Foliar nectar enhances plant–mite mutualisms: the effect of leaf sugar on the control of powdery mildew by domatia-inhabiting mites

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INTRODUCTION

Mite domatia are specialized plant traits that can house predacious or fungivorous mites on leaves in return for protection against natural enemies, such as pathogenic fungi and small herbivores (Lundström, 1887; O’Dowd and Willson, 1991; Romero and Benson, 2005). They are taxonomically widespread, have evolved many times across the plant tree of life, and occur in economically important crop plants such as cherry, coffee, and avocado (Brouwer and Clifford, 1990). Because their only known function is to defend plants via the retention of mutualistic mite bodyguards, mite domatia can serve as a model trait for understanding the evolution and ecology of mutualistic defence. However, while mite domatia frequently house predacious or fungivorous mites, they can also contain a myriad of other small arthropods, including plant parasites, and studies testing for impacts of domatia-inhabiting mites on plant enemies have produced mixed results (Walter et al., 1995; Walter, 1996). Because of the context dependency of these interactions, clarifying how mite domatia interact with other plant traits to reduce plant damage, as well as what other phenotypes impact the evolutionary distribution of domatia across plants, is of interest for disentangling what factors govern mutualism gain, loss and maintenance over ecological contexts and evolutionary time.

One widespread plant trait hypothesized to impact the selective benefit of mite domatia is extraloral nectar; a sugary substance secreted from non-floral plant glands (called extraloral nectaries) that attracts and feeds beneficial arthropods (Koptur, 1992). Although extraloral nectar has typically been studied for its role in attracting ant bodyguards to plants (Bentley, 1977; Heil, 2008), several recent studies have suggested that extraloral nectar may also enhance plant–mite defence mutualisms by facilitating larger standing populations of mites on leaves (Walter et al., 1995; Walter, 1996; van Rijn and Tanigoshi, 1999; Weber et al., 2012). In work on the castor oil plant Ricinus communis, predacious mites were observed visiting extraloral nectaries, and the presence of extraloral nectar was experimentally demonstrated to increase population growth of predacious mites (Walter, 1996; van Rijn and Tanigoshi, 1999). Walter et al. (1995) found that experimentally excising extraloral nectaries from leaves reduced the number of...
domatia-dwelling fungivorous mites. Finally, in a common garden of phylogenetically diverse species of Viburnum, the full factorial experimental manipulation of both domatia and extrafloral nectaries revealed that extrafloral nectaries on the lamina increase the abundance of fungus-eating mites inhabiting domatia (Weber et al., 2012). In this last study, the observed additive effect of housing (domatia) and food (extrafloral nectar) rewards on mite abundance was hypothesized to have driven, in part, the macro-evolutionary correlation of extrafloral nectaries and domatia across the Viburnum genus.

Despite evidence that extrafloral nectar can increase the abundance of domatia-dwelling mites, currently no research has directly evaluated whether this increase in mite abundance translates into a decreased pathogen or pest load for the plant, a key requirement if the interaction is to have a selective benefit. To fill this gap, we conducted an empirical test of the hypothesis that foliar sugar indirectly decreases leaf pathogen load by increasing the abundance of mutualistic mites inhabiting domatia. We use two species of wild grape, Vitis munsoniana and V. riparia (Vitaceae), and their naturally occurring mutualistic mite communities as study systems. Both grape species have domatia that are inhabited almost exclusively by mutualistic (fungivorous and predacious) mites rather than phytophagous mites (Walter and Denmark, 1991; Karban et al., 1995; O’Dowd and Willson, 1997). Furthermore, high levels of domatia-inhabiting mites have been demonstrated to reduce powdery mildew growth effectively in the riverbank grape V. riparia and in the cultivated grape V. vinifera, but these impacts were dependent upon sufficiently high abundances of mite mutualists on leaves, which are not always present in natural systems (Norton et al., 2000; English-Loeb et al., 2005).

