

Article type : Reports

Similarity in volatile communities leads to increased herbivory and greater tropical forest diversity

Tara Joy Massad^{1,2,3}

Marcílio Martins de Moraes³

Casey Philbin⁴

Celso Oliveira Jr.⁴

Gerardo Cebrian Torrejon³

Lydia Fumiko Yamaguchi³

Christopher S. Jeffrey⁴

Lee A. Dyer⁵

Lora A. Richards⁵

Massuo Jorge Kato³

¹Corresponding author, tmassad77@gmail.com

²Rhodes College, Biology Department, 2000 N. Parkway, Memphis, TN 38103, USA

³Instituto de Química, Universidade de São Paulo, Av. Prof. Lineu Prestes, 748, Bloco 11 Térreo, 05508-000, São Paulo, SP, Brasil

⁴Department of Chemistry, University of Nevada, Reno 89557, USA

⁵Department of Biology, University of Nevada, Reno 89557, USA

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ecy.1875

This article is protected by copyright. All rights reserved.

Abstract

A longstanding paradigm in ecology is that there are positive associations between herbivore diversity, specialization, and plant species diversity, with a focus on taxonomic diversity. However, phytochemical diversity is also an informative metric, as insect herbivores interact with host-plants not as taxonomic entities, but as sources of nutrients, primary metabolites, and mixtures of attractant and repellent chemicals. The present research examines herbivore responses to phytochemical diversity measured as volatile similarity in the tropical genus *Piper*. We quantified associations between naturally occurring volatile variation and herbivory by specialist and generalist insects. Intraspecific similarity of volatile compounds across individuals was associated with greater overall herbivory. A structural equation model supported the hypothesis that plot level volatile similarity caused greater herbivory by generalists, but not specialists, which led to increased understory plant richness. These results demonstrate that using volatiles as a functional diversity metric is informative for understanding tropical forest diversity and indicate that generalist herbivores contribute to the maintenance of diversity.

Key words: chemical community similarity, diversity, generalist herbivore, specialist herbivore, volatiles

Introduction

Diverse plant communities may be maintained partly by insect herbivory or, conversely, increased plant diversity may lead to reductions in herbivore damage (Janzen 1970, Connell 1971, Tahvanainen and Root 1972, Barbosa 2009; Dyer et al. 2010; Alvarez-Loayza and Terborgh 2011). Such plant-herbivore interactions are highly influenced by plant chemistry, including phytochemical diversity, an important axis of functional diversity that

Accepted Article

influences multitrophic interactions (Richards et al. 2015). Plant chemical defenses are extremely diverse, particularly in the tropics (Coley and Barone 1996); similarly plant communities can be chemically diverse (Courtois et al. 2009, 2016; Salazar et al. 2016), and variation in this diversity has consequences for species interactions. Even within species, there is measurable chemical variation that can affect an individual's palatability to herbivores (Langenheim and Stubblebine 1983; Sanchez-Hidalgo et al. 1999; Glassmire et al. 2016). Studying chemical community diversity is uncommon, but it is an information rich approach to understanding relationships between herbivory and tropical forest diversity.

Volatiles are an important component of the broad classes of plant compounds that influence herbivory, partly because many insects detect their host plants through volatile chemical signaling (e.g., Carroll et al. 2008; Magalhães et al. 2012). Qualitative and quantitative changes in these bouquets can influence herbivory (Halitschke et al. 2008; Magalhães et al. 2012; Xiao et al. 2012). Mixtures of plant volatiles are not only diverse, but individual volatiles and different subsets of volatile mixtures can have both positive and negative effects on plants; associational resistance and associational susceptibility (Tahvanainen and Root 1972; Barbosa et al. 2009) can thus be difficult to predict. The associational resistance and susceptibility hypotheses originate from a taxonomic perspective of mono- versus polycultures, but herbivores' perception of potential host plants depends partly on volatile emissions, which are characterized by high variance in functional diversity that exceeds plant species diversity (Kleine and Mueller 2011).

Few studies have explicitly considered associational resistance and plant chemistry simultaneously. Recent work shows that herbivory declines with increasing intraspecific diversity of fixed (non-volatile) defenses among populations in a common garden. At the same time herbivory decreases, the abundance of herbivores increases, indicating that host searching is not affected by fixed chemical diversity but feeding behavior is (Bustos-Segura

et al. 2017). An olfactometer study with tropical species shows volatiles are important host-location signals (García-Robledo and Horvitz 2009), further emphasizing the importance of volatile chemistry in herbivore host selection.

