esa ECOSPHERE

Induced resistance mitigates the effect of plant neighbors on susceptibility to herbivores

KATHERINE D. HOLMES † AND ANURAG A. AGRAWAL

Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York 14853 USA

Citation: Holmes, K. D., and A. A. Agrawal. 2021. Induced resistance mitigates the effect of plant neighbors on susceptibility to herbivores. Ecosphere 12(1):e03334. 10.1002/ecs2.3334

Abstract. At small spatial scales, attraction or deterrence of herbivores by plant neighbors can alter the susceptibility of plants to damage (i.e., associational effects). Given the patchy nature of plants and insect herbivory, we hypothesized that induced resistance may play an important role in mitigating such spatial variability. To test this notion, we first documented neighbor effects between two closely related and co-occurring plant species in natural populations, and second, we measured how these effects changed after inducing plant resistance in a common garden. In wet fields and marshes of Northeastern North America, boneset (*Eupatorium perfoliatum*) is the primary host for the herbivorous beetle *Ophraella notata*. Across two years of surveys at multiple sites, we found that Joe Pye weed (*Eutrochium maculatum*) was a secondary host to *O. notata* and was more likely to receive beetle eggs when it grew near boneset, constituting a negative neighbor effect (associational susceptibility) for Joe Pye weed. Reciprocally, there were trends of reduced susceptibility for boneset when it grew near Joe Pye weed (a positive neighbor effect), but this pattern was less consistent over space and time. In the common garden, we manipulated patches, each with a center (focal) and surrounding (neighbor) plants, with focal plants of each species either induced by the plant hormone jasmonic acid or left as controls. While neighbor effects prior to induction mirrored the pattern in surveys, induction was most effective in reducing beetle oviposition on focal plants in heterospecific groups. This effectively eliminated negative neighbor effects (susceptibility) for Joe Pye weed, the less preferred plant species. However, in conspecific patches, induction had minimal effect on either species' susceptibility to beetles. Given the importance of spatial variation generally and the ubiquity of neighbor effects in plant communities, we suggest that inducible resistance may be an important mechanism to cope with spatial heterogeneity in susceptibility to herbivores.

Key words: associational effects; Asteraceae; herbivory; indirect interactions; inducible responses; neighbor effects; patch dynamics; plant defenses; plant–herbivore; plasticity.

Received 14 July 2020; accepted 21 July 2020; final version received 16 November 2020. Corresponding Editor: Debra P. C. Peters.

Copyright: © 2021 The Authors. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

† **E-mail:** kdh73@cornell.edu

INTRODUCTION

Organisms that live in close proximity often affect each other's susceptibility to enemies. In plant communities, neighboring plants can increase or decrease herbivore occurrence and damage on nearby plants (termed associational susceptibility vs. resistance, respectively; Tahvanainen and Root 1972, Brown and Ewel 1987). While these neighbor effects typically occur at small spatial scales, they can lead to substantial changes in herbivore populations (Russell and Louda 2005) and have been used to protect crops from insect pests (Khan et al. 2011). Neighbor effects occur by a variety of mechanisms, including reduced herbivory on a primary host plant caused by inferior neighbors (Atsatt and O'Dowd 1976, Hambäck and Beckerman 2003,

Agrawal et al. 2006, Barbosa et al. 2009). However, if neighbors serve as alternative hosts or offer resources such as physical structures for herbivores, they may increase herbivore occurrence on a focal plant, leading to associational susceptibility (Futuyma and Wasserman 1980, Agrawal 2004). Accordingly, neighbor effects can result in positive or negative indirect interactions between plants. Strong associations with certain neighbors have been proposed to yield evolutionary changes in plant traits related to herbivore resistance (Zangerl and Berenbaum 2003), facilitation between species (Graff et al. 2007) and contribute to species coexistence (Callaway et al. 2003).

Given the prevalence of neighbor effects, plasticity in plant defenses may be important in communities where neighbors impact the intensity of herbivory (Alpert and Simms 2002). Indeed, plants typically respond to herbivory by upregulating physical and chemical defenses (Edwards et al. 1986, Karban and Baldwin 1997, Thaler et al. 2001). This defense plasticity may mitigate the extremes of herbivore susceptibility by reducing damage (increasing resistance) in the presence of neighbors that confer susceptibility, while reducing the costs of defense investment in the absence of such neighbors (Coverdale et al. 2018). While induced defense is known to affect herbivore movement and spatial dispersion among host plants (Underwood et al. 2005, Morrell and Kessler 2017), how induced responses interact with associational effects is unclear. For instance, the effectiveness of induced responses in deterring herbivores could depend on whether the neighbor environment permits detection of, or response to, changes in focal plant defenses. If induction increases the phenotypic contrast between focal plants and neighbors, allowing herbivores to better detect and respond to differences in host quality, this may mitigate associational effects (Huang et al. 2016). However, if induced responses reduce the neighbor contrast, it may increase the intensity of neighbor effects.

Recent work shows defensive traits can play an important role in associational effects. For example, Ida et al. (2018) recently demonstrated that intraspecific (constitutive) variation in alkaloids can generate associational resistance in tobacco. Conversely, Coverdale et al. (2018) found that associational resistance reduced the expression of both constitutive and induced defenses in spiny African shrubs. Given that plant defenses can be both the cause and consequence of neighbor effects, we sought to test whether a manipulation of defense (through induced responses) could modify natural patterns of neighbor effects.

We investigated the impact of induced responses on neighbor effects in two co-occurring wetland Asteraceae, *Eupatorium perfoliatum* (hereafter "boneset"), and *Eutrochium maculatum* (hereafter "Joe Pye weed"; both in tribe Eupatorieae). These species share a suite of herbivores including the leaf beetle *Ophraella notata*, a specialist on species in Eupatorieae. In upstate New York, *O. notata* has been anecdotally reported as occurring in small numbers on Joe Pye weed only in the presence of boneset (Futuyma 1990). For two years, we surveyed natural populations of Joe Pye weed and boneset to test for a natural pattern of neighbor effects. Nonetheless, field observations of neighbors may be confounded by differences in plant relatedness, abiotic conditions, and other factors that correspond with neighbor proximity. Accordingly, we planted a common garden of Joe Pye weed and boneset in conspecific or heterospecific groupings to test (1) the repeatability of the neighbor effects under controlled conditions, and (2) whether early-season induction of responses would modify neighbor effects for focal plants at the center of groups. We expected that induced responses of focal plants would repel insects from the center of groups and onto neighbors or away from the patch. Given this expectation and our observations from the first year of study, we predicted that induction of the focal plants would enhance associational resistance for the preferred host, boneset, and reduce associational susceptibility for the secondary host, Joe Pye weed.