We used experimental field manipulations to evaluate the hypothesis that foliar nectar enhances domatia-mediated mite–plant mutualisms. In particular, we asked the following two questions. (1) Is mutualistic mite abundance higher on leaves supplemented with foliar sugar compared with leaves without sugar? (2) If so, does this increase in domatia-inhabiting mites translate into a decreased load of powdery mildew (Erysiphe necator) on grape leaves? To assess the general co-occurrence of extrafloral nectar and leaf domatia across plant clades and to identify candidate clades for future study, we paired these experiments with a broad survey of the frequency with which extrafloral nectaries and mite domatia occur in the same groups across vascular plants.

MATERIALS AND METHODS

Experimental manipulations

We simulated the presence of extrafloral nectar in field experiments on two species of wild grape: V. munsoniana and V. riparia. Neither species naturally possesses extrafloral nectar, but close relatives (members of the genus, Cissus) have extrafloral nectaries (Bentley, 1977; Baker et al., 1978; Schupp and Feener, 1991; Fiala and Linsenmair, 1995; Mexzon and Chincilla, 1999; Díaz-Castelazo et al., 2004). Extrafloral nectar was simulated by applying two small drops (approx. 40–50 μL) of sugar solution to the abaxial surface of leaves (Fig. 1) daily between 0700 and 1000 h (hereafter referred to as the ‘foliar sugar’ treatment). Controls received the same amount of physical handling as treatment leaves, but no foliar sugar solution was applied. The sugar solution consisted of 60 % volume honey, 30 % volume water and 10 % volume artificial nectar (Songbird Essentials, Inc.: contains dextrose, sucrose, fructose and sodium salts). We chose this composition to mimic the high sugar concentration and complex composition (typically a mix of sugars and amino acids) common to extrafloral nectars (Bentley, 1977; Koptur, 1992).

Vitis munsoniana manipulation

Vitis munsoniana experiments were conducted at the Archbold Biological Station south of Lake Placid, Florida, USA, from 1 April 1 to 9 April 2014. A total of 40 leaves were haphazardly chosen from multiple grapevines and assigned to treatment (foliar sugar added, n = 20 leaves) or control (leaves handled but no foliar sugar added, n = 20 leaves). All leaves were mature (fully expanded), intact whole leaves of similar stages. After 9 d (to allow mite numbers to acclimate), leaves were destructively harvested, placed in moist paper towels, transported to the lab on ice, and surveyed for mites under a dissecting scope. Mites found on leaves were counted and scored according to morphospecies. Representatives from each morphospecies were mounted in Hoyers solution on microscope slides and identified to taxonomic family (Krantz and Walter, 2009). Because mites within families typically have a conserved diet range (i.e. mycophagous, predaceous, herbivorous) (Krantz and Walter, 2009), we used family as an indication of their interactions with the plant. For each leaf, we recorded the total number of domatia present, the number of domatia occupied by mites, the total abundance of mites per leaf and the relative abundance of all trophic guilds of mites (mutualistic vs. parasitic). Because larger leaves may have higher abundances of organisms simply due to their size, we controlled for differences in leaf size by recording leaf length measured as the distance from the base of the leaf to the leaf tip. While area is a more direct measure of size, several studies have shown that length is linearly related to total area in grape leaves (Elsner and Jubb, 1988; Blom and Tarara, 2007).

