11m from the 3rd nearest neighbor with aphid infestation in the previous year—more so for neighbors with early-season aphid infestation (~90%) than for neighbors with late-season aphid infestation (~37%) (Fig. 9c,d). This indicates that the distribution of aphid infestation in 2012 clustered more closely around plants with early-season aphid-infestation in 2011 than around plants with late-season aphid infestation in 2011.

Discussion

I found strong evidence that plant phenology, spatial distribution, and demography influence aphid population dynamics from the bottom up. Aphid population phenology followed a consistent seasonal schedule consisting of an early-season peak of winged morphs followed by a mid-season peak in overall aphid infestation. The peak of winged morphs roughly corresponded to peak flowering of the host. When a spring burn shifted peak flowering in one population, the peak of winged morphs also shifted relative to unburned populations. This suggests that plant phenology plays a greater role in the production of winged morphs than seasonal abiotic conditions, such as temperature or photoperiod.

Aphid infestation was not uniformly distributed among plants within the experimental plot. Overall, aphid infestation was more frequent on flowering plants than on basal plants and more frequent on large basal plants than small basal plants. Aphid infestation had a clustered distribution within a season; plants located near early-season infested plants were more likely to host aphids during the summer than those located farther away, regardless of their size or flowering status. However, further away from initial clusters of aphid infestation, flowering plants were more likely to host aphids than basal plants, suggesting that aphids disperse longer distances to flowering plants (scenarios 4 and 5, Table 1). I also found that plants located near plants with early or late-season aphid infestation in 2011 were more likely to host aphids during the summer of 2012 than those located further away. This relationship was stronger for neighbors infested in early 2011 than for neighbors infested later in 2011. This suggests that patterns of aphid infestation within a season may depend on patterns from previous years.

I did not find strong evidence that aphids influence individual plant performance from the top-down. Neither manipulated nor naturally-occurring aphid infestation led to increased mortality or decreased plant size from 2011 to 2012. In contrast, manipulated aphid infestation was weakly associated with a gain in basal leaf count and naturally-occurring aphid infestation was strongly associated with flowering and increasing length of the longest basal leaf. While the combined experimental and observational results suggest a potential benefit of aphid infestation, it is important to note that naturally-occurring aphid abundance increased with plant size and that large plants were more likely to flower than smaller plants. Therefore, the association between naturally-occurring aphid infestation and gains in flowering likely resulted from plant size, rather than a beneficial influence of aphids. Considering *Echinacea* is a long-lived perennial, it is possible that negative effects of aphids accumulate over multiple years. Host fitness measured

after the second year of aphid addition and exclusion in 2012 may reveal effects that were not apparent after the first year.

Manipulated aphid infestation appeared to decrease foliar herbivore damage and to accelerate leaf senescence. In 2011, there were a greater proportion of damaged plants and damaged leaves per plant in the exclusion treatment than in the addition treatment. In 2012, the proportion of senesced leaves per plant was greater in the addition treatment than in the exclusion treatment. However, the opposite relationship occurred between aphid infestation and foliar herbivore damage in unmanipulated plants. An older cohort from the same experimental plot (1997; Fig.1) showed a positive association between aphid infestation and foliar herbivore damage over five years. One likely reason for the opposing experimental and observational results is that the aphid addition and exclusion treatments created patterns of aphid infestation that would not occur with natural dispersal. When aphids dispersed naturally in the experimental plot, they spread in a cluster and were more likely to land on large plants. The experiment controlled for plant size and location by randomly assigning plants into the aphid addition and exclusion treatments. The negative relationship between aphid infestation and foliar herbivore damage in the experiment suggests that aphids deterred other herbivores. However, factors that shape a plant's susceptibility to aphid infestation—e.g. plant size and location—may play a more important role in foliar herbivore damage than any deterring influence of aphids.

Top-down influence of aphids on plants:

The lack of any relationship between aphid infestation and a decline in plant performance suggest that *Aphis echinaceae* does not cause serious harm to host plants. The negative association between aphid infestation and foliar herbivore damage in the experiment indicates that the cost of aphid infestation may be balanced by reduced damage from other herbivores.

This is consistent with results highlighted in a recent 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 (Siquera-Neves et al. 2011). This study also found that aphids and ants reduced the abundance of other herbivores and that their effects were additive. Because ants commonly occurred with aphids in the experimental plot, my results reflect the net influence of aphids and aphid-tending ants. In order to separate effects of aphids and ants, it would be necessary to conduct an experiment with a crossed design that manipulated both aphids and ants. However, a treatment that included aphids and excluded ants would not represent a realistic scenario for *A. echinaceae*, since aphids typically co-occur with ants.

It is important to note that the experiment and long-term observational data showed an opposing relationship between aphid infestation and foliar herbivore damage—negative in the experiment and positive in the observational data. This suggests that characteristics of the host plant play a more important role in shaping a plant's susceptibility to herbivores than any top-down influence of aphids or aphid-tending ants. Relatively few manipulative experiments on aphid-plant interactions have compared manipulative experiments with results from naturally-occurring aphid infestation (e.g. Foster 1984, Kobayashi et al. 2008). While manipulative experiments like Siquera-Neves et al. (2011) have found compelling evidence that aphids and ants deter other herbivores, experiments alone cannot reveal whether such mechanisms play an important role in natural populations. The difference between my experimental and observational results suggests that manipulative experiments are fundamentally limited in their ability to

estimate the influence of aphid-ant interactions on herbivore behavior and plant performance in natural populations.

The higher rate of leaf senescence in the aphid addition treatment suggests that *A. echinaceae* induces premature senescence in *E. angustifolia*. While the premature senescence may reflect mortality due to aphid stress, it may also represent an induced defense that protects plants against herbivore damage. A study on *Arabidopsis thaliana* found that the green peach aphid (*Myzus persicae*) induces premature senescence by increasing expression of senescence genes in host plants (Pegadaraju et al. 2005). The genes associated with premature senescence also increased plants' resistance to aphids and mitigated losses in seed production due to aphid feeding. This suggests that premature senescence in *Arabidops*is an adaptive response protecting host plants against aphid damage (Pegadaraju et al. 2005). In *E. angustifolia* induced defenses may explain the rapid decline of aphid infestation that occurs in late-summer. A large body of research has examined mechanisms of herbivore-induced plant defenses—particularly responses of plants to different herbivore types (Agrawal and Karban 1999, Utsumi 2011). There is strong evidence that plants respond differently to different herbivore feeding guilds (e.g. chewing vs. sap-feeding insects) and lesser evidence that the degree of herbivore specialization influences plant response (Ali and Agrawal 2012). Considering *Aphis echinaceae* is a specialist sap-feeder, it likely induces different responses in host plants than chewing herbivores or less specialized sap-feeders.

I found striking differences in aphid infestation among the six remnant populations I surveyed in the summer of 2012—with heavy infestation in EELR and SPPW, moderate infestation in ALF and SPPE, and little or no aphid infestation in NNWLF or NESS (Fig. 10). Annual observations of flowering plants showed consistent differences in relative rates of aphid

infestation within these populations from year to year (Fig. S4). EELR maintained high levels of aphid infestation relative to other sites, while NNWLF showed little or no aphid infestation in the past three years. The low level of aphid infestation in NNWLF suggests that dispersal to this population is limited. For heavily infested populations, repeated aphid infestation may lead to fitness costs that were not apparent over the two-year manipulative experiment. However, if aphids deter other herbivores, the loss of aphids in a plant population may mean greater pressure from generalist herbivores, which are thought to impose greater harm on host plants than specialists (Zvereva et al. 2010).

Bottom-up influence of plants on aphid population dynamics

Based on the observation that abundances of winged aphids peaked occurred earlier than overall aphid infestation in two seasons, it is clear that *Aphis echinaceae* follows a seasonal schedule in the production of winged morphs. Winged morph production in aphids is phenotypically plastic and depends on a combination of genetic, biotic, and abiotic factors (Braendle et al. 2006). It is common for aphid species to produce winged morphs on a seasonal schedule. For instance, some species have an early-season peak of winged morphs as aphids disperse, then a late-season peak of winged sexual morphs as aphids undergo a generation of sexual reproduction (Dixon 1998). The factors influencing winged morph production vary among species. Mehparvar et al. (2013) compared density-dependent and density-independent factors influencing winged morph production in two species of specialist Tansy aphids, one of which is tended by ants. They found that density-independent factors, such as seasonal and generational timing, were important for both species, However, only the non-ant-tended species responded to density-dependent factors (crowding and predator presence) in the production of winged morphs (Mehparvar et al. 2013). The relative timing of peaks in winged morphs and

flowering phenology across years and sites suggest that density-independent factors of seasonal timing and plant phenology play influence the production of winged morphs in *Aphis echinaceae.* It is unclear to what extent density-dependent factors play a role in winged morph production.

The observation that flowering plants were more likely than basal plants to host aphids further away from initial clusters of aphid infestation suggests that aphids disperse longer distances to flowering plants. This may explain why aphid infestation was rare on basal plants compared to flowering plants in a 2010 survey of 10 remnant populations (Rath and Dysktra, *unpub. data*). It is likely that the presence of flowering heads helps winged morphs to disperse to new hosts. I often observed winged aphids on the undersides of flowering heads in the experimental plot and repeatedly removed them during my exclusion treatments in 2012. Considering burning promotes flowering in *Echinacea* (Wagenius *pers. obs.*), managed burns may support aphid populations by facilitating their dispersal within and among populations.

Plants were more likely to host aphids in the summer of 2012 if they were located near plants infested in the previous year. This relationship was stronger for proximity to plants infested early in 2011 than late in 2011. One potential explanation for this pattern is that aphid infestation begins in highly susceptible plants and spreads to other plants. I did not find differences in aphid susceptibility among plants in the aphid addition experiment (Fig. S5, Table S6). However, a previous study in the experimental plot found differences in aphid infestation among three genetic cross-types: progeny of full or half-siblings (I), progeny of parents from the same remnant population (W), and progeny of parents originating from different remnant populations (B). While early-season aphid infestation was nearly twice as frequent on B and I offspring as on W offspring, these differences went away in mid-summer (Ridley et al. 2011).

Foliar herbivore damage showed a similar relationship to genotype, with a greater occurrence of chew marks and holes among B and I offspring than W offspring. These results suggest that genotype plays an important role in *Echinacea's* susceptibility to aphid infestation and foliar herbivore damage. However, any genotype-based differences in aphid susceptibility were obscured as aphids dispersed throughout the experimental plot and aphid abundances became too high to capture variation based on our categorical measure. Therefore, any attempt to characterize genotype-based differences in aphid susceptibility in *Echinacea* should consider early-season aphid infestation over multiple years.

While host genotype may explain differences in aphid infestation among individual plants, it does not account for patterns of aphid infestation within populations. Studies in other Asteraceae systems have found that the genetic diversity of host populations plays an important role in aphid population dynamics. Two recent field experiments found that aphid abundance was greater in mixed-genotype plots than in single-genotype-plots of *Solidago altissima* (Utsumi et al. 2011) and *Baccharis salicifolia* (Moreira and Mooney 2013). Utsumi et al. also found that the difference in abundance between mixed-and single-genotype plots was greater than expected based on individual genotypes. They proposed that aphids spread from highly susceptible genotypes (sources) to less susceptible genotypes (sinks), thus raising the level of aphid infestation in the population beyond what would be predicted from each plant's genetic susceptibility. Ridley's observation that genotype-based differences in aphid infestation disappear in mid-summer, combined with my observation that aphid infestation clustered around early-season-infested plants, suggests similar source-sink dynamics in *Echinacea*.

My observation that aphid infestation clusters around plants infested early in the season suggests that aphids disperse short distances within a plant population. Because these results

come from an experimental plot with plentiful host plants, they may not accurately reflect dispersal patterns in remnant populations, where host plants may be sparse. While it is likely that aphids fly between nearby remnant populations, we do not know how far aphids disperse across the landscape or to what extent dispersal contributes to the genetic structure of aphid populations. Another study of specialist aphids on Tansy (Asteraceae) found a high degree of genetic heterogeneity among subpopulations within several kilometers (Loxdale et al. 2011), which is similar to the scale of *Echinacea* populations in Douglas County. The level of genetic heterogeneity on this scale for Tansy aphids was similar to worldwide genetic heterogeneity for the generalist crop aphid *Myzus persicae*. This study points to an essential difference between specialist and generalist herbivores that may influence their sensitivity to habitat fragmentation: due to their narrow range of hosts, specialists carry a greater risk in long-distance dispersal and may be more "reluctant to fly" than generalists (Loxdale et al. 2011). While the specialization-disturbance hypothesis predicts that ecological specialists are more prone to negative effects of habitat disturbance than generalists due to loss of suitable hosts (Holt et al. 2012), dispersal behavior may also contribute to the loss of specialist species in fragmented ecosystems.