Materials and Methods

Study system

Boneset and Joe Pye weed frequently co-occur in wet fields and marshes and along creeks, and inhabit similar geographic ranges across eastern North America (Schmidt and Schilling 2000). While boneset and Joe Pye weed produce some similar defensive compounds in their leaves (particularly guaianolide sesquiterpene lactones;

Hensel et al. 2011), Joe Pye weed additionally produces pyrrolizidine alkaloids (Wiedenfeld et al. 2009). The two species also differ in physical defenses; boneset leaves and stems have dense trichomes while Joe Pye weed stems and leaves produce only moderate to low densities of trichomes (K. D. Holmes, *personal observation*). The leaf beetle *O. notata* is the only species in its genus to feed on host plants from the tribe Eupatorieae (Funk et al. 1995). Preliminary data show that *O. notata* larvae can mature and pupate while feeding exclusively on Joe Pye weed, although larvae feeding on boneset have higher mass (K. D. Holmes, *unpublished data*). Adult *O. notata* tend to lay eggs singly (Futuyma 1990), although clusters are occasionally observed (K. D. Holmes, *personal observation*). Larvae hatch at a very small size and skeletonize leaves, migrating up the stem to damage the meristem and younger foliage, and move to nearby stems at later instars (K. D. Holmes, *personal observation*). Information on adult dispersal is sparse; while the congener *Ophraella communa* is invasive in Asia and disperses significant distances (Tanaka and Yamanaka 2009), it must also disperse from ephemeral populations in its native range (Futuyma 1990). Other *Ophraella*, including *O. notata*, appear persistent in the populations of their perennial hosts (Futuyma 1990). In an open field, released *O. notata* adults have been observed to make repeated short burst flights of <30 cm between non-hosts in movement toward host plants (K. D. Holmes, *personal observation*).

Field surveys

To document *O. notata* presence on these two species in natural habitats, pairs of focal stems (either Joe Pye weed or boneset) were selected for observation in emergent wetland habitats and wet fields near Ithaca, New York, USA. Eight sites were visited in July 2016, and three sites were returned to in June 2017 (Fig. 1). The subset of sites surveyed in the second year was chosen for their accessibility, moderate to high density of both species, and because they represented sites with high, low, and equal proportions of Joe Pye weed to boneset in the community (Appendix S1: Fig. S1). Stems of either species of at least 25 cm in height were chosen every 3 m along transects. Once a focal stem was identified, a neighboring stem was located that was adjacent to, but not within 30 cm of the initial focal stem. This distance was chosen to prevent selecting neighboring stems that would grow in close physical contact (fully expanded leaf length is approximately 15–20 cm). As both Joe Pye weed and boneset clonally produce ramets, we could not differentiate stems that were from the same or different genetic individuals in the field. Transects were walked until at least six of each focal pair type (conspecific Joe Pye weed, conspecific boneset, or heterospecific pairs) were found in 2016, and until ten were found at each site in 2017 (Appendix S1: Table S1).

Two measures of herbivore presence and neighborhood were collected over the two survey years. In the first year, only presence/absence data were collected on *O. notata* eggs. Given that *O. notata* was one of the most abundant herbivores found on both species in the first year, in 2017 we counted the number of eggs on each focal stem. To test whether neighbor effects found in the first year of surveys were reflected in a broader spatial environment than the nearest stem, in the second year we also recorded the total number of either Joe Pye weed or boneset within 1 m of focal plants. Data on leaf damage were also collected both years. The youngest three whorls of leaves on each focal stem were surveyed for herbivory by visual estimation of damage in quarter intervals (e.g., 25% of a given leaf).

Common garden

To experimentally manipulate plant neighbors while controlling for proximity in a common abiotic environment, we planted a common garden of Joe Pye weed and boneset in a plowed field in Dryden, New York, USA. In 2016, we cold-stratified locally collected seeds of Joe Pye weed and boneset (The Plantsmen Nursery, Groton, New York, USA) for four weeks in damp soil before germinating them in a greenhouse in mid-May. Seedlings were transplanted into the field at the end of June. Seedlings were planted in groups of four, with 2 m between the center of each group (~1.5 m between group edges). Each group was arranged in one of four neighborhoods randomized within the common garden: Joe Pye weed or boneset monocultures, or two different heterospecific arrangements (n = 22 per neighborhood) for a total of 88 groups (Appendix S1: Fig. S6). Heterospecific groups consisted of a