Vitis riparia manipulation

We conducted a second experiment on a population of V. riparia in Ithaca, NY from 14 June to 6 July 2014. For this experiment, we manipulated a total of 40 leaves (20 = foliar sugar addition, 20 = control). Treatment leaves received two small drops (approx. 40–50 μL) of nectar solution on the abaxial surface daily between 0700 and 1000 h. Nine days after the start of the experiment, 20 leaves (ten control and ten treatment) were destructively sampled and surveyed for mutualistic mite abundance and domatia traits as described above. The remaining 20 leaves, however, were immediately inoculated with a laboratory-maintained powdery mildew (E. necator) population originating from V. vinifera leaves of cultivated grape in the NY Experimental Station in Geneva, NY. Erysiphe is a group of widespread and virulent plant pathogens, impacting many plant taxa globally. Erysiphe necator, the grapevine powdery mildew, is a major pathogen of Vitaceae species with devastating effects
on both wild and cultivated populations. While highly virulent, *E. necator* can have a patchy distribution in both time and space. As such, we chose to inoculate with *E. necator* rather than use natural levels of the pathogen to ensure the presence of the disease on our experimental leaves. Two types of powdery mildew inoculation were applied to ensure high colonization of leaves with conidia. First, we depressed the lower, abaxial leaf surface of an infected *V. vinifera* leaf to the lower leaf surface of each experimental *V. riparia* leaf. Secondly, to ensure infection further, a suspension of *E. necator* conidia in distilled water was misted directly onto the abaxial leaf surface of each experimental leaf. We estimated the severity of mildew infection 8 d after inoculation by scoring the presence or absence of mycelia on the abaxial and adaxial surfaces at six, 1 cm diameter circles at standard locations across the leaf surface.

**STATISTICAL ANALYSES**

All analyses were carried out in R version 3.0.1 (R Development Core Team, 2015). The effects of the experimental addition of foliar sugar on mite abundance and/or domatia occupancy, and the relationship between mite abundance and powdery mildew growth (for the *V. riparia* experiment only), were assessed using generalized linear models using the glm function in R. Because response variables were count data, Poisson error distributions were utilized for all GLM analyses. One outlier was identified in the *V. riparia* data set using the Bonferroni outlier test in the car package (Fox and Weisberg, 2010) and subsequently removed. To disentangle the relevance of experimental treatment and leaf size for predicting response variables, we compared models based on Akaike’s information criterion (AIC) using the ‘dredge’ function in the ‘MuMIn’ package.

**SURVEY OF CO-OCCURRENCE OF EXTRAFLORAL NECTARIES IN DOMATIA-BEARING CLADES**

To determine how commonly extrafloral nectaries are known to occur in clades with mite domatia-bearing species, we cross-referenced the Annotated List of Domatia-Bearing Plants (Brouwer and Clifford, 1990; only includes acarodomatia, excludes ant domatia) with the World List of Plants with Extrafloral Nectararies (data as of 20 February 2015; Weber et al., 2015). Each entry in both lists was first corrected for taxonomic name changes, synonyms, and misspellings using the packages Taxize (Chamberlain and Szöcs, 2013) using the following pipeline. First, genus and species names were queried against The Plant List (www.ThePlantList.org) using the ‘TPL’ function in taxize. This function resolves genus and species names by replacing synonyms with accepted names, removing orthographical errors and assigning genera to families using their current placement according to the Angiosperm Phylogeny Group. We then assigned the resolved families to the correct taxonomic order according to the NCBI Taxonomy Database as of 6 June 2015 (http://www.ncbi.nlm.nih.gov/taxonomy) using the ‘tax_name’ function in taxize. Species that were not assigned to higher groups were hand checked for spelling errors and placed in the correct currently assigned clade according to Angiosperm Phylogeny Group classifications. Because many plants have not been scored for extrafloral nectary (Weber and Keeler, 2013) or mite domatia presence or absence at the species level, the corrected names and classifications were cross-referenced at the scale of family and genus. We visualized the distribution of groups reported to have both extrafloral nectaries and mite domatia across vascular plant groups using a rooted vascular plant mega-tree published by Zanne et al. (2013). We asked whether the presence of extrafloral nectaries and mite domatia was correlated across the plant family phylogeny using the phylogeny of Zanne et al. (2013), trimmed so that each family was represented by a single tip using the drop.tip function in the R package ape (Paradis et al., 2004). We tested for non-random patterns of overlap in extrafloral nectaries and mite domatia using Pagel’s discrete test (Pagel, 1994) implemented in the phytools package (Revell, 2012). Because both mite domatia and extrafloral nectaries have originated many times independently, this data set is not susceptible to issues associated with using Pagel’s discrete test single (or low) replication traits (Maddison and FitzJohn, 2014).
Experimental manipulations

Mites found on leaves almost exclusively belonged to the largely mutualistic (mycophagous or predatory) family Tydeidae, but also included a small proportion of predatory Phytoseiidae mites, and one velvet mite Trombidiopidea (on V. munsoniana). All mites found on leaves during the study were thus treated as mutualistic (no herbivorous mites were encountered).