Conservation implications

Most studies on plant-herbivore interactions take place in an agricultural or theoretical context (Mooney and Singer 2013 in Press). Considering that trophic interactions are crucial for maintaining the productivity, stability, and biodiversity of communities (Worm and Duffy 2003), studies on plant-herbivore interactions have an important place in conservation research. Restoration ecology recognizes the importance of plant diversity for promoting pollinator communities (Menz et al. 2011). Prairie restorations in West-Central Minnesota often omit the native *E. angustifolia* in favor of non-native *Echinacea* species, such as *E. pallida* and *E.*

purpurea (Wagenius, *pers. obs.*). Since *Echinacea* is pollinated by generalist bees, substituting non-native *Echinacea* species may promote the same pollinators; but it excludes *Aphis echinaceae.* Ant-aphid interactions are considered a keystone mutualism for arthropod communities and host plants (Styrsky and Eubanks 2007) and they may provide a valuable ecosystem service to plants by reducing damage from generalist herbivores. Considering Asteraceae hosts the largest number of aphid species of any plant family—including many species-specialists (Peccoud et al. 2010)—restorations should take care to include a high diversity of forb species native to the restored area in order to promote the diversity of specialist herbivores.

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Table 1: Scenarios describe potential mechanisms explaining variation in aphid infestation among plants in the experimental plot. I tested each scenario with binomial GLMs of the presence or absence of aphids on plant during a given time period as a function of isolation from previously infested plants (***I***) (k = 3rd nearest neighbor), a category combining plant size and flowering status (***S***) (flowering or basal with 1-3, 4-5, or >5 leaves), and the interaction between these two terms (***I X S***).

	Model terms ($p<0.05$)		
Scenarios	***I***, isolation from previously-infested neighbors	***S***, host status	***I X S***
1. Long-distance dispersal, plants statuses equally susceptible	Yes	No	No
2. Short-distance dispersal, plant status influences susceptibility	Yes	Yes	No
3. Short-distance dispersal, plants statuses equally susceptible	No	No	No
4. Short-distance dispersal, plant status influences susceptibility	No	Yes	No
5. Short-distance dispersal, dispersal distance varies by plant status	No	Yes	Yes

Table 2: Ant species observed tending aphids in 2012

All ant species were collected from aphid-infested plants between July and August 2012 from six remnant populations (Fig. 1).

Species	*No. collected*	*Sites*
Lasius alienus	38	ALF, EELR, NESS, SPPE, SPPW
Formica obscuripes	24	ALF, EELR, NWLF
Myrmica spp. 3	6	ALF, SPPW
Myrmica spp. 2	2	EELR
Myrmica spp. 1	1	SPPW
Formica subsericea	1	EELR
Brachymyrmex depilis	1	ALF

Table 3: Co-occurrence of aphids and ants in the experiment

Cell values represent the percentage of plants with or without aphids that did or did not harbor ants in the manipulative field experiment. The range of values represents all visits in 2011 (n = 100) and 2012 (n = 97). The co-occurrence of aphids and ants was significant on all visit dates at $p < 0.01$ (Fisher test).

	ants absent	*ants present*
aphids absent	95-100%	0-5%
aphids present	7-77%	23-93%

Table 4: Description of remnant populations

In the summer of 2012 I surveyed aphid infestation on flowering plants from the current and previous year, some of which were basal in 2012. At the Staffanson Prairie Preserve and East of Elk Lake Road, my observations were limited to a random sample of up to 60 plants. Precise GPS coordinates of all flowering plants at each site were recorded with a TopCon GRS-1 as part of an annual census. At the Staffanson Prairie preserve, this census is limited to a 600m long, 5m-wide transect extending east to west. Locations of each site are presented in Fig. 1.

Site name	Abbr.	# plants observed in 2012 basal / flowering	Total # flowering plants at site 2011 / 2012	Density of flowering plants † 2011 / 2012	description
Nessman's	NESS	2 / 4	3 / 8	4m */ 4m	unmanaged roadside remnant (ditch)
Northwest of Landfill	NWLF	9 / 10	10 / 18	15m / 9m	unmanaged roadside remnant (ditch)
North-Northwest of Landfill	NNWLF	3 / 6	6 / 7	8m / 5m	unmanaged roadside remnant (ditch)
Around Landfill	ALF	24 / 40	51 / 103	17m / 10m	unmanaged roadside remnant (ditch and hill)
East Elk Lake Road	EELR	23 / 37	44 / 56	10m / 5m	unmanaged roadside remnant (ditch and hill)
Staffanson East Unit	SPPE	11 / 17	38 / 59	15m / 5m	half of a 96 acre prairie preserve; burned in 2010
Staffanson West Unit	SPPW	8 / 32	69 / 234	7m / 3m	half of a 96 acre prairie preserve; burned in 2012

† Mean distance (m) to the 3rd nearest flowering plant.

* Because there were 3 flowering plants in 2011, this value represents the mean distance to the 2nd nearest flowering plant.

Table 5: Relationship between aphid infestation and plant performance

P-values give the results of statistical tests of the relationship between predictors of aphid infestation or plant size and responses of plant performance in the manipulative experiment (**a**) and the observational study in the experimental plot (**b**). Aphid infestation was defined based on addition or exclusion treatment, highest observed aphid abundance (in categories of 0, 1-10, 11-80, and >80), presence of aphids at any time during the study, and presence of aphids at the beginning of the study (observational study only). Plant performance was defined based on change in basal leaf count, rosette count, status, and length of the longest basal leaf. With the exception of leaf length, which was the numeric difference between the length of the longest basal leaf in 2012 and 2011, plant responses were defined categorically based on whether they increased, decreased, or remained the same between 2011 and 2012. Plants that flowered in 2012 were categorized as having an increase in basal leaf count and plants that died were categorized as having a decrease in basal leaf count and rosette count. Fisher tests were performed for all contingency tables with cell values of less than 5. Up arrows refer to a significant association between aphid infestation or plant size and a gain in plant performance. The boxes with both an up and a down arrow indicate that large plants were more likely increase or decrease in rosette and leaf count. Grey boxes signify no significant association with plant performance. Contingency tables for basal leaf count, rosette count, and status and ANOVA results tables for leaf length are included in the Appendix (Table S1)

a. Experiment (n = 100)

Predictor	n	Δ Basal leaf count	ΔRosette count	ΔLeaf length (cm)	ΔStatus
Treatment	addition (n = 50) exclusion (n = 50)	p = 0.63 χ^2 test	p = 1 χ^2 test	p = 0.47 linear model	p = 0.64 Fisher test
Maximum aphid abundance	0 (n = 37) 1-10 (n = 16) 11-80 (n = 34) >80 (n = 13)	p = 0.35 Fisher test	p = 0.84 Fisher test	p = 0.83 linear model	p = 0.80 Fisher test
Aphid presence (overall)	present (n = 63) absent (n = 37)	**p = 0.06** χ^2 test ↑	p = 0.31 Fisher test	p = 0.4 linear model	p = 0.17 Fisher test
Plant size	1-3 lvs (n = 34) 4-5 lvs (n = 28) >5 lvs (n = 38)	p = 0.55 Fisher test	**p = 0.02 *** Fisher test ↑↓	p = 0.52 linear model	**p = 0.01 *** Fisher test ↑

b. Observational data (experimental plot, n=484)

Sample size refers to plants that were basal in 2011.

Predictor	*n*	ΔBasal leaf count	ΔRosette count	ΔLeaf length	ΔStatus
Maximum aphid abundance	0 (n = 301) 1-10 (n = 54) 11-80 (n = 75) >80 (n = 54)	p = 0.45 χ^2 test	p = 0.89 χ^2 test	p = 0.2 linear model	**p = 0.004**** Fisher test ↑
aphid presence (overall)	present (n = 189) absent (n = 295)	p = 0.31 χ^2 test	p = 0.49 χi^2 test	**p = 0.05*** linear model ↑	**p = 0.003**** χ^2 test ↑
aphid presence (early-season)	present (n = 35) absent(n = 449)	p = 0.10 χ^2 test	p = 0.10 Fisher test	p = 0.11 linear model	p = 0.41 Fisher test
Plant size	1-3 lvs (n = 198) 4-5 lvs (n = 173) >5 lvs (n = 113)	**p = 0.001**** χ^2 test ↑↓	**p < 0.0001***** χ^2 test ↓↑	p = 0.96 linear model	**p < 0.0001***** Fisher test ↑

Table 6: Contingency table of the association between plant size and aphid abundance
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 observational study in the experimental plot **(a)**, but not in the experiment **(b)**.

a. Observational study (experimental plot) $p < 0.001$, χ^2 test.

plant size	*0 aphids*	*1-10 aphids*	*11-80 aphids*	*>80 aphids*	*n*
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: p = 0.45, Fisher test

plant size	*0 aphids*	*1-10 aphids*	*11-80 aphids*	*>80 aphids*	*n*
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	0.80	38

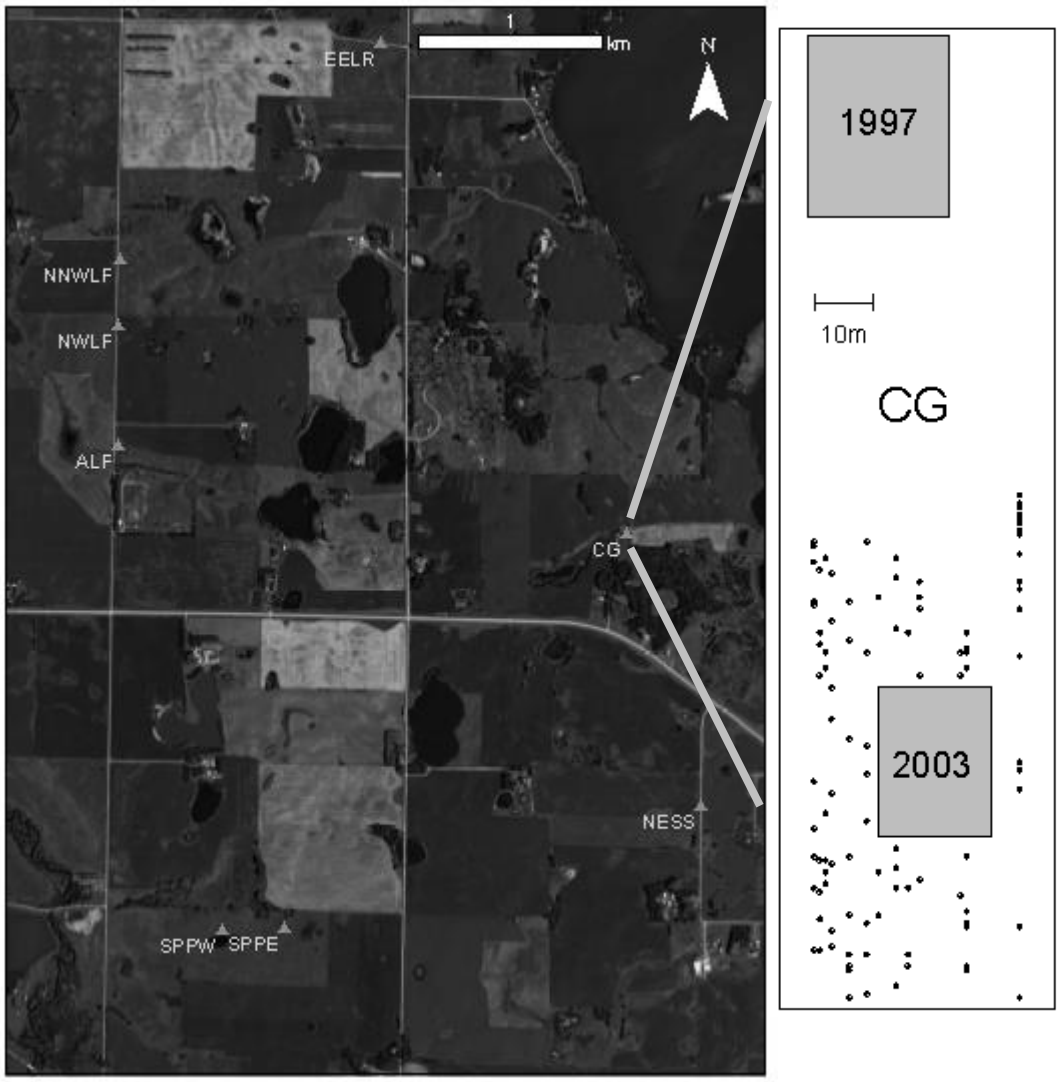


Figure 1: Map of study area

Triangles represent approximate locations the 6 remnant populations and experimental plot (CG) in Douglas County, MN. Black dots within the experimental plot indicate plants in the manipulative field experiment (n = 100) and boxes 2003 and 1997 indicate locations of the two observational studies. I observed aphid infestation on the 2003 cohort in the summers of 2011 and 2012 (n = 548 in 2011) and analyzed observational data from the 1997 cohort from 2004 to 2008 (n = 398 in 2004).