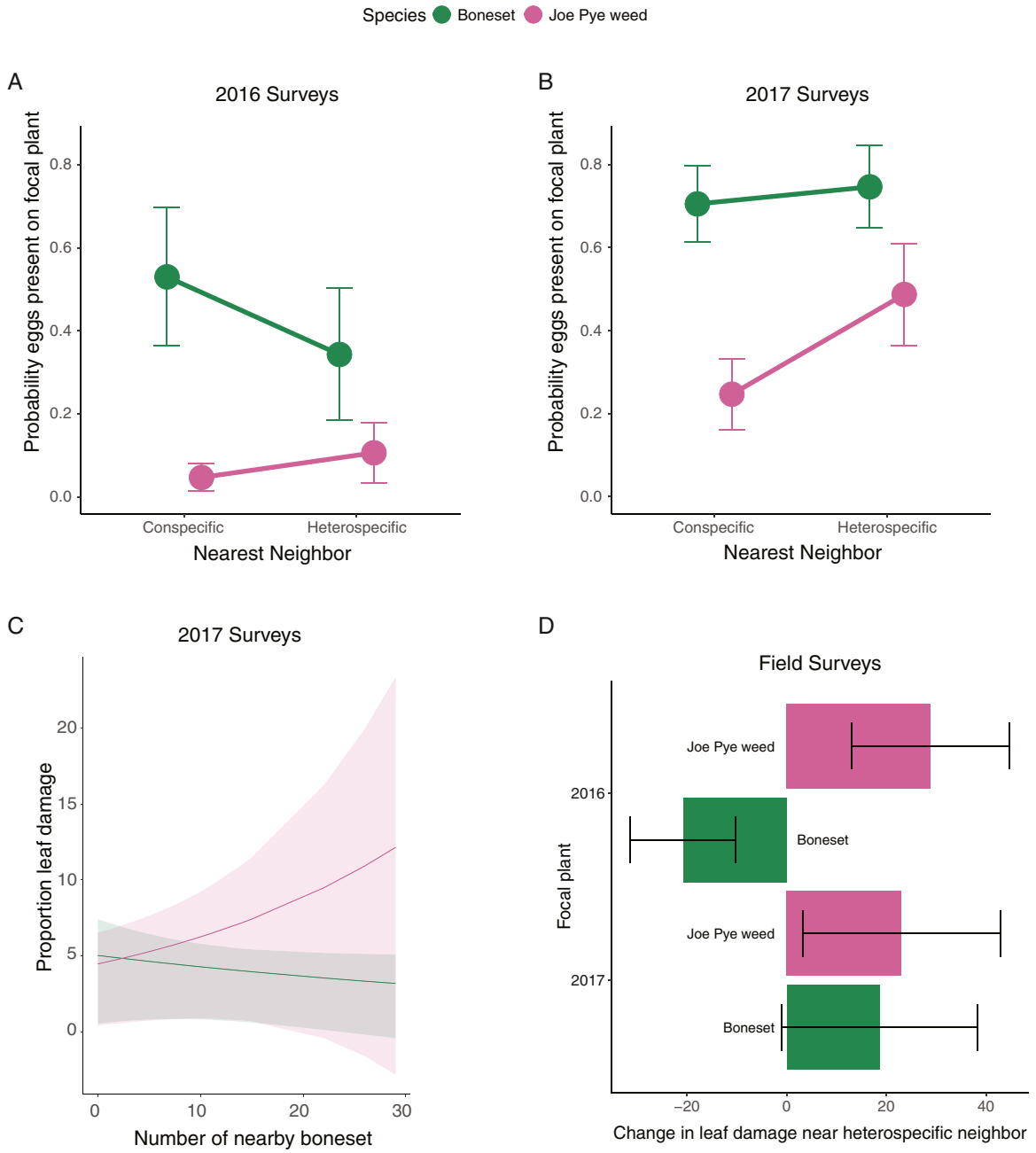

Fig. 1. Two years of surveys of plant neighbors impacting the likelihood of *Ophraella notata* egg presence on Joe Pye weed (green) and boneset (red) focal stems (A, B), or leaf damage (C, D). Values represent estimated marginal means and regression (A, B), regression (C), or percent change (D) from mixed effects models ± 1 standard error. Effects of the neighboring stem (C, D) and the number of boneset within a meter (C) on focal plant damage are also shown.

focal plant in the center of the group, surrounded by three heterospecific neighbors. Conspecific groups were arranged in the same spatial pattern of focal and neighbor plants (Fig. 2A). We allowed plants to establish in 2016 and by the following summer, each plant had grown multiple ramets as is common in both species (Fig. 2B). Hereafter, "stems" will be used to refer to plants

surveyed in the field, or known individuals in the common garden; and "ramets" will be used to describe the multiple stems known to belong to the same genetic individual in the common garden.

In early June 2017, we sprayed each focal plant with either 1.45 mL of a 5% aqueous solvent (95% ethanol) control, or a 10.5% solution of jasmonic acid (0.5 mmol/L). We estimate that less than 1 mL of spray reached the leaves. Jasmonic acid is a plant hormone that is upregulated following herbivory and induces a cascade of metabolic responses that widely increase plant resistance to herbivores (Thaler et al. 1996, Benevenuto et al. 2018), including increased production of sesquiterpene lactones and pyrrolizidine alkaloids (Abd El-Mawla 2010, Wang et al. 2010). Treatments were randomized spatially in 6 columns and 15 rows and subsequently adjusted to ensure there were no more than three adjacent groups of a given induction treatment or group type in a row or column. All plants in the common garden were surveyed for unhatched beetle eggs in May (pre-treatment) and June (two weeks post-treatment), when eggs were most abundant on plants (Appendix S1: Fig. S2). Plant height was also measured pre- and post-treatment (5 d after each egg survey), with ramet numbers also counted at the end of May. To assess leaf damage due to herbivory, half of ramets of each plant were sampled in a cross-section in early July. Only half of ramets were surveyed due to sampling effort limitations; the common garden included 88 groups with over 3787 total ramets. In this measure, leaf damage was assessed using a scale of 0–100% damage (starting from 0%, 5%, 10% and thereafter in intervals of 10%) following observer training by comparing visual observations with leaf area loss measurements in Image J (Schneider et al. 2012).

Statistical analyses

All data analysis was conducted in R (v. 3.5.2, Free Software Foundation, Inc. Boston, Massachusett, USA). Data on the presence or absence of beetle eggs on plants at field sites in 2016 were analyzed using binomial mixed-effect models (glmer; Bates et al. 2015). All models of field survey data included random effects for (1) site and (2) stem pair number embedded within site. Pair number was used as a random effect to account for the potential non-independence caused by proximity of stems in a pair. Counts of beetle egg numbers in the second year of field surveys and the common garden were analyzed using negative binomial models (glmmTMB; Brooks et al. 2017). Counts of leaf quarters lost to herbivore damage were also analyzed using negative binomial models. To account for the fact that the two plant species differ in the number of leaves in each whorl, total leaf number was included in models of leaf damage. The species identity of focal and neighbor plants (and their interaction) was treated as predictors in all models of leaf damage and egg number. Post hoc tests were performed using the function emmeans (emmeans; Lenth 2016).