The total abundance of mutualistic mites found on leaves increased with the presence of foliar sugar in all experiments. For V. munsoniana, leaves with foliar sugar experimentally added had approx 80% more mites than control leaves (z_{1.40} = 2.49, P = 0.013; Fig. 2A), and foliar sugar increased the number of domatia occupied by mites by 38% (z_{1.40} = 1.97, P = 0.048; Fig. 2B). For V. riparia, foliar sugar addition increased mite abundance by approx. 58% and approx. 69%, respectively, for counts conducted before mildew inoculation (z_{1.18} = 6.5, P < 0.001; Fig. 3B). For V. munsoniana, mite abundance and the number of domatia occupied by mites were both best explained by foliar sugar presence (Table 1): alternative models containing leaf size (Δ-AICc ≥1.6), and the size by sugar addition interaction term (Δ-AICc ≥3.9), had substantially worse fit. In V. riparia, mite abundance was best explained by a model that included both foliar sugar treatment and leaf length (Δ-AICc ≥9.27; Table 2). Finally, in V. riparia powdery mildew inoculation experiments, increased mite abundance was correlated with a decrease in the number of powdery mildew colonies found on V. riparia leaves (z_{1.18} = -2.171, P = 0.03; Fig. 3C).

Survey of occurrence of extrafloral nectaries and leaf domatia across plant groups

Cross-referencing the database of species with extrafloral nectaries (Weber et al., 2015) with the database of species with leaf domatia (Brouwer and Clifford, 1990), we found that approx. 61% of the 87 plant families documented to contain species with mite domatia were also reported to have species with extrafloral nectaries. These 53 families contained 78 genera that were reported on both lists, approx. 45% of which contained species reported to have both traits. Extrafloral nectaries and leaf domatia occurred together in the same plant families across the angiosperm phylogeny more than expected at random (Pagel’s discrete: likelihood ratio = 52.6, P < 0.0001). The plant groups that contain both extrafloral nectaries and leaf domatia were widely dispersed across the Eudicot portion of the plant phylogeny (Fig. 4), suggesting that they repeatedly evolved in the same plant clades over evolutionary history.

RESULTS

Morphological structures on plant leaves can modify interactions between plants, plant enemies and the third trophic level. Despite the prevalence and well-recognized ecological importance of leaf traits that mediate these interactions, we know remarkably little about how different morphological leaf traits interact with one another to attract beneficial arthropods to plants or to retain them (Heil, 2008). Recent work has led to the hypothesis that extrafloral nectar, a widespread plant trait known for its role in attracting ant bodyguards, may also enhance mite–plant mutualisms by increasing the number of mites present in mite domatia (Walter et al., 1995; Walter, 1996; van Rijn and Tanigoshi, 1999; Weber et al., 2012). We found that experimentally adding foliar sugar to plant leaves increased the number of mutualistic mites inhabiting leaf domatia in two species of wild grape, Vitis munsoniana and V. riparia. Furthermore, in a simulated outbreak of powdery mildew on V. riparia leaves, this increased mite abundance negatively correlated with the extent of powdery mildew establishment on leaves. Our study is the first to demonstrate experimentally that foliar sugar can enhance the efficacy of plant–mite mutualisms by increasing the abundance of mutualistic mites inhabiting domatia, indirectly reducing pathogen load on plant leaves.