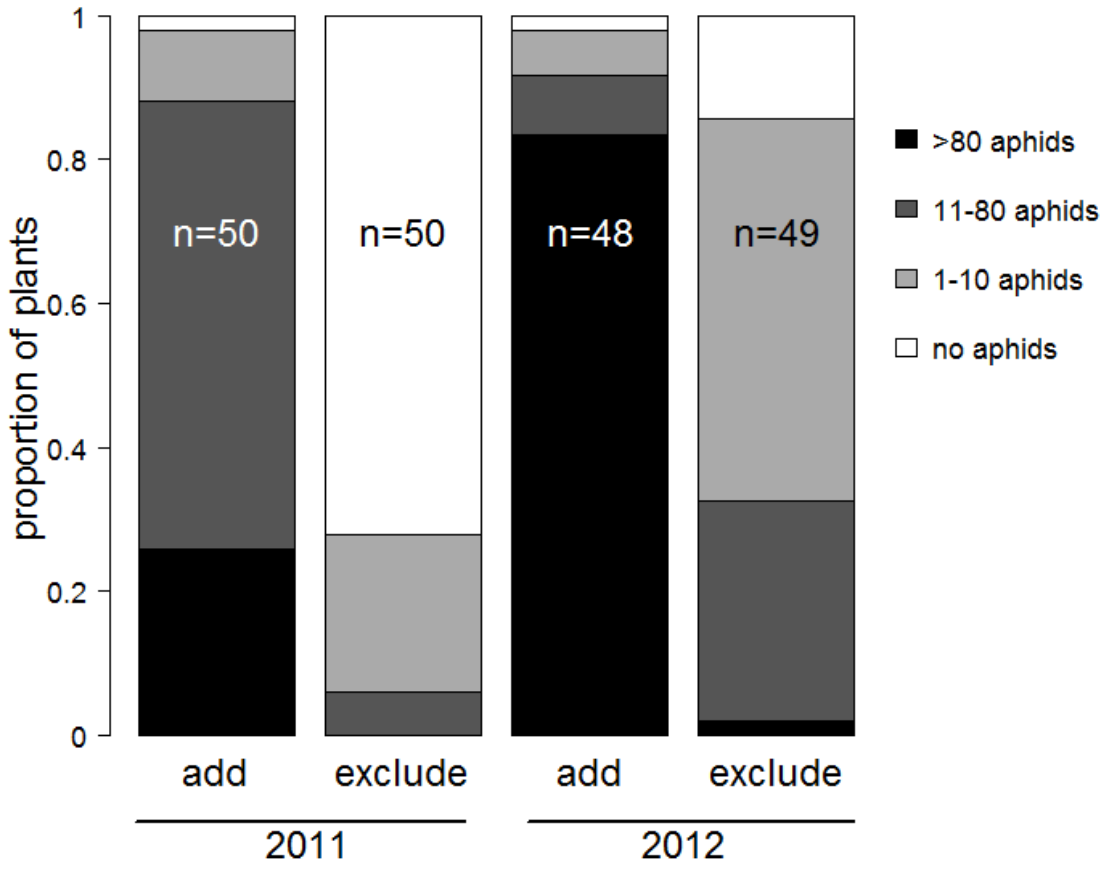
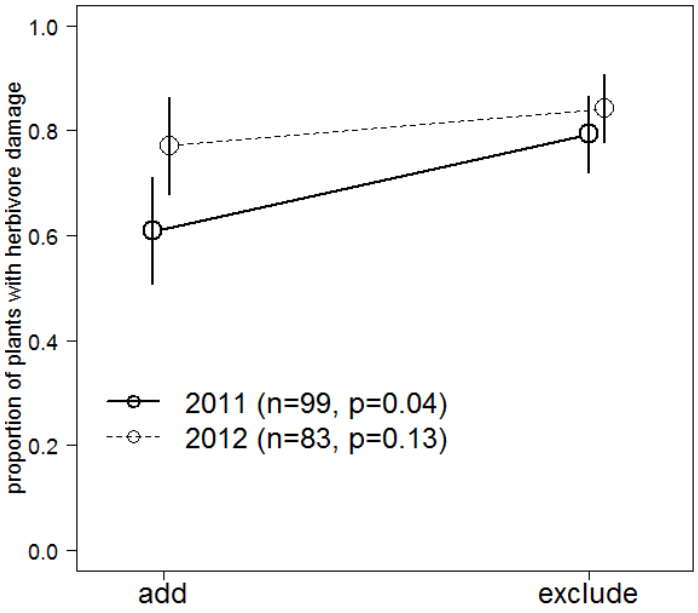


Figure 2: Effectiveness of addition and exclusion treatments in 2011 and 2012

Add and exclude refer to the aphid addition and exclusion treatments. Abundance categories (>80, 11-80, 1-10, no aphids) refer to the highest recorded aphid abundance for each plant in the summers of 2011 and 2012.

a.



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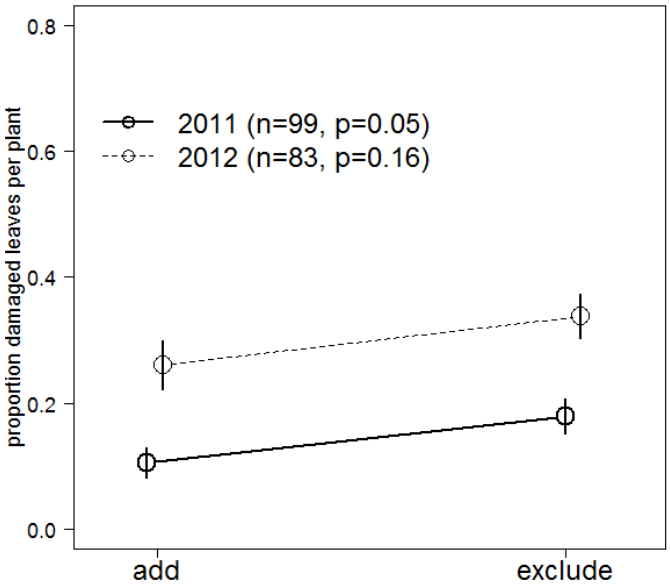

Figure 3: Differences in the occurrence and severity of foliar herbivore between the aphid addition and exclusion treatments in 2011 and 2012.

Herbivore damage was recorded after the decline of aphid infestation (Sep. 4, 2011 and Aug. 25 2012). The occurrence of foliar herbivore damage (**a**) was defined as the proportion of plants

with chew marks or holes. The severity of foliar herbivore damage (**b**) was defined as the proportion of leaves with chew marks or holes per plant. In 2011, the severity of foliar herbivore damage included chew marks, but not holes. Values are fitted means and standard error for a plant with modal leaf count (> 5 leaves) from a GLM with binomial response and quasibinomial error that accounted for plant size (basal leaf count). Minimal adequate models were chosen by backwards elimination from maximal models that included treatment, plant size (basal leaf count in categories of 1-3, 4-5, and > 5), and the interaction between these two terms (results analysis of deviance tables provided in Table S3A.2).

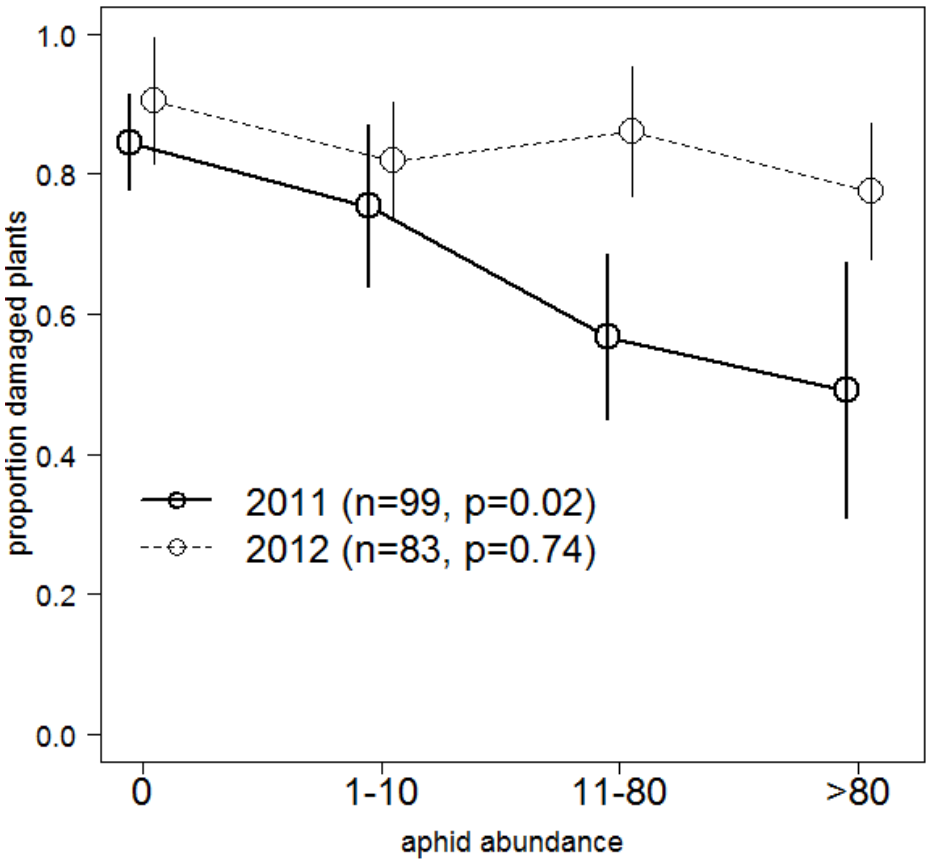

Figure 4: The relationship between foliar herbivore damage and aphid abundance in the experiment.

Herbivore damage was recorded on September 4th, after the seasonal decline of aphid infestation. Categories refer to highest observed aphid abundance between June 24th and September 4th 2011. Values are fitted means and standard errors for a plant with modal leaf count (>5 leaves) from a quasibinomial GLM that accounted for plant size (basal leaf count). Minimal adequate models were chosen by backwards elimination from maximal models that included aphid abundance, plant size (basal leaf count in categories of 1-3, 4-5, and > 5), and the interaction between these two terms (results ANOVA, Table S3A.3). The rate of foliar herbivore damage increased with plant size (2011: $p = 0.032$).

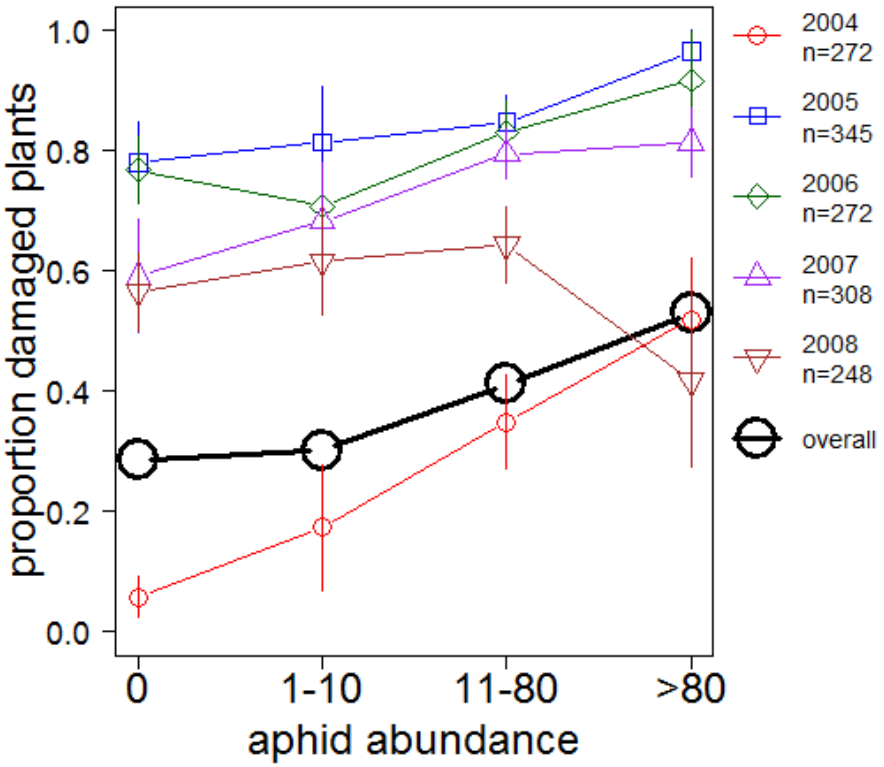

Figure 5: The relationship between foliar herbivore damage and aphid abundance in the long-term observational data.