In the common garden, the effects of species, neighborhood (conspecific or heterospecific group), and position in the group (center vs. neighbor) on plant susceptibility to *O. notata* eggs were tested in May, prior to the jasmonic acid treatment. Group number was added to all pre-treatment models as a random effect. Data collected following the JA treatment in June were analyzed for the effects of treatment and group type on focal plant susceptibility to *O. notata* oviposition and total insect leaf damage. The number of ramets and prior egg counts for each plant was also added as a covariate to all models predicting egg number and leaf damage to control for variation in plant size and early oviposition. Species were found to differ significantly in size (see *Results*), and so estimated marginal means and standard errors for treatment, position, and neighbor effects presented graphically and in the text are derived from single-species models (emmeans; Lenth 2016). The exception is our analysis of neighborhood type differences in May, when egg counts on neighbor were collected as pooled data; here, ramet number is controlled for at the level of center and neighbor plants. Statistics for the significance of predictors are reported from likelihood ratio tests between models (with one degree of freedom) unless otherwise noted (anova; R Core Team 2020). Any additional post hoc statistical tests (e.g., percent differences) were conducted using pairwise comparisons of estimated marginal means. Where appropriate, correlation coefficients of factors within a model were calculated using the functions vcov and cov2cor (R Core Team 2020).

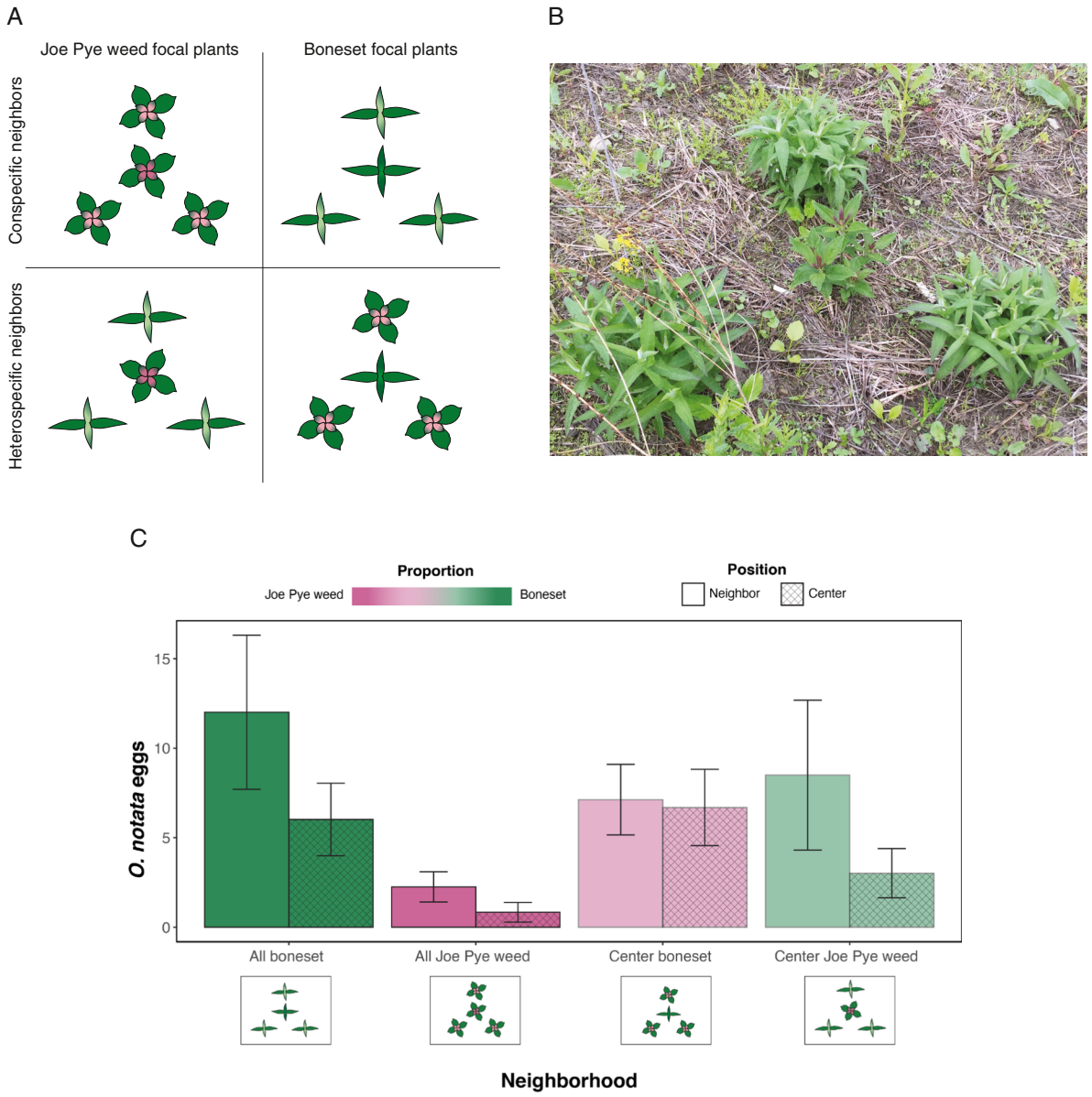

Fig. 2. Common garden design and pre-treatment neighbor effects. (A) Groups were arranged with one Joe Pye weed or boneset plant in the center surrounded by three neighbors of the same or other species. (B) A photograph of a group with Joe Pye weed surrounded by boneset. Estimated marginal means (±1 standard error) from a model of early-season (May) *Ophraella notata* egg counts controlling for plant size (ramet number) are shown in C. Means of aggregated egg counts for neighbor plants in each group are shown in solid bars, while focal plant averages are displayed in hatched bars.

Results

Field surveys

Q1: How do neighbors affect susceptibility to a specialist herbivore?—Across two years, *O. notata* eggs, larvae, or adults were found on boneset at all eight sites, and on Joe Pye weed at seven of the same sites. 79% of eggs, 70% of larvae, and 86% of adults were found on boneset (n = 358 Joe Pye weed stems and 342 boneset stems). Neighbor effects on the presence of *O. notata* eggs were strong and differed by species (focal

species × neighbor species: $D = 766.7$, $\chi^2 = 8.79$, $P = 0.004$). For Joe Pye weed, the probability of *O. notata* egg presence rose from 8% to 18% when it grew near boneset relative to conspecifics across both years ($D = 353.9$, $\chi^2 = 9.10$, $P = 0.003$, Fig. 1A), while there was no overall effect of neighbor identity for boneset (Fig. 1B, $D = 406$, $\chi^2 = 1.20$, $P = 0.27$); a trend of associational resistance for boneset growing near Joe Pye weed in the first year of surveys was not statistically significant ($D = 272$, $\chi^2 = 2.79$, $P = 0.09$). Our larger-scale measure of neighborhood—the number of Joe Pye weed or boneset growing within 1 m of focal plants—did not predict the number of *O. notata* eggs on either species in 2017 (Joe Pye weed stems $\chi^2 = 0.90$, $P = 0.34$; boneset stems $\chi^2 = 2.68$, $P = 0.10$).