DISCUSSION

Fig. 2. The effect of foliar sugar solution addition on mite abundance and the number of domatia occupied by mites in V. munsoniana. For control leaves (white) and leaves with experimentally added foliar sugar solution (black), the mean (± s.e.) is shown for mite abundance (A) and the number of domatia occupied by mites (B). An asterisk indicates significance < 0.05.
Our results revealed that the presence of foliar sugar solution facilitated larger standing populations of mutualistic mites on leaves, which in turn reduced subsequent plant infection and damage in a downstream outbreak. Because pathogen outbreaks can be strong selective agents in plants, the results of our study suggest that selection may favour leaf morphologies that attract and retain larger standing populations of mutualistic mites before outbreaks occur. In particular, it may be selectively beneficial for plants to have both domatia (as mite housing) and extrafloral nectar (as a non-fungal food alternative for mites) together on the same leaf. While adding traits to plants that naturally lack them is a powerful and long-practiced experimental approach to testing for potential trait effects, future studies evaluating the ecological impact of foliar nectar on plant–mite mutualisms should be conducted in systems that naturally possess extrafloral nectaries. These studies will directly address how

### Table 1. Models explaining mite variables according to AICc selection for V. munsoniana manipulations

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<th>Rank</th>
<th>Intercept</th>
<th>Leaf length</th>
<th>Foliar sugar</th>
<th>Leaf length × foliar sugar</th>
<th>d.f.</th>
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<th>AICc</th>
<th>ΔAICc</th>
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The ‘delta’ column shows the difference between a model’s AICc and that of the highest ranked model (rank = 1). The best fitting models (ΔAICw >2) are in bold. Model coefficients are shown for leaf length and plus signs are shown for factor variables.
extrafloral nectaries impact mite defence in the ecological and trait backgrounds in which they naturally occur. Additionally, one caveat of our study is that our experimental design did not allow us to distinguish which aspect of the foliar sugar solution impacted mite numbers. Follow-up studies that disentangle the relative contributions of leaf water, sugars and amino acids on mite populations will be fruitful.

By cross-listing the current list of taxa known to have extrafloral nectaries with the current list of taxa known to have mite domatia, we found that the two traits occur non-randomly in the same clades across Eudicots. While the co-occurrence of these two traits is generally correlated evolutionarily. To our knowledge, a detailed survey of nectaries amongst mite domatia-bearing crops is worth further consideration, as the benefits of co-occurrence for these traits may extend to a community scale.

**Conclusion**

Many striking defence mutualisms occur on plants that reward their mutualists with several distinct beneficial traits (Heil, 2008; Weber et al., 2012). Here we tested for an interaction between two common plant traits that are independently known to mediate plant-arthropod defence mutualisms. By experimentally manipulating the presence of foliar sugar solution on leaves and documenting the effects of mutualist abundance and pathogen load, this study provides the previously missing link in testing the hypothesis that extrafloral nectaries can enhance plant–mite mutualisms by increasing mite abundance in domatia, indirectly decreasing pathogen load.

**SUPPLEMENTARY DATA**

Supplementary data are available online at www.aob.oxfordjournals.org and consist of Table S1: plant genera where both extrafloral nectaries and leaf domatia have been documented (not necessarily in the same species).

**ACKNOWLEDGEMENTS**

We thank Greg Loeb and David Gadoury for methodological advice and help with the grape–domatia–powdery mildew system; Harry Greene, Jed Sparks and the Cornell EEB graduate field course for facilitating the Florida manipulations; Nick Mason and Nick Fletcher for access to the New York field site; Sharon Strauss for advice on figures; and Anurag Agrawal for helpful discussion throughout the project.

**LITERATURE CITED**


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**Table 2. Models explaining mite and mildew variables according to AICc selection for *V. riparia* manipulations**

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<th>Leaf length × Foliar sugar</th>
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The ‘delta’ column shows the difference between a model’s AICc and that of the highest ranked model (rank = 1). The best fitting models (ΔAICc >2) are in bold. Model coefficients are shown for leaf length, and plus signs are shown for factor variables.

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Fig. 4. Extrafloral nectaries (EFNs) and domatia have evolved in multiple lineages across the angiosperm tree of life. A phylogeny of angiosperm families modified from Zanne et al. (2013) with families reported in the literature to have both extrafloral nectaries and domatia are in pink, and families reported to have either extrafloral nectaries or domatia are in green or blue, respectively.