Aphid abundances were recorded annually on each plant between mid-July and mid-August in each year. Values are fixed effects of aphid abundance from a binomial mixed-effects model (GLMM) encompassing 5 years of observational data ($p<0.0001$) and excluding flowering plants. Large points represent fitted values for 2004 from a GLMM that accounted for row, position, plant size (basal leaf count), and year. Smaller points represent fitted means and standard errors from single-year models (binomial GLMs) that accounted for row, position, plant size (basal leaf count), and observation date. Minimal adequate models were chosen by backwards elimination from models with all explanatory variables and relevant interaction terms (ANOVA results, see Table S3D). For the single-year models, aphid abundance was positively associated with foliar herbivore damage in 3 out of 5 years (2004, $p < 0.0001$; 2005, $p = 0.071$; 2006, $p = 0.26$; 2007, $p = 0.031$; 2008, $p = 0.46$; Table S3D.2).

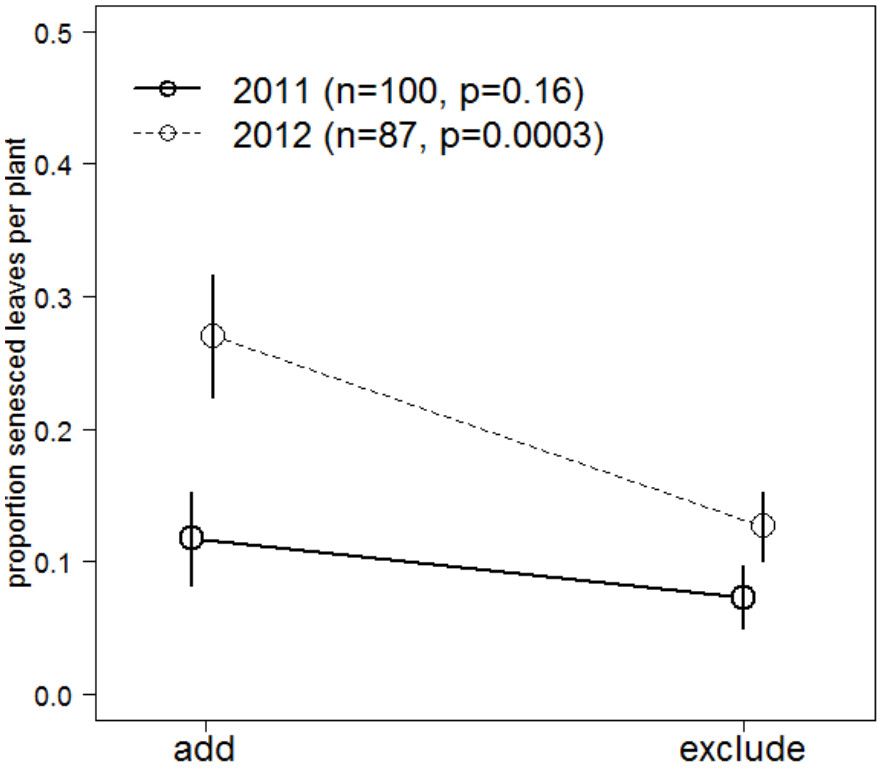

Figure 6: Differences in the proportion of senesced leaves in addition and exclusion treatments

Senescence was measured as the proportion of yellow, purple, or crisp leaves present in late-summer (Sep. 9th 2011, Aug. 25th 2012). Values are fitted means and standard error for a plant with the modal leaf count (>5 leaves) from a quasibinomial GLM. Minimal adequate models were chosen by backwards elimination from maximal models that included treatment, plant size (basal leaf count in categories of 1-3, 4-5, and > 5), and the interaction between these two terms (Table S4). Plant size significantly contributed to senescence in 2012 ($p = 0.018$), but not in 2011 ($p = 0.61$).

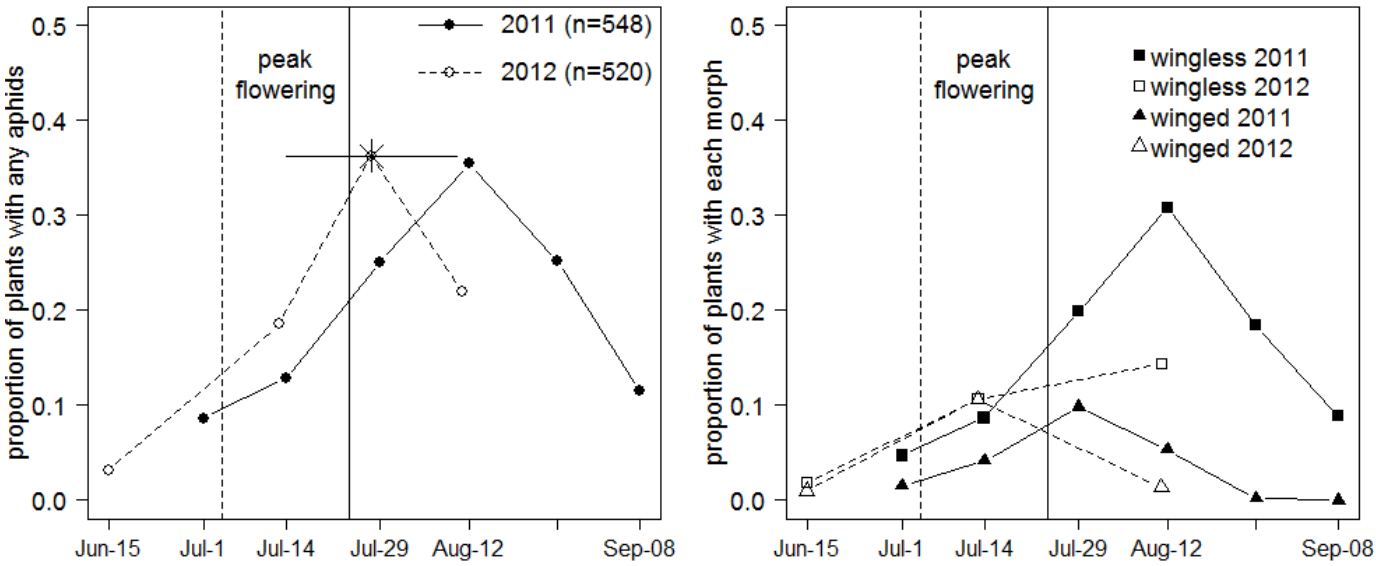

Figure 7: Phenology of aphids vs. flowering in the experimental plot

The peak of aphid infestation occurred earlier in 2012 than 2011 (left), and winged morphs peaked before wingless morphs in both years (right). Vertical lines refer to peak flowering dates in 2011 and 2012 (Jul. 24th, 2011, Jul. 4th, 2012). Peak flowering occurred earlier than peak aphid infestation in both years. The estimated peak frequency in 2012 (indicated by asterix and x-error bar) is the percentage of plants with live or dead aphids on August 10th in the observational study in the experimental plot. Based on aphid abundances in the manipulative experiment, I estimated that the peak of aphid infestation in 2012 occurred near July 21st. The peak of aphid infestation in 2011 occurred near Aug. 12th, the date on which I observed the highest proportion of plants with aphids in the observational study in the experimental plot.

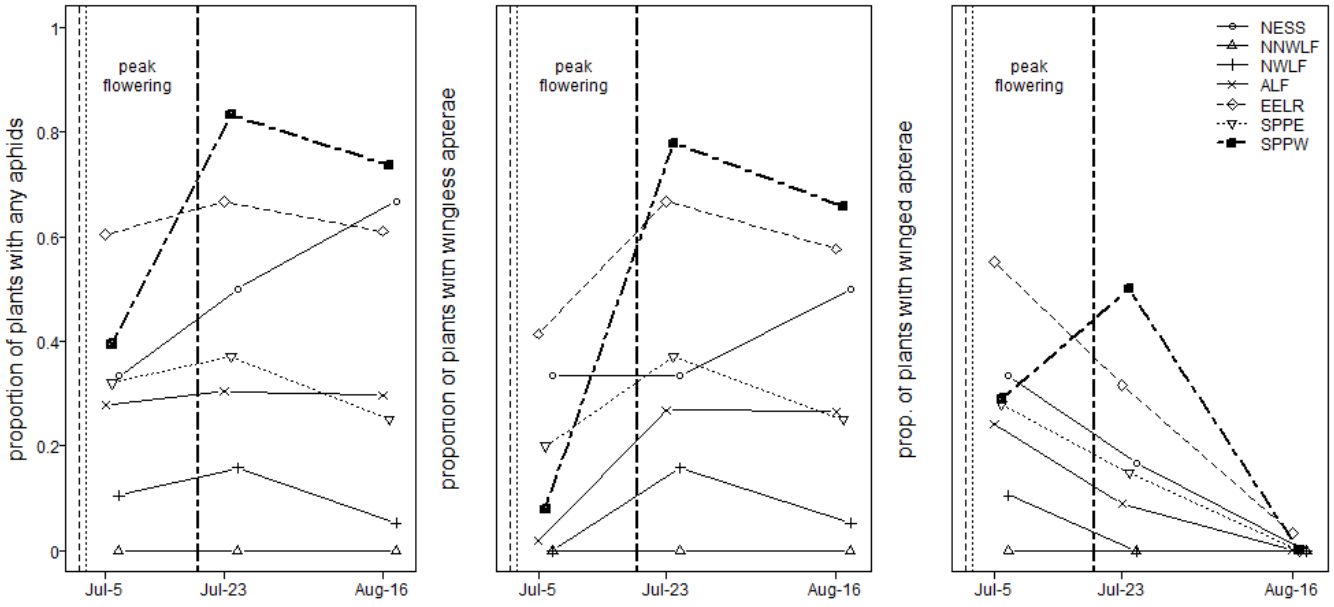

Figure 8: Phenology of aphids vs. flowering in remnant populations

Proportion of plants with any aphids (left), wingless morphs (center), and winged morphs (right) in 6 remnant populations. Plants at SPPW (bold) experienced delayed emergence and flowering due to a spring burn. Vertical lines indicate peak flowering dates at EELR, SPPE, and SPPW (bold). The proportion of plants with winged morphs declined after Jul. 5th at all sites except for SPPW, which increased between Jul. 5th and Jul. 23rd. Sample sizes for each observation date, along with aphid abundances in each population, are included in Fig. 9.