Q2: How do neighbors affect susceptibility to herbivore damage?.—Joe Pye weed and boneset experienced the same amount of total leaf herbivory over both years; across sites, both species lost an average of 17% leaf area to herbivores in 2016 and 13% in 2017 (species: 2016 $\chi^2 = 0.002$, $P = 0.97$; 2017 $\chi^2 = 0.17$, $P = 0.68$). In 2016, the effects of the nearest neighbor on leaf damage differed by plant species (focal species × neighbor $\chi^2 = 7.33$, $P = 0.007$, Fig. 1C). When Joe Pye weed grew near boneset, there was 29% more leaf damage (neighbor $\chi^2 = 4.10$, $P = 0.043$), while there was a trend for 21% less leaf damage on boneset growing near Joe Pye weed (neighbor $\chi^2 = 3.10$, $P = 0.079$). In 2017, the identity of the neighboring focal stem did not predict herbivory (neighbor $\chi^2 = 2.62$, $P = 0.11$; species × neighbor $\chi^2 = 0.004$, $P = 0.95$), but leaf damage on Joe Pye weed did increase with the addition of each boneset stem growing within a meter, while damage on boneset was reduced (focal species × boneset stems $\chi^2 = 5.64$, $P = 0.02$; Fig. 1D).

Common garden

Q1: Are neighbor effects independent of site-specific factors?—In the common garden, the effects of neighbors on plant susceptibility to *O. notata* oviposition were remarkably similar to those observed in field surveys. Early in the growing season, the number of *O. notata* eggs across all plants in a patch was predicted by the combination of plant species and neighbor identity (group type × species $\chi^2 = 8.44$, $P = 0.004$, Fig. 2C). As in field surveys, the strongest neighbor effect was on Joe Pye weed, where oviposition increased by 238% on all plants in groups with boneset (group type $\chi^2 = 7.30$, $P = 0.007$). But group type did not impact boneset susceptibility to *O. notata* eggs (group type $\chi^2 = 0.24$, $P = 0.63$). Plants at the center of groups had 42% fewer eggs on average than neighbors (position $\chi^2 = 3.88$, $P = 0.049$), irrespective of species (species × position $\chi^2 = 0.28$, $P = 0.60$, Fig. 2C) or group type (position × group type $\chi^2 = 0.92$, $P = 0.34$). However, boneset at the center of groups still had more eggs than Joe Pye weed at the center of groups, consistent with field host use patterns (species $\chi^2 = 15.50$, $P < 0.001$, Fig. 2C).

Q2: Does jasmonate induction modify the effect of neighbors on susceptibility?—The jasmonic acid treatment had a similar effect on both species (species × treatment $\chi^2 = 1.58$, $P = 0.21$), reducing the number of *O. notata* eggs on focal plants in heterospecific groups, but not in conspecific groups (group × treatment, $\chi^2 = 6.36$, $P = 0.01$). For Joe Pye weed, induction decreased oviposition by 78% when it was next to boneset neighbors, but increased oviposition 10-fold near Joe Pye weed neighbors. However, counts remained low for the latter (Fig. 3A). Neighbor-dependent responses to induction were also observed for boneset, with a 60% reduction in egg number on boneset surrounded by Joe Pye weed, but no effect of induction in the midst of boneset neighbors (Fig. 3B). Induction of focal plants with jasmonic acid did not affect the surrounding neighbors' susceptibility to eggs for any patch type (treatment $\chi^2 = 0.01$, df = 1, $P = 0.90$).

In the common garden, plant height was also positively correlated with egg number on boneset ($\chi^2 = 9.84$, $P = 0.002$), but not Joe Pye weed ($\chi^2 = 1.05$, $P = 0.31$). Both species grew taller in heterospecific groups than conspecific groups, particularly Joe Pye weed focal plants (species × group type × position $F = 2.97$, $P = 0.09$, Appendix S1: Fig. S3). Boneset was taller than Joe Pye weed across environments, and in heterospecific groups had more ramets (species × mixed $F = 12.22$, df = 1, $P < 0.001$, Appendix S1: Fig. S4). Thus, Joe Pye weed grew taller amidst larger boneset neighbors. The jasmonic acid treatment suppressed boneset focal plant growth when it was surrounded by Joe Pye