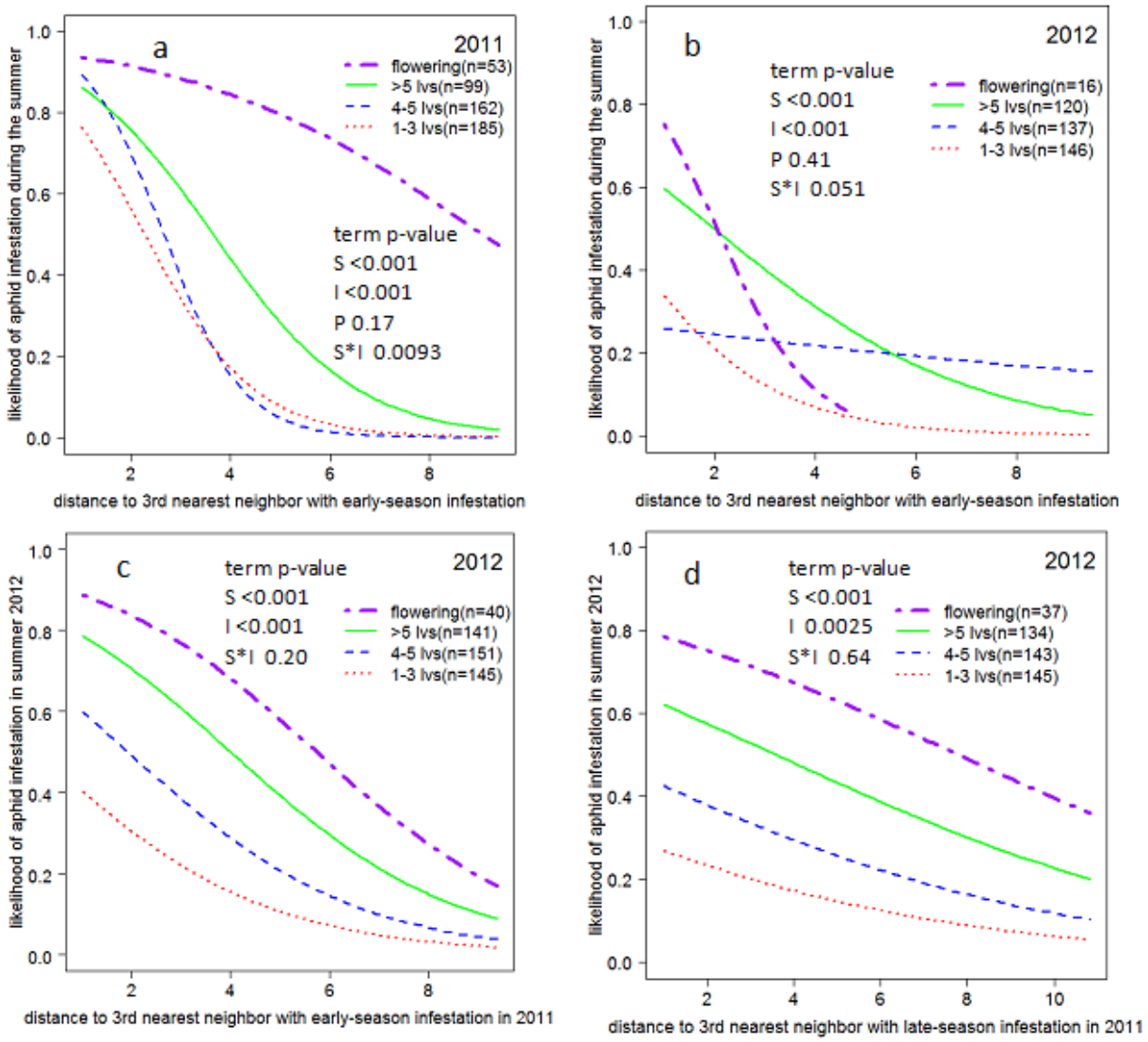

Figure 9: Within and across-season aphid dispersal: I observed aphid infestation during the summers of 2011 (Jul. 1st-Sept 8th) and 2012 (Jun. 15-Aug. 12) in a 20 x 20m area in the experimental plot containing plants from a 2003 cohort (Fig. 1). Prediction lines give a plant's likelihood of hosting aphids during the summers of 2011 or 2012 as a function of its proximity to previously infested plants (***I***) and its status (**S**), according to a binomial GLM. Aphid infestation was quantified as the presence or absence of aphids at any time during the survey period. including dead aphid colonies observed on Aug. 10th, 2012. Plant status (***S***) was quantified as a

category with 4 levels: flowering (dot-dashed line), large basal (>5 leaves, solid line), medium basal (4-5 leaves, dashed line), and small basal (1-3 leaves, dotted line). Isolation (***I***) was quantified as the distance (m) to a plant's 3rd nearest neighbor with previous aphid infestation, not including plants outside the 20x20m study area. For within-season dispersal, ***I*** represents isolation from neighbors infested earlier in the season (**a**, Jul. 1, 2011; **b**, Jul. 12th, 2012). For across-season dispersal, ***I*** represents neighbors infested early (**c**) or late (**d**) in the previous year (Jul. 1st or Sep. 9th, 2011). Minimal adequate models were chosen by backward elimination from maximal models with S, I, and the interaction between S and I (ANOVA results, table S5). Within-year models (**a, b**) also included a term for plant clustering (***P***), as well as the interaction between ***S*** and ***P***. Plant clustering was quantified as the distance (m) to the 14th nearest plant, including plants outside the survey area. Sample sizes are the number of plants from each category of size and flowering status included in the model, excluding plants with previous aphid infestation. Plant size and flowering status significantly contributed to rates of aphid infestation in both years ($p<0.0001$), with higher rates among flowering plants, followed by basal plants with >5 leaves, 4-5 leaves, and 1-3 leaves. Plant clustering did not significantly contribute to rates of aphid infestation in either 2011 ($p = 0.39$) or 2012 ($p = 0.41$), nor did the interaction between P and S (2011, $p = 0.39$; 2012, $p = 0.62$).

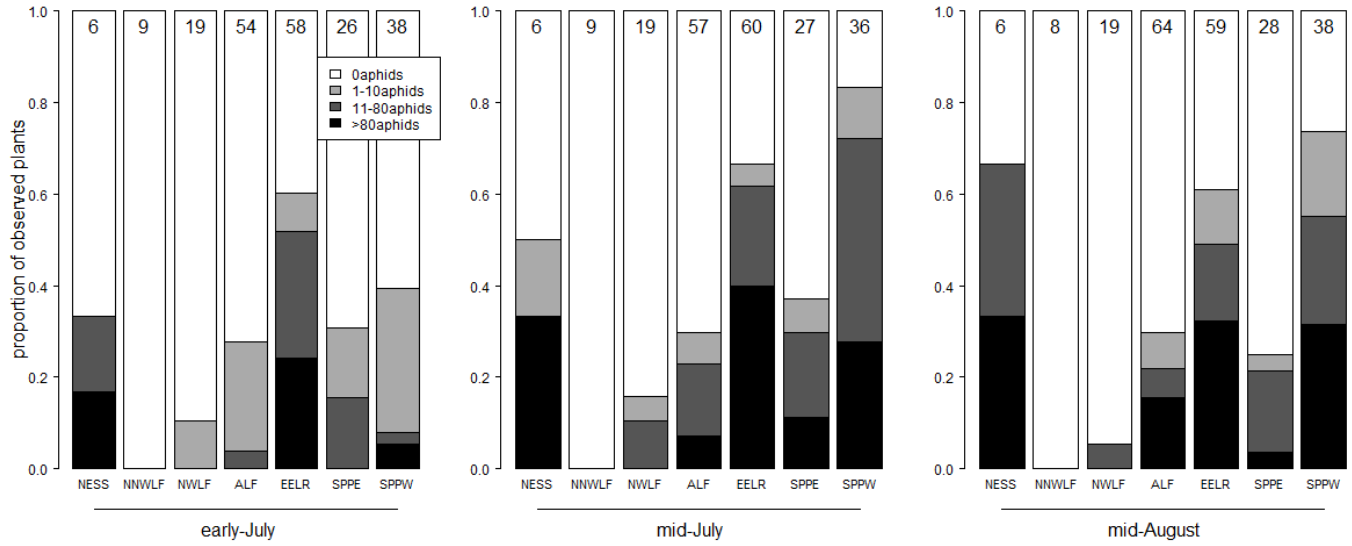

Figure 10: Aphid abundance in six remnant populations during summer 2012

Aphid abundance was observed three times on plants that flowered in 2011 and 2012. Numbers at the top of bars indicate number of plants observed on each survey date (early-July = Jul. 5-7, mid-July = Jul. 23-35, mid-August = Aug. 15-17). The sample included all flowering plants at ALF, NESS, NWLF, and NWLF and a random subset of plants at EELR, SPPW, and SPPE. SPPE and SPPW refer to burn units within a managed prairie preserve (SPP). SPPW was burned in the spring of 2012 and SPPE was last burned in 2010.

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Figure S5: Susceptibility to aphid infestation among plants in the aphid addition treatment (n=48)..... 24

Table S1: Contingency tables of the relationship between aphid infestation and plant size and plant performance.

The numbers –1, 0, and 1 indicate whether each plant trait increased, decreased, or remained the same between 2011 and 2012. Cell values give the proportion of plants within each category of aphid infestation or plant size that exhibited each trait response (sample sizes provided in the last column on the left) Plants that flowered in 2012 were categorized as having an increase in basal leaf count. Plants that died were categorized as having a decrease in basal leaf count and rosette count. Aphid presence and abundance refer to presence and highest recorded abundance of aphids at any point during the study. I performed F tests for all contingency tables with a value of < 5 in any cell.

A. Experiment

	Δbasal leaf count				Δ rosette count			status 2012			
a. treatment	p = 0.63, X^2 = 0.92, df = 2				No difference			p = 0.64, Fisher test			
		-1	**0**	**1**	**-1**	**0**	**1**	**basal**	**dead**	**flowering**	**n**
	addition	0.28	0.12	0.60	0.14	0.72	0.14	0.84	0.04	0.12	50
	exclusion	0.30	0.18	0.52	0.14	0.72	0.14	0.9	0.02	0.08	50
b. aphid abundance	p = 0.35, Fisher test				p=0.84, Fisher test			p = 0.80, Fisher test			
		-1	**0**	**1**	**-1**	**0**	**1**	**basal**	**dead**	**flowering**	**n**
	0	0.38	0.22	0.41	0.19	0.73	0.08	0.95	0.03	0.03	37
	1-10	0.25	0.06	0.69	0.12	0.69	0.19	0.75	0	0.25	16
	11-80	0.26	0.12	0.62	0.12	0.71	0.18	0.85	0.03	0.12	34
	>80	0.15	0.15	0.69	0.08	0.77	0.15	0.85	0.08	0.08	13
c. aphid presence	p = 0.055, X^2 = 5.8, df = 2				p=0.31, Fisher test			p = 0.17, Fisher test			
		-1	**0**	**1**	**-1**	**0**	**1**	**basal**	**dead**	**flowering**	**n**
	absent	0.38	0.22	0.41	0.19	0.73	0.08	0.95	0.03	0.03	37
	present	0.24	0.11	0.65	0.11	0.71	0.17	0.83	0.03	0.14	63
d. basal leaf count, 2011	p = 0.55, Fisher test				p = 0.018*, Fisher test			p=0.014*, Fisher test			
		-1	**0**	**1**	**-1**	**0**	**1**	**basal**	**dead**	**flowering**	**n**
	1-3 lvs	0.24	0.24	0.53	0.06	0.88	0.06	0.88	0.06	0.06	34
	4-5 lvs	0.29	0.11	0.61	0.11	0.79	0.11	0.96	0.04	0	28
	>6 lvs	0.34	0.11	0.55	0.24	0.53	0.24	0.79	0	0.21	38

B. Observational study in the experimental plot

Tables only include plants that were basal in 2011.

	Δ basal leaf count				Δrosette count			status 2012			
a. aphid abundance	p = 0.45, X^2=5.8,df = 6				p = 0.89 X^2=2.3, df =6			p = 0.004, Fisher test			
		-1	0	1	-1	0	1	basal	dead	flowering	n
	0	0.31	0.25	0.45	0.12	0.74	0.14	0.9	0.07	0.03	301
	1-10	0.37	0.19	0.44	0.15	0.69	0.17	0.91	0.06	0.04	54
	11-80	0.24	0.24	0.52	0.15	0.73	0.12	0.8	0.04	0.16	75
	>80	0.31	0.15	0.54	0.15	0.67	0.19	0.87	0.04	0.09	54
b. aphid presence (overall)	p = 0.31 X^2 = 2.4, df = 2				p = 0.49 X^2 = 1.4, df = 2			p = 0.003 X^2 = 11.9, df = 2			
		-1	0	1	-1	0	1	basal	dead	flowering	n
	absent	0.31	0.25	0.44	0.12	0.74	0.14	0.89	0.07	0.03	295
	present	0.3	0.2	0.5	0.15	0.7	0.15	0.86	0.04	0.1	189
c. aphid presence (July 1st)	p = 0.10 X^2 = 4.5, df = 2				p = 0.10, Fisher test			p = 0.41, Fisher test			
		-1	0	1	-1	0	1	basal	dead	flowering	n
	absent	0.29	0.24	0.47	0.12	0.73	0.14	0.88	0.06	0.06	449
	present	0.46	0.14	0.4	0.26	0.63	0.11	0.83	0.11	0.06	35
d. basal leaf count, 2011	p = 0.001 X^2 = 17.8, df = 4				p < 0.0001 X^2 = 49.2, df = 4			p <0.0001, Fisher test			
		-1	0	1	-1	0	1	basal	dead	flowering	n
	1to3	0.26	0.3	0.43	0.13	0.81	0.06	0.87	0.12	0.01	198
	4to5	0.3	0.23	0.47	0.08	0.78	0.14	0.95	0.02	0.03	173
	>5	0.38	0.1	0.5	0.23	0.49	0.28	0.79	0.02	0.19	113

Table S2: Analysis of variance tables for the relationships between aphid infestation or plant size and change in length of the longest basal leaf.
Results refer to plants in the manipulative experiment (A) and the observational study in the experimental plot (B). All linear models excluded plants that flowered or died in 2012. Basal leaf count was quantified in categories of 1-3, 4-5, and >5 basal leaves. Basal leaf length was measured in cm. Aphid infestation was defined by treatment (1), highest observed aphid abundance (2), and the presence of aphids during the study period (3). For the observational study. Minimal adequate models excluded all terms that resulted in an ANOVA p-value of > 0.1 when removed from the maximal model.