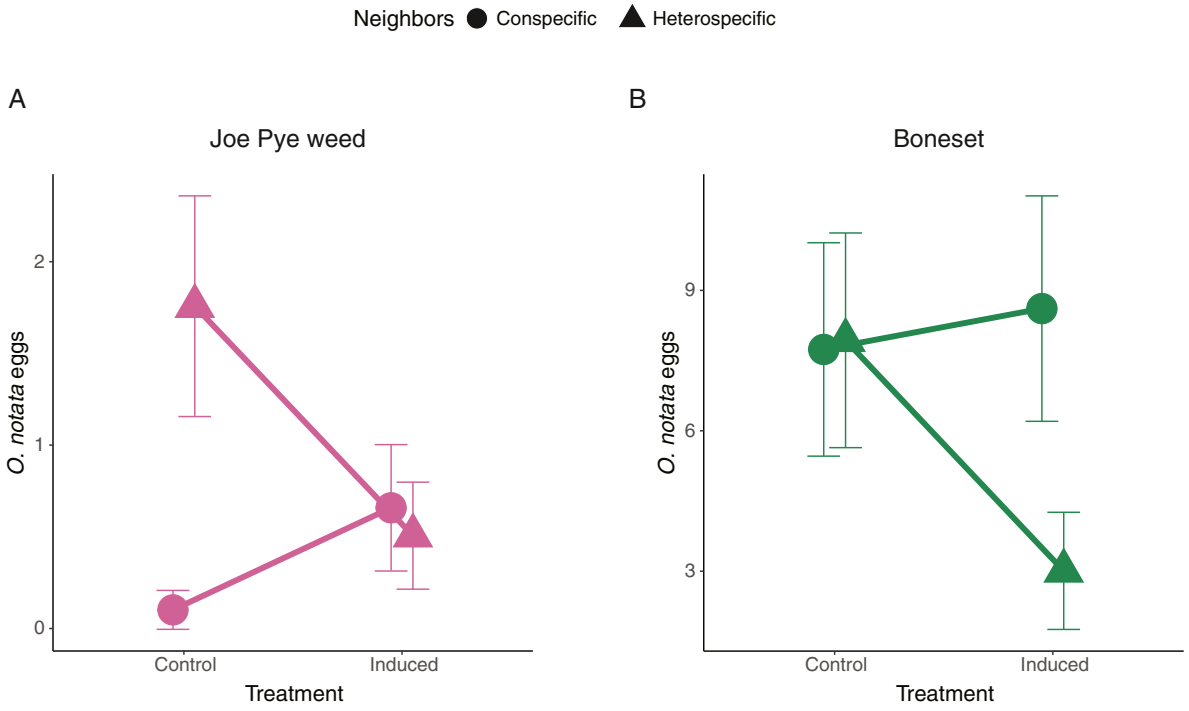

Fig. 3. Effects of jasmonic acid treatment on *Ophraella notata* oviposition on focal plants in the common garden for (A) Joe Pye weed (red) and (B) boneset (green). Estimated marginal means and ±1 standard error are shown.

weed (treatment × group type $F = 4.48$, $P = 0.041$; Appendix S1: Fig. S5).

Q3: Does jasmonate induction modify the effect of neighbors on damage?.—The average percent leaf tissue lost to herbivore damage on each plant was not related to jasmonic acid induction (treatment $\chi^2 = 1.71$, $P = 0.19$). Plant height was positively correlated with leaf damage on Joe Pye weed ($\chi^2 = 8.82$, $P = 0.003$), but not boneset ($\chi^2 = 0.33$, $P = 0.57$).

Discussion

We found consistent negative neighbor effects for Joe Pye weed over two years of field surveys and in a common garden, where plants were more susceptible to beetle oviposition and leaf damage near the beetle's primary host, boneset. In addition, we found that neighbor effects changed when we experimentally manipulated induced responses to herbivory, a widespread form of trait plasticity in plants (Karban and Baldwin 1997). Our manipulation resulted in changes in susceptibility consistent with induced resistance, modifying associational effects; in particular, jasmonate induction reduced insect oviposition on Joe Pye weed when near boneset, effectively mitigating associational susceptibility.

While associational resistance in the form of reduced herbivory is more commonly documented in the literature, associational susceptibility is frequently reported in studies that measure impacts on insect herbivores (Barbosa et al. 2009). Proximity to preferred hosts is known to cause spillover of insect herbivores onto plants and is likely the mechanism for boneset's negative effect on Joe Pye weed (Futuyma and Wasserman 1980, White and Whitham 2000, Russell and Louda 2005, Stenberg and Ericson 2015). We found that Joe Pye weed's associational susceptibility to *O. notata* occured at the small scale of a nearest neighbors in both 2016 and 2017; and in 2017, an increasing number of boneset within a square meter corresponded with greater leaf damage. This greater scale of susceptibility is likely due to the presence of additional damaging boneset specialists at the field sites, particularly a Eupatorium plume moth

(*Oidaematophorus* spp.). Boneset, however, experienced less consistent neighbor effects (associational resistance) growing near Joe Pye weed across our two years of field surveys. It is noteworthy that neighbor effects were not strongly reciprocal in our study and that the effect of neighbor identity on herbivore oviposition and damage was strongest and most consistent on the less preferred host. In general, the patterns we observed best fit classic definitions of associational resistance and susceptibility (Champagne et al. 2016).

Neighbor effects were mirrored in the common garden, and jasmonate induction caused complex ecological effects. Application of the plant hormone to focal plants did not reduce leaf damage. Damage was measured one month following the JA treatment, and early effects on herbivory by *O. notata* or other damaging insects may have diminished. However, egg-laying *O. notata* were repelled by the jasmonic acid treatment, particularly in mixed-species neighbor environments. Effectively, induction created associational resistance to *O. notata* for boneset growing near Joe Pye weed and mitigated the pattern of associational susceptibility for Joe Pye weed near boneset.

The differential effect of induction on both species in conspecific vs. heterospecific groups (Fig. 3A, B) appears to be due to the combination of individual and group-level attraction. Same-species groups of boneset and Joe Pye weed may have been perceived as the highest and lowest quality patches by egg-laying *O. notata*. In these groups, induction effects were either non-existent (in the case of boneset) or even positive (for Joe Pye weed). In the latter case, average rates of *O. notata* oviposition were low even in induced groups. In mixed species groups however, induction of both boneset and Joe Pye weed focal plants substantially reduced susceptibility to oviposition. It seems likely that induction increased the phenotypic contrast between poor quality Joe Pye weed focal plants and high-quality boneset neighbors, mitigating associational susceptibility. But for boneset, induction appeared to reduce the contrast between focal and neighbor plants, yielding associational resistance as seen in the field. This corresponded with changes in height; our treatment suppressed boneset height among Joe Pye weed neighbors, and attractiveness to ovipositing females decreased. Changes in boneset height likely corresponded with other JA-induced physiological changes that deterred oviposition, such as an increase in sesquiterpene lactone compounds in plant tissues (Wang et al. 2010).

We did not detect effects of treatment on overall group susceptibility, likely due to the relatively low occurrence of *O. notata* on focal plants relative to the three neighbors in each group (which also tended to be larger than center plants). Instead, treatment effects were primarily on focal plants and led us to conclude that an increase in contrast between focal and neighbor plants reduced associational susceptibility for a poor quality host; a decrease in the focal-neighbor contrast for yielded associational resistance for our high-quality host. Others have also found that presence and direction of associational effects depend on the contrast between focal and neighbor plants (Huang et al. 2016). Assessing the degree to which induction amplifies or reduces the contrast between focal and neighbor plants in different groups contributes to our understanding of how plant neighborhood affects host plant choice in herbivores. Considering susceptibility in this context is also more inclusive of herbivore life-history and behavior, as the degree to which herbivores detect phenotypic contrast between potential hosts will depend on herbivore traits such as sensory ability, dispersal mode and degree of specialization (Hambäck et al. 2014, Kim 2017, Ida et al. 2018, Verschut et al. 2018).

The phenotypic contrast between focal and neighbor plants, and the impact of defense plasticity on that contrast, may also depend on other plant–plant interactions that affect phenotype. Associational effects are expected to depend upon the relative density and frequency of neighbors of the same or different species, but such variation is also likely to contribute to differences in competition for resources between plants and corresponding differences in herbivore resistance (Kim 2017). In this study, both species grew taller in heterospecific groups than conspecific groups, suggesting that the neighbor-dependent effects of our jasmonic acid treatment may have corresponded with differences in competitive environment and associated changes in plant physiology. In other study systems, resource

competition has frequently been found to affect plant physiological responses to signals of herbivore damage, often by suppressing defense induction (Cipollini and Bergelson 2001, Izaguirre et al. 2006, 2013, Jones et al. 2006). There is also evidence for differential plant induction in response to conspecific vs. heterospecific neighbors (Broz et al. 2010, Kigathi et al. 2019). In our case, we found that plant height affected boneset susceptibility and corresponded with results expected from defense induction; induced plants were shorter and less susceptible to oviposition. Although studies of associational effects rarely assess competitive dynamics (but see Graff et al. 2007), variable levels of resource competition with neighbors and subsequent effects on plant defenses could act as important drivers or sources of variation in neighbor effects in the field.

The interaction between associational effects and induced resistance has received comparatively little study, but some evidence suggests that plasticity may allow plants to maximize fitness across associational contexts (Coverdale et al. 2018). Since the production of defenses in the absence of herbivores can be costly (Agrawal 1999), plasticity (i.e., defense relaxation) may allow plants to optimize resource allocation toward growth or defense in different neighbor environments (Züst and Agrawal 2017). In our system, plasticity may allow plants to minimize the deleterious effect of associational susceptibility by increasing defense in areas of high herbivory. This result raises important questions about the role of inducibility in reducing spatial heterogeneity in herbivore risk, the interaction of defense plasticity with other neighbor-dependent plant interactions, and the mechanisms by which induction affects herbivore behavior in the context of local communities.

Acknowledgments

We thank Meghan Hayden for assistance with data collection, Zachary Stoessel for help planting the common garden, and Erika Mudrak for guidance in statistical analysis. Amy Hastings, Lina Arcila Hernandez, Tyler Coverdale, Monica Geber, Katherine Eisen, Aubrie James, and Gregor Siegmund provided helpful comments on this manuscript. This research was supported by the Cornell Kieckhefer Student Research Grant and NSF IOS-1907491.

Literature Cited

Abd El-Mawla, A. M. A. 2010. Effect of certain elicitors on production of pyrrolizidine alkaloids in hairy root cultures of *Echium rauwolfii*. Die Pharmazie 65:224–226.

Agrawal, A. A. 1999. Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. Ecology 80:1713–1723.

Agrawal, A. A. 2004. Resistance and susceptibility of milkweed: competition, root herbivory, and plant genetic variation. Ecology 85:2118–2133.

Agrawal, A. A., J. A. Lau, and P. A. Hambäck. 2006. Community heterogeneity and the evolution of interactions between plants and insect herbivores. Quarterly Review of Biology 81: 349–376.

Alpert, P., and E. L. Simms. 2002. The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? Evolutionary Ecology 16:285–297.

Atsatt, P. R., and D. J. O'Dowd. 1976. Plant defense guilds. Science 193:24–29.

Barbosa, P., J. Hines, I. Kaplan, H. Martinson, A. Szczepaniec, and Z. Szendrei. 2009. Associational resistance and associational susceptibility: Having right or wrong neighbors. Annual Review of Ecology, Evolution, and Systematics 40:1–20.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Benevenuto, R. F., S. J. Hegland, J. P. Töpper, K. Rydgren, S. R. Moe, C. Rodriguez-Saona, and T. Seldal. 2018. Multiannual effects of induced plant defenses: Are defended plants good or bad neighbors? Ecology and Evolution 8:8940–8950.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R Journal 9:378–400.

Brown, B. J., and J. J. Ewel. 1987. Herbivory in complex and simple tropical successional ecosystems. Ecology 68:108–116.

Broz, A. K., C. D. Broeckling, C. De-la-Peña, M. R. Lewis, E. Greene, R. M. Callaway, L. W. Sumner, and J. M. Vivanco. 2010. Plant neighbor identity influences plant biochemistry and physiology related to defense. BMC Plant Biology 10:115.

Callaway, R. M., S. C. Pennings, and C. L. Richards. 2003. Phenotypic plasticity and interactions among plants. Ecology 84:1115–1128.

Champagne, E., J.-P. Tremblay, and S. D. Côté. 2016. Spatial extent of neighboring plants influences the

strength of associational effects on mammal herbivory. Ecosphere 7:e01371.

Cipollini, D. F., and J. Bergelson. 2001. Plant density and nutrient availability constrain constitutive and wound-induced expression of trypsin inhibitors in *Brassica napus*. Journal of Chemical Ecology 27:593–610.

Coverdale, T. C., J. R. Goheen, T. M. Palmer, and R. M. Pringle. 2018. Good neighbors make good defenses: Associational refuges reduce defense investment in African savanna plants. Ecology 99:1724–1736.

Edwards, P. J., S. D. Wratten, and S. Greenwood. 1986. Palatability of British trees to insects: constitutive and induced defences. Oecologia 69:316–319.

Funk, D. J., D. J. Futuyma, G. Orti, and A. Meyer. 1995. A history of host associations and evolutionary diversification for *Ophraella* (Coleoptera: Chrysomelidae): New evidence from mitochondrial DNA. Evolution 49:1008–1017.

Futuyma, D. J. 1990. Observations on the taxonomy and natural history of *Ophraella* Wilcox (Coleoptera: Chrysomelidae), with a description of a new species. Journal of the New York Entomological Society 98:163–186.