Maximal model: Δ leaf length ~ treatment + basal leaf count + treatment X basal leaf count.

A. Experiment

1. Aphid infestation defined by treatment

factor	Res. Df	RSS	Df	Sum of Sq.	F	P
	86	4330.3				
treatment	85	4303.4	1	26.97	0.52	0.47
basal leaf count	83	4236.4	2	66.93	0.64	0.53
treatment X basal leaf count	81	4207.5	2	28.95	0.28	0.76

2. Aphid infestation defined by highest observed aphid abundance (0, 1-10, 11-80, >80 aphids)

factor	Res. Df	RSS	Df	Sum of Sq.	F	P
	86	4330.3				
basal leaf count	84	4266.1	2	64.23	0.63	0.54
aphid abundance	81	4219.9	3	46.18	0.30	0.83
aphid abundance X basal leaf count	75	3843.2	6	376.71	1.23	0.30

3. Aphid infestation defined by presence or absence of aphids during the study period

		RSS	Df	Sum of Sq.	F	P
	86	4330.3				
aphid presence	85	4293.3	1	37.02	0.72	0.40
basal leaf count	83	4230.0	2	63.36	0.61	0.55
aphid presence X basal leaf count	81	4194.1	2	35.86	0.35	0.71

B. ***Observational study in experimental plot***

1. Aphid infestation defined as the highest observed aphid abundance (0, 1-10, 11-80, >80)

factors		RSS	Df	Sum of Sq.	F	P
	452	40492				
aphid abundance	449	40073	3	418.58	1.55	0.20
basal leaf count	447	40067	2	6.69	0.037	0.96
aphid abundance X basal leaf count	441	39768	6	298.23	0.55	0.77

2. Aphid infestation defined as the presence of aphids during the study period

factors		RSS	Df	Sum of Sq.	F	P
	452	40492				
basal leaf count	451	40147	1	344.63	3.84	0.051 .
aphid presence	449	40144	2	3.00	0.017	0.98
aphid presence X basal leaf count	447	40118	2	26.26	0.15	0.86

3. Aphid infestation defined as the presence of aphids on the first survey date (Jul. 1st, 2011)

factors		RSS	Df	Sum of Sq.	F	P
	452	40492				
aphid presence	451	40264	1	227.75	2.54	0.11
basal leaf count	449	40262	2	1.898	0.011	0.99
aphid presence X basal leaf count	447	40159	2	103.13	0.57	0.56

Table S3: Analysis of deviance tables for the relationship between aphid infestation and foliar herbivore damage for the manipulative experiment (**A**), the observational study in the 2003 cohort (B), and the long-term data from the 1997 cohort (**B**). Single-year models were quasibinomial GLMs (2,4) and multi-year models were binomial GLMMs (1,3) with random effects of individual plants. Foliar herbivore damage was defined as the presence of chew marks and holes in leaves. Basal leaf count was quantified as a categories of 1-3, 4-5, and >5 leaves. In the experiment, the presence of foliar herbivore damage was recorded in mid- and late-summer 2011 (2a, 2b) and 2012 (2d, 2e) and early-summer 2012. The number of damaged leaves was recorded only in late summer in both years (4a, 4b). In 2011, the number of damaged leaves did not include leaves with holes. Minimal adequate models excluded all terms that resulted in an F-test p-value of > 0.1 when removed from the maximal model.

A. Experiment

1. Maximal model: Presence of chew marks or holes on plant ~ treatment + basal leaf count + year + basal leaf count X treatment + 1 | plant ID

September 4th, 2011 (n=99) and August 25th, 2011 (n=83)

predictors	df	AIC	BIC	logLik.	χ^2	χ^2 df	P
1 | plant ID	2	243.47	249.88	-119.74			
year	4	234.96	247.78	-113.48	12.51	2	0.0019 **
basal leaf count	5	230.82	246.84	-110.41	6.14	1	0.013 *
treatment	6	228.51	247.74	-108.26	4.31	1	0.038 *
treatment X basal leaf count	8	231.98	257.61	-107.99	0.53	2	0.77

2. Maximal model: Presence of chew marks or holes on plant ~ treatment + basal leaf count + basal leaf count X treatment

a. August 10th, 2011 (n=100)

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	99	138.63				
basal leaf count	1	97	130.15	1	8.48	3.98	0.022 *
treatment	2	96	126.70	1	3.45	3.24	0.075
treatment X basal leaf count	3	94	126.44	1	0.26	0.12	0.88

b. September 4th, 2011 (n=99)

Sample excludes 1 plant that became crisp between Aug. 10th and Sep. 4th.

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	99	136.42				
basal leaf count	1	97	128.79	1	7.63	3.85	0.032 *
treatment	2	96	124.34	1	4.45	4.18	0.043 *
treatment X basal leaf count	3	94	123.95	1	0.39	0.18	0.83

c. June 24th, 2012 (n=87)

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	86	120.5				
basal leaf count	1	84	117.0	2	3.51	1.63	0.20
treatment	2	83	116.0	1	0.99	0.93	0.34
basal leaf count X treatment	3	81	115.17	2	0.83	0.39	0.68

d. July 14th, 2012 (n=87)

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	86	119.21				
basal leaf count	1	84	105.69	2	13.52	6.29	0.0029 **
treatment	2	83	104.38	1	1.31	1.22	0.27
basal leaf count X treatment	3	81	101.05	2	3.33	1.55	0.22

e. August 25th, 2012, n=83)

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	82	95.99				
basal leaf count	1	80	91.38	2	4.61	2.14	0.12
treatment	2	79	90.61	1	0.77	0.71	0.40
treatment X basal leaf count	3	89	89.96	2	0.65	0.30	0.74

3. Maximal model: Presence of chew marks or holes on plant ~ aphid abundance + basal leaf count + year + basal leaf count X aphid abundance + 1 | plant ID

September 4th, 2011 (n=99) and August 25th, 2012 (n=83)

predictors	df	AIC	BIC	logLik.	χ2	χ2 df	P
1 | plant ID	2	243.47	249.88	-119.74			
year	4	234.96	247.78	-113.48	12.51	2	0.0019**
basal leaf count	5	230.82	246.84	-110.41	6.14	1	0.013 *
aphid abundance	8	227.26	252.90	-105.63	9.55	3	0.027 *
basal leaf count X aphid abundance	14	238.44	283.29	-105.22	0.83	6	0.99

4. Maximal model: Presence of chew marks or holes ~ aphid abundance + basal leaf count + aphid abundance X basal leaf count

Aphid abundance refers to highest recorded aphid abundance during the experiment, in categories of 0, 1-10, 11-80, and >80.

a. September 4th, 2011 (n=99)

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	98	136.42				
aphid abundance	1	95	124.65	3	11.77	3.45	0.02 *
basal leaf count	2	93	118.33	2	6.31	2.77	0.068
aphid abundance X basal leaf count	3	87	115.65	6	2.69	0.39	0.88

b. August 25th, 2012 (n=83)

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	82	95.995				
basal leaf count	1	80	91.38	2	4.61	2.05	0.14
aphid abundance	2	77	90.03	3	1.36	0.40	0.75
aphid abundance X basal leaf count	3	71	86.49	6	3.54	0.52	0.79

5. Maximal model: Proportion of leaves with chew marks or holes ~ treatment + basal leaf count + treatment X basal leaf count + 1 | plant ID

September 4th, 2011 (n=99), August 25th, 2012 (n=83)

predictors	df	AIC	BIC	logLik.	χ2	χ2 df	P
1 | plant ID	2	338.69	345.10	-167.34			
year	3	296.70	306.31	-145.35	43.99	1	<0.0001 ***
treatment	4	292.31	305.12	-142.15	6.39	1	0.011 *
basal leaf count	6	294.76	313.98	-141.38	1.55	2	0.46
treatment X basal leaf count	8	297.77	323.40	-140.88	0.99	2	0.61

6. Maximal model: Proportion of leaves with chew marks or holes ~ treatment + basal leaf count + treatment X basal leaf count

a. September 4th, 2011 (n=99)—includes only chew marks

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	98	166.62				
treatment	1	97	159.98	1	6.64	3.95	0.05 *
basal leaf count	2	95	159.61	2	0.37	0.11	0.89
treatment X basal leaf count	3	93	159.01	2	0.59	0.18	0.84

b. August 25th, 2012 (n=83)—chew marks and holes

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	82	161.21				
treatment	1	81	157.72	1	3.49	2.03	0.16
basal leaf count	2	79	157.37	2	0.34	0.1	0.91
treatment X basal leaf count	3	77	156.60	2	0.77	0.22	0.799

7. Maximal model: Proportion of leaves with chew marks or holes ~ aphid abundance + basal leaf count + basal leaf count X aphid abundance

a. September 4th, 2011 (n=99)

Does not include leaves with holes.

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	98	166.62				
aphid abundance	1	95	153.09	3	13.53	2.92	0.04 *
basal leaf count	2	93	152.75	2	0.34	0.11	0.89
aphid abundance X basal leaf count	3	87	139.55	6	13.20	1.43	0.21

b. August 25th, 2012 (n=83)—chew marks and holes

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	82	161.21				
aphid abundance	1	79	155.34	3	5.87	1.06	0.37
basal leaf count	2	77	155.05	2	0.29	0.078	0.92
aphid abundance X basal leaf count	3	71	152.74	6	2.31	0.21	0.97

B. Observational study in the experimental plot (2003 cohort, August 2012) (n = 242)

The model excludes flowering plants, n=472. P-values were obtained from ANOVA F-tests comparing models with and without each factor. Aphid infestation was quantified as the presence or absence of aphids at any point during the season, including dead aphid colonies observed on August 10th, 2012. Foliar herbivore damage refers to the presence of chew marks or holes on the final survey (Aug. 25th, 2012)

Maximal model: Presence of chew marks or holes ~ basal leaf count + aphid presence + row + position + basal leaf count X aphid presence

	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	471	626.52				
basal leaf count	1	469	590.51	2	36.01	17.69	<0.0001 ***
row	2	468	589.69	1	0.82	0.8066	0.37
position	3	467	589.64	1	0.047	0.047	0.83
aphid presence	4	466	589.61	1	0.036	0.036	0.85
basal leaf count X aphid presence	5	464	584.67	2	4.93	2.42	0.09

C. Observational study in remnant populations (n=209)

Models include all plants that flowered in 2011 and 2012 and exclude the site NNWLF, which was removed by a road maintenance project in early August. Rosette count was quantified in categories of 1, 2, 3 and >3 rosettes. Foliar herbivore damage and aphid abundance was defined as the presence of chew marks or holes and the highest recorded aphid abundance between July 7th and August 16th.

Maximal model:
presence of chew marks or holes ~ aphid abundance + rosette count + site + aphid abundance X rosette count + aphid abundance X site + rosette count X site

predictors	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	208	262.21				
site	1	203	250.57	5	11.64	2.196	0.058
rosette count	2	200	242.82	3	7.75	2.44	0.067
aphid abundance	3	197	235.72	3	7.10	2.23	0.087
aphid abundance X site	4	185	222.28	12	13.44	1.06	0.40
rosette count X site	5	171	207.70	14	14.58	0.98	0.47
aphid abundance X rosette count	6	162	202.42	9	5.29	0.55	0.83

D. Long-term data

Foliar herbivore damage and aphid abundance were recorded once each year in mid-July or early August. Basal leaf count was quantified categories of 1-3, 4-5, and >5 basal leaves. The models exclude flowering plants.

1. Multi-year model (GLMM)

The model accounts for random effects of individual plants (1 | plant ID) as well as 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 measure year.

Maximal model:

Presence of chew marks or holes ~ basal leaf count + aphid abundance + basal leaf count X aphid abundance + year + (1 | plant ID)

predictors	df	AIC	BIC	logLik.	χ^2	χ^2 df	P
1 | plant ID	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 X aphid abundance	17	1732.1	1821.8	-849.07	7.32	6	0.29

2. Single-year models (GLM binomial)

Maximal model:

Presence of chew marks or holes ~ basal leaf count + aphid abundance + basal leaf count X aphid abundance + measure date + row + position

a. 2004 (n = 272)

predictors	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	P
	0	271	301.44			
aphid abundance	1	268	261.67	3	39.76	<0.0001***
row	2	267	256.43	1	5.24	0.022 *
position	3	266	253.81	1	2.63	0.11
basal leaf count	4	264	252.06	2	1.74	0.42
measure date	5	260	250.87	4	1.198	0.88
basal leaf count X aphid abundance	6	254	242.88	6	7.98	0.24

b. 2005 (n = 345)

factors	model terms	Resid.df	Resid. Deviance	Test. df	Deviance	P
	0	344	445.8			
basal leaf count	1	342	414.54	2	31.27	<0.0001***
row	2	341	403.13	1	11.41	0.00073**
aphid abundance	3	338	396.1	3	7.03	0.071
measure date	4	333	386.72	5	9.38	0.095
position	5	332	386.24	1	0.47	0.49
basal leaf count X aphid abundance	6	327	378.33	5	7.91	0.16

3. 2006 (n=272)

factors	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	P
	0	271	369.26			
basal leaf count	1	269	318.76	2	50.495	<0.0001***
aphid abundance	2	266	314.73	3	4.03	0.26
measure date	3	259	303.22	7	11.51	0.12
row	4	258	302.86	1	0.36	0.55
position	5	257	302.83	1	0.025	0.87
basal leaf count X aphid abundance	6	253	298.08	4	4.75	0.31

4. 2007 (n=308)

factors	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	P
	0	307	418.83			
basal leaf count	1	305	374.51	2	44.32	<0.0001***
row	2	304	353.03	1	21.48	<0.0001***
aphid abundance	3	301	344.17	3	8.86	0.031 *
measure date	4	294	334.03	7	10.14	0.18
position	5	293	331.71	1	2.32	0.13
basal leaf count X aphid abundance	6	287	330.9	6	0.82	0.99

5. 2008 (n=248)

factors	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	P
	0	247	343.66			
basal leaf count	1	245	321.61	2	22.04	<0.0001***
row	2	244	319.30	1	2.31	0.13
measure date	3	236	308.91	8	10.39	0.24
aphid abundance	4	233	306.68	3	2.23	0.53
position	5	232	306.60	1	0.078	0.78
basal leaf count X aphid abundance	6	228	304.03	4	2.57	0.63

Table S4. Summary of backwards model selection for factors influencing the premature senescence in the experiment.

Model selection for a GLM quasibinomial. Basal leaf count is in categories of 1-3, 4-5, and >5 leaves. For 2012, I included separate models including and excluding flowering plants.

1. Maximal model: proportion of yellow, purple, or crisp leaves ~ treatment + basal leaf count + treatment X basal leaf count

a. September 9th, 2011 (n=100)

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	99	200.28				
treatment	1	98	195.53	1	4.74	2.03	0.16
basal leaf count	2	97	193.82	1	1.72	0.74	0.39
treatment X basal leaf count	3	96	193.74	1	0.08	0.034	0.85

b. August 25th, 2012 (n=96) (flowering plants included)

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	95	241.51				
treatment	1	94	211.76	1	29.75	15.86	0.00013 **
total leaf count	2	93	205.62	1	6.14	3.27	0.074
treatment X total leaf count	3	92	204.66	1	0.96	0.51	0.48

c. August 25th, 2012 (n=87) (flowering plants excluded)

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	86	212.43				
treatment	1	85	187.36	1	25.07	14.15	0.00032**
basal leaf count	2	83	172.56	2	14.80	4.18	0.019 *
treatment X basal leaf count	3	81	170.13	2	2.43	0.69	0.51

2. Maximal model: proportion of yellow, purple, or crisp leaves ~ aphid abundance + basal leaf count + aphid abundance X basal leaf count

a. September 9th, 2011 (n = 100)

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	99	200.28				
basal leaf count	1	98	197.5	1	2.78	1.15	0.29
aphid abundance	2	95	193.53	3	3.97	0.55	0.65
aphid abundance X leaf count	3	92	185	3	8.54	1.18	0.32

b. August 25th, 2012 (n = 96) (flowering plants included)

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	99	200.28				
total leaf count	1	98	197.5	1	2.78	1.15	0.29
aphid abundance	2	95	193.53	3	3.97	0.55	0.65
aphid abundance X total leaf count	3	92	185	3	8.54	1.18	0.32

c. August 25th, 2012 (n = 87) (flowering plants excluded)

factor	model terms	Resid.df	Resid. Deviance	Test.df	Deviance	F	P
	0	86	212.43				
basal leaf count	1	84	191.38	2	21.05	5.36	0.0067**
aphid abundance	2	81	180.97	3	10.42	1.77	0.16
aphid abundance X basal leaf count	3	75	170.83	6	10.14	0.86	0.53

Table S5: Results tables for within and across-season aphid dispersal in the experimental plot (2003 cohort).

Models predict a plant's likelihood of hosting aphids within a 20 x 20m area of the experimental plot at a given time period (summer 2011 or 2012, peak aphid infestation 2011, peak of winged aphids 2011) based on its status (***S***) and isolation (***I***) from previously infested plants. Isolation was quantified as the distance (m) to its 3rd nearest neighbor with previous aphid infestation in the same season (A, B, E, and F) and the previous season (C, D), not including plants outside the survey area. For across-season dispersal, ***I*** refers to plants with early-season (C) or late-season (D) infestation in 2011. Status was quantified as a category of flowering or basal with >5, 4-5, or 1-3 leaves. Models for within-season dispersal also include a term for plant clustering (***P***) to account for variation in plant density throughout the plot, which was quantified as distance (m) to the 14th nearest plant.

A. Within-season, 2011: Isolation from plants with early-season infestation (Jul. 1st, 2011)

factor	Resid. Df	Resid. Deviance	Df	Deviance	P
	498	671.18			
I	497	549.09	1	122.09	< 0.0001 ***
S	494	476.06	3	73.03	< 0.0001 ***
P	493	474.19	1	1.87	0.17
S X I	490	462.69	3	11.498	0.0093 **
S X P	487	459.71	3	2.98	0.39

B. Within-season, 2012: Isolation from plants with early-season infestation (Jul. 12th, 2012)

factor	Resid. Df	Resid. Deviance	Df	Deviance	P
	418	471.79			
I	415	445.33	3	26.45	< 0.0001 ***
S	414	427.26	1	18.08	< 0.0001 ***
P	413	426.58	1	0.67	0.41
S X I	410	418.82	3	7.76	0.051
S X P	407	417.06	3	1.76	0.62

C. Across-season: Isolation from early-infested plants from 2011 (Jul. 1st)

factor	Resid. Df	Resid. Deviance	Df	Deviance	P
	476	632.27			
S	473	569.89	3	62.38	< 0.0001 ***
I	472	526.44	1	43.45	< 0.0001 ***
S X I	469	521.80	3	4.64	0.20

D. Across-season: Isolation from late-infested plants from 2011 (Sep. 8th)

factor	Resid. Df	Resid. Deviance	Df	Deviance	P
	458	607.19			
S	455	550.41	3	56.78	< 0.0001 ***
I	454	541.28	1	9.13	0.0025 **
S X I	451	539.60	3	1.67	0.64

E. Within-season dispersal at peak aphid infestation (Aug. 12th, 2011): Isolation from early-infested plants (Jul. 1st, 2011)

factor	Resid. Df	Resid. Deviance	Df	Deviance	P
	498	618.36			
S	495	572.80	3	45.56	< 0.0001 ***
I	494	463.47	1	109.33	< 0.0001 ***
P	493	460.31	1	3.16	0.075
S X I	490	450.52	3	9.79	0.02 *
S X P	487	447.66	3	2.85	0.41

F. Within-season dispersal at the peak of winged morphs (Jul. 29st, 2011) Isolation from early-infested plants (Jul. 1st, 2011)

Quasibinomial GLM

factor	Resid. Df	Resid. Deviance	Df	Deviance	F	P
	491	496.80				
S	488	434.32	3	62.475	32.93	< 0.0001 ***
I	487	318.31	1	116.018	183.48	< 0.0001 ***
P	486	317.81	1	0.497	0.79	0.38
S X I	483	302.84	3	14.965	7.89	<0.0001 ***
S X P	480	301.31	3	1.532	0.81	0.49

Table S6: Susceptibility to aphid infestation among plants in the addition treatment (n=48)
On each visit of the addition treatment, I visually estimated the count of aphids on all aboveground parts of the plant before adding aphids. This count was bounded at 80 in 2011. Plant size was quantified as a category of mean leaf count between 2011 and 2012 (1-3, 4-5, >5).

Mean aphid abundance
Maximal model: Mean aphid count in 2012 ~ row + position + basal leaf count + mean aphid count in 2011 + basal leaf count X mean aphid count in 2011

factor		RSS	Df	Sum of Sq.	F	P
	41	108222				
plant size	39	78573	2	29649.1	9.16	0.00066 **
row	38	66330	1	12242.8	7.56	0.0095 **
position	37	56460	1	9870.1	6.097	0.019 *
mean aphid count 2012	36	56436	1	24.0	0.0148	0.90
basal leaf count X mean aphid count in 2011	34	55039	2	1397.0	0.43	0.65

Maximum aphid abundance
Maximal model: Maximum aphid count in 2012 ~ row + position + basal leaf count + maximum aphid count in 2011 + basal leaf count X maximum aphid count in 2011

factor		RSS	Df	Sum of Sq.	F	P
	41	2706701				
plant size	39	2128140	2	578561	5.63	0.0077 **
position	38	1932669	1	195471	3.81	0.059
row	37	1791457	1	141212	2.75	0.11
maximum aphid count 2011	36	1785103	1	6354	0.12	0.73
basal leaf count X maximum aphid count in 2011	34	1746265	2	38838	0.38	0.69

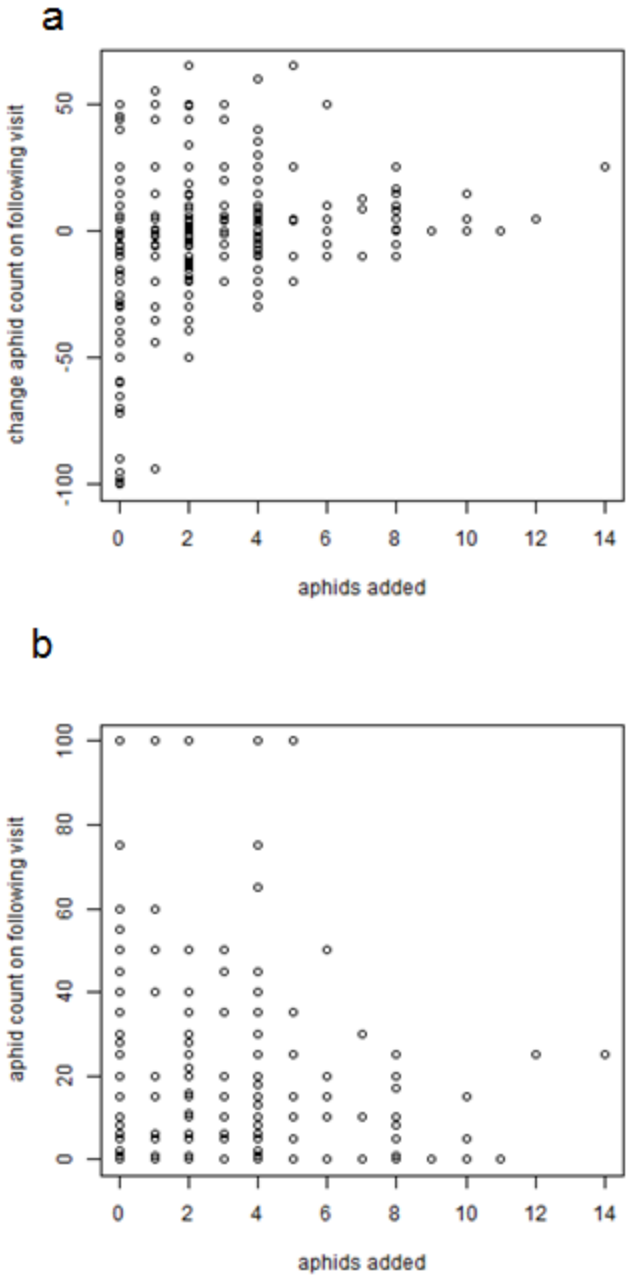

Figure S1: Aphid addition in 2011

In 2011 I varied the number of aphids I added to each plant in the addition treatment based on the abundance of aphids present on the plant. I recorded the approximate number of aphids present on each plant before the next aphid addition 3-7 days later (**b**) and calculated the change in aphid abundance between the two visits (**a**). Because my estimation of aphid abundance was bounded at >80, I converted all observations of >80 aphids to an integer value (100) for this plot.