Futuyma, D. J., and S. S. Wasserman. 1980. Resource concentration and herbivory in oak forests. Science 210:920–922.

Graff, P., M. R. Aguiar, and E. J. Chaneton. 2007. Shifts in positive and negative plant interactions along a grazing intensity gradient. Ecology 88:188–199.

Hambäck, P. A., and A. P. Beckerman. 2003. Herbivory and plant resource competition: a review of two interacting interactions. Oikos 101:26–37.

Hambäck, P. A., B. D. Inouye, P. Andersson, and N. Underwood. 2014. Effects of plant neighborhoods on plant–herbivore interactions: resource dilution and associational effects. Ecology 95:1370–1383.

Hensel, A., M. Maas, J. Sendker, M. Lechtenberg, F. Petereit, A. Deters, T. Schmidt, and T. Stark. 2011. *Eupatorium perfoliatum* L.: phytochemistry, traditional use and current applications. Journal of Ethnopharmacology 138:641–651.

Huang, Y., L. Wang, D. Wang, D.-H. Zeng, and C. Liu. 2016. How does the foraging behavior of large herbivores cause different associational plant defenses? Scientific Reports 6:20561.

Ida, T. Y., K. Takanashi, M. Tamura, R. Ozawa, Y. Nakashima, and T. Ohgushi. 2018. Defensive chemicals of neighboring plants limit visits of herbivorous insects: Associational resistance within a plant population. Ecology and Evolution 8:12981–12990.

Izaguirre, M. M., C. A. Mazza, M. S. Astigueta, A. M. Ciarla, and C. L. Ballaré. 2013. No time for candy: Passionfruit (*Passiflora edulis*) plants down-regulate damage-induced extra floral nectar production in response to light signals of competition. Oecologia 173:213–221.

Izaguirre, M. M., C. A. Mazza, M. Biondini, I. T. Baldwin, and C. L. Ballaré. 2006. Remote sensing of future competitors: impacts on plant defenses. Proceedings of the National Academy of Sciences USA 103:7170–7174.

Jones, T., S. Kulseth, K. Mechtenberg, C. Jorgenson, M. Zehfus, P. Brown, and D. H. Siemens. 2006. Simultaneous evolution of competitiveness and defense: induced switching in *Arabis drummondii*. Plant Ecology 184:245–257.

Karban, R., and I. T. Baldwin. 1997. Induced responses to herbivory. University of Chicago Press, Chicago, Illinois, USA.

Khan, Z., C. Midega, J. Pittchar, J. Pickett, and T. Bruce. 2011. Push—pull technology: a conservation agriculture approach for integrated management of insect pests, weeds and soil health in Africa. International Journal of Agricultural Sustainability 9:162–170.

Kigathi, R. N., W. W. Weisser, M. Reichelt, J. Gershenzon, and S. B. Unsicker. 2019. Plant volatile emission depends on the species composition of the neighboring plant community. BMC Plant Biology 19:58.

Kim, T. N. 2017. How plant neighborhood composition influences herbivory: Testing four mechanisms of associational resistance and susceptibility. PLOS ONE 12:e0176499.

Lenth, R. 2016. lsmeans: least-squares means. R package version 2.20-23. https://www.jstatsoft.org/article/view/v069i01

Morrell, K., and A. Kessler. 2017. Plant communication in a widespread goldenrod: keeping herbivores on the move. Functional Ecology 31:1049–1061.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Russell, F. L., and S. M. Louda. 2005. Indirect interaction between two native thistles mediated by an invasive exotic floral herbivore. Oecologia 146:373–384.

Schmidt, G. J., and E. E. Schilling. 2000. Phylogeny and biogeography of *Eupatorium* (Asteraceae:Eupatorieae) based on nuclear its sequence data. American Journal of Botany 87:716–726.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675.

Stenberg, J. A., and L. Ericson. 2015. Raspberry inflicts associational susceptibility to meadowsweet in a complex food web. Entomologia Experimentalis et Applicata 157:68–73.

Tahvanainen, J. O., and R. B. Root. 1972. The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). Oecologia 10:321–346.

Tanaka, K., and T. Yamanaka. 2009. Factors affecting flight activity of *Ophraella communa* (Coleoptera: Chrysomelidae), an exotic insect in Japan. Environmental Entomology 38:235–241.

Thaler, J. S., M. J. Stout, R. Karban, and S. S. Duffey. 1996. Exogenous jasmonates simulate insect wounding in tomato plants (*Lycopersicon esculentum*) in the laboratory and field. Journal of Chemical Ecology 22:1767–1781.

Thaler, J. S., M. J. Stout, R. Karban, and S. S. Duffey. 2001. Jasmonate-mediated induced plant resistance affects a community of herbivores. Ecological Entomology 26:312–324.

Underwood, N., K. Anderson, and B. D. Inouye. 2005. Induced vs. constitutive resistance and the spatial distribution of insect herbivores among plants. Ecology 86:594–602.

Verschut, T. A., B. D. Inouye, and P. A. Hambäck. 2018. Sensory deficiencies affect resource selection and associational effects at two spatial scales. Ecology and Evolution 8:10569–10577.

Wang, H., C. Ma, Z. Li, L. Ma, H. Wang, H. Ye, G. Xu, and B. Liu. 2010. Effects of exogenous methyl jasmonate on artemisinin biosynthesis and secondary metabolites in *Artemisia annua* L. Industrial Crops and Products 31:214–218.

White, J. A., and T. G. Whitham. 2000. Associational susceptibility of cottonwood to a box elder herbivore. Ecology 81:1795–1803.

Wiedenfeld, H., G. Hösch, E. Roeder, and T. Dingermann. 2009. Lycopsamine and cumambrin B from *Eupatorium maculatum*. Die Pharmazie 64:415–416.

Zangerl, A. R., and M. R. Berenbaum. 2003. Phenotype matching in wild parsnip and parsnip webworms: causes and consequences. Evolution 57:806–815.

Züst, T., and A. A. Agrawal. 2017. Trade-offs between plant growth and defense against insect herbivory: an emerging mechanistic synthesis. Annual Review of Plant Biology 68:513–534.

Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3334/full