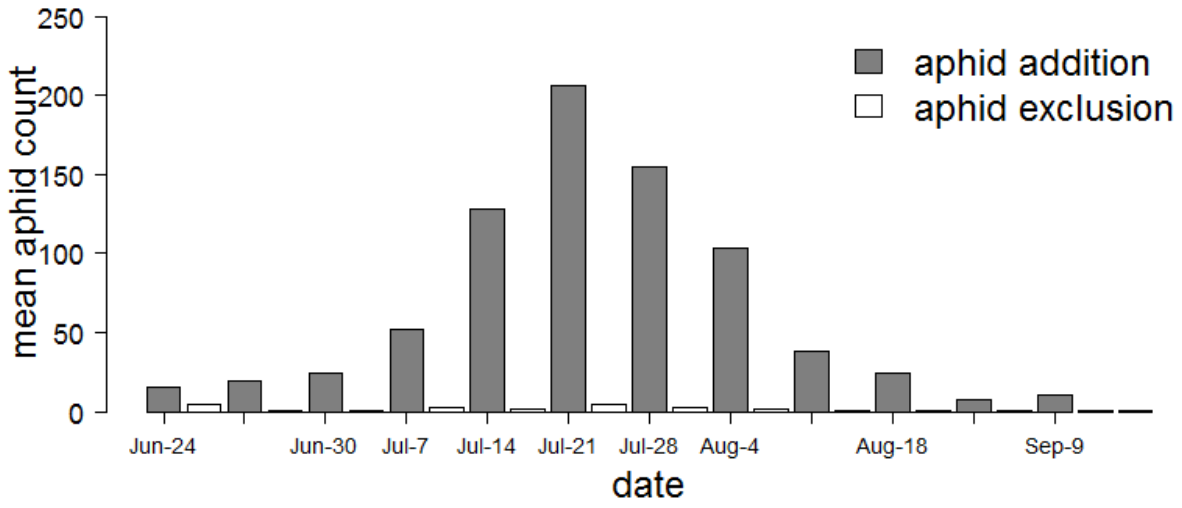

Figure S2: I estimated peak date of aphid infestation in 2012 based on changes in aphid abundance among plants in the aphid addition (n = 48) and exclusion (n = 49) treatments in 2012 (mean). During each visit, I estimated the count of aphids on the aboveground parts of each plant by visual inspection. Based on these observations, I estimated that the peak date of aphid infestation in 2012 occurred on Jul. 12th.

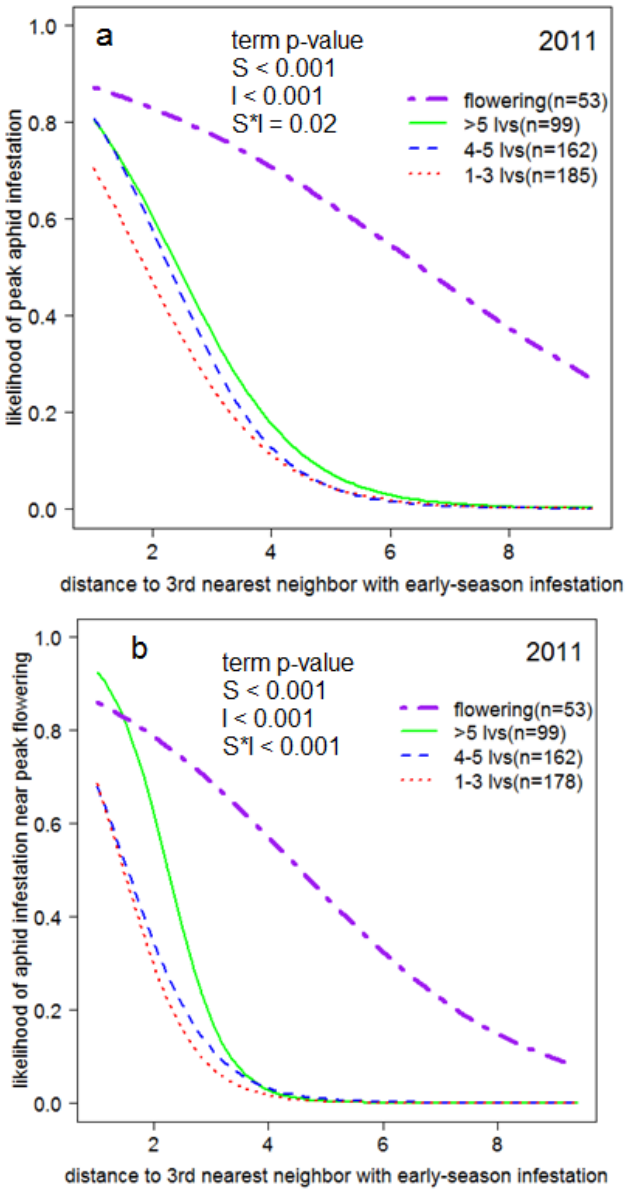

Figure S3: Within-season aphid dispersal in 2011

Lines represent a plant's predicted likelihood of hosting aphids at peak aphid infestation (**a,** August 12th 2011, n=499) and at the peak of winged morphs (**b,** July 29th, 2011) based on binomial GLMs. All models included isolation (***I***) from previously infested plants, plant status (**S**), and their interaction (***SXI***). ***I*** was quantified as the distance (m) from the 3rd nearest neighbor with aphid infestation on Jul. 1st and ***S*** was a 4-level category: flowering (dot-dashed line), basal with >5 leaves (solid line), basal with 4-5 leaves (dashed line), and basal with 1-3 leaves (dotted line). Sample sizes are the number of plants in each status category, excluding plants with aphid infestation on Jul. 1st, 2011.

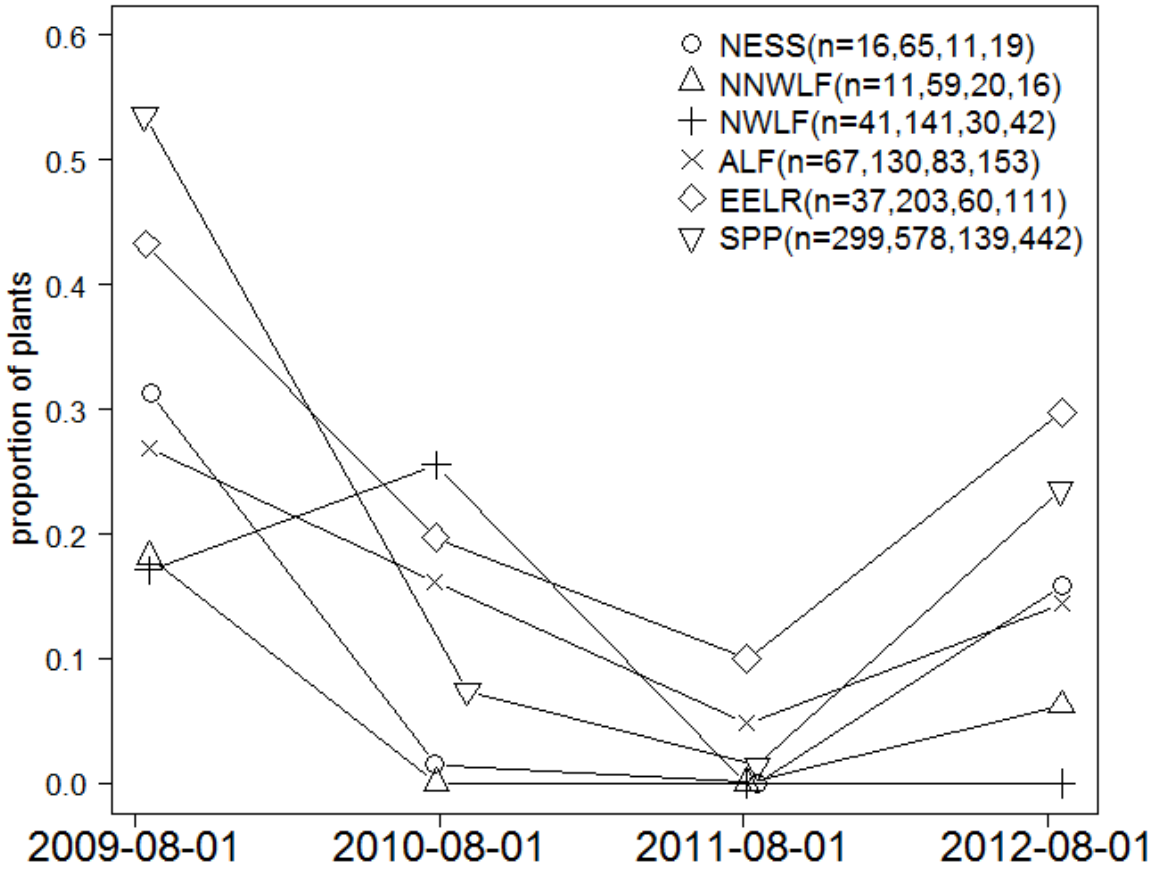

Figure S4: Rates of aphid infestation on flowering plants in six remnant populations across four years

The presence of aphids was recorded on all populations once per year between late July and mid-August. Sample sizes are the number of plants observed in each subsequent year (separated by a comma). The site SPP includes two burn units SPPW and SPPE, which were surveyed separately in my observational study (Fig. 8).

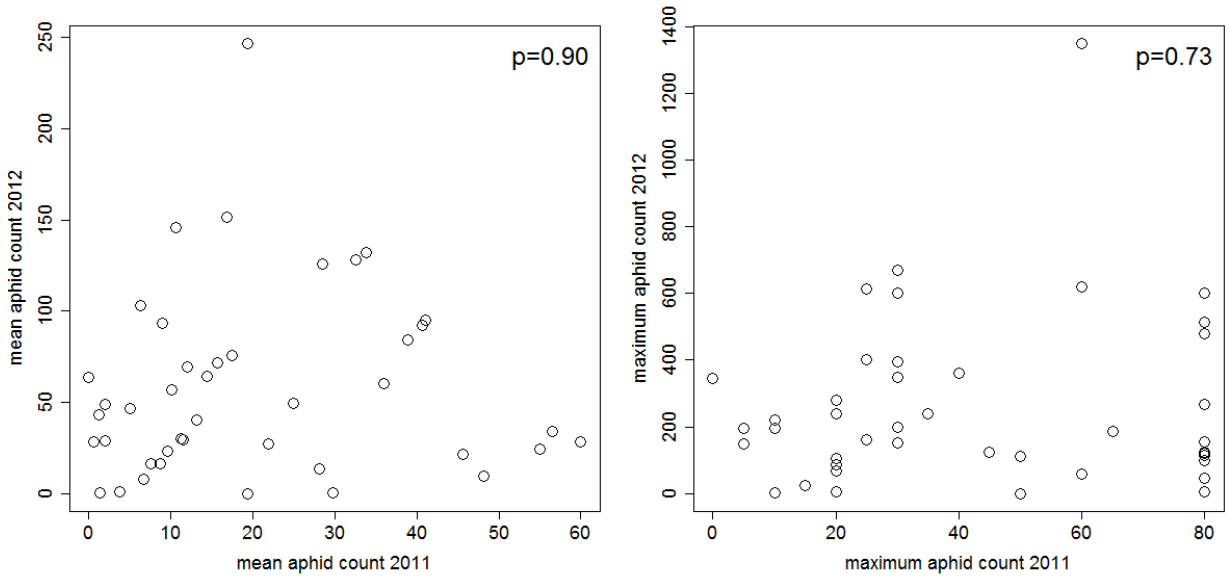

Figure S5: Susceptibility to aphid infestation among plants in the aphid addition treatment (n=48)

Each dot represents an individual plant in the aphid addition treatment. On each visit of the addition treatment, I visually estimated the count of aphids on all aboveground parts of the plant before adding aphids. In 2011, the estimate of aphid count was bounded at 80. A positive correlation between aphid abundance in 2011 and 2012 would suggest that individual plants varied in their innate susceptibility to aphid infestation. A negative correlation would suggest that hosting high aphid abundance in 2011 led plants to become more resistant in 2012. There was no correlation between an individual's mean ($p = 0.90$) or maximum ($p = 0.73$) aphid abundance in 2011 and 2012, according to a linear model that accounted for plant size and location in the experimental plot (Table S6). Plant size—as quantified by a category of mean leaf count between 2011 and 2012 (1-3, 4-5, >5)—did significantly contribute to aphid abundance in 2012 (mean aphid count: $p=0.00066$, maximum aphid count: $p=0.0077$).