The Janzen-Connell (J-C) hypothesis has also been viewed as a variant of associational susceptibility in that patches of forest with a high density of conspecific seedlings may be more apparent to herbivores, leading to increased specialist herbivory, reduced seedling survival, and increased opportunity for heterospecific plant recruitment. The outcome of these density-herbivore interactions is a negative density dependence that yields greater plant richness. The Langenheim and Stubblebine (1983) hypothesis connects the J-C hypothesis with phytochemical variation, suggesting differences in chemistry between parent trees and seedlings may lead to higher than expected herbivore recruitment. Distinct chemotypes of seedlings have, in fact, been found to survive more successfully near their parent tree (Sanchez-Hidalgo et al. 1999). Most interpretations of the J-C hypothesis focus on the role of specialists (Carson et al. 2008), nevertheless, generalist herbivores can cause increases in plant diversity via J-C mechanisms (Dyer et al. 2010). Specialist and generalist herbivores respond differently to plant defenses, and hypotheses about their effects on plant diversity are also different (Dyer et al. 2004; Richards et al. 2010; Massad et al. 2011). Similarly, specific volatile compounds and mixtures may be stronger cues for more specialized herbivores, which search-out specific host-plants in complex communities (Bernays 2001).

Understanding plant volatile diversity and its relationships with herbivory and taxonomic diversity is an important, almost entirely uninvestigated step in deciphering mechanisms maintaining tropical forest diversity. We seek to close this knowledge gap by investigating the genus, *Piper* (Piperaceae) to address hypotheses regarding volatile community diversity, herbivory, and taxonomic diversity. Neotropical forest understories are

Accepted Article

comprised of a high richness and abundance of *Piper* shrubs and vines, and about 47 species occur in the mid-Atlantic Rainforest region of Brazil where the present study was conducted (E. Tepe, University of Cincinnati, *pers. comm.*). *Piper* is chemically defended by a range of compounds, and a single species can produce metabolites as diverse in biosynthesis as phenolics, amides, and terpenoids (Parmar et al. 1997; Kato and Furlan 2007); most species also have complex volatile chemistry (Andrade et al. 2008; Mayer et al. 2008; Marques and Kaplan 2011). *Piper* hosts a high diversity of herbivores, including specialists in the genus *Eois* (Lepidoptera: Geometridae) and generalist caterpillars and orthopterans (Letourneau 2004). *Eois* are restricted to *Piper*, but a given species may feed on more than one species of *Piper* (Dyer and Palmer 2004; Bodner et al. 2010). Herbivory on *Piper* can have community-wide effects; in Costa Rica, reduced generalist herbivory on *Piper* led to lower understory plant diversity (Dyer et al. 2010).

We hypothesized that specialist herbivory on *Piper* in diverse volatile communities would be lower compared to individuals in chemically more similar communities because of reduced host-searching success in chemically complex environments. Specialists may be adapted to find specific hosts, but volatile complexity may disrupt their host searching more than it does for generalists because generalists may not use volatiles to find a desired host. We also hypothesized generalist herbivory would be less affected by volatile community diversity than specialist herbivory, as generalists may not be cue in on specific host-plant volatiles to the same degree. Lastly, we hypothesized generalist herbivory on *Piper* would have a diversifying effect on understory plant communities as was found by Dyer et al. (2010).

Material and methods

Field methods. Twenty-one circular 10 m diameter plots were studied across four remnants of Mata Atlântica forest in the states of São Paulo and Rio de Janeiro between January and May 2014. These plots included both primary and naturally regenerating secondary forest in Itatiaia National Park, São Bento de Sapucaí, Intervales State Park, and the Picinguaba sector of the Serra do Mar State Park. Plots ranged from 18-1513 m a.s.l. and were all in sub-tropical humid forests (Köppen climate classification; Table S1).

Plots were temporarily established at least 10 m from any forest edge. The center of each plot was anchored at a randomly selected *Piper* individual, and all *Piper* individuals within the plots were marked and identified to morphospecies or species. All leaves within reach were exhaustively searched for herbivores. Herbivore damage is inflicted by a variety of insects, including tettigoniids, specialist *Eois* (Geometridae) caterpillars, other Lepidoptera, and many juvenile and adult coleopterans. Herbivory was measured on each individual by visually estimating the area consumed by herbivores relative to the total leaf area that would be present without herbivory. Specialist and generalist insect damage were distinguished as much as possible (for a key to herbivore damage, see Dyer et al. 2010). In general, damage beginning at leaf margins originates from generalist insects; damage in the center of leaves and the characteristic ‘windows’ created by *Eois* feeding were categorized as specialist herbivory. The number of leaves on each plant was recorded, and ten leaves per species per plot were randomly selected and measured to determine the leaf area of each individual in a plot. All understory morphospecies other than *Piper* with leaves between 1-1.5 m aboveground were counted in each plot, and canopy openness was measured using a convex spherical crown densitometer (Forestry Suppliers, Model A). Three randomly located soil samples from the top 10 cm of soil were collected from each plot and were analyzed for Ca, Na, Mg, K, P, C, and N in the Analytical Center of the Institute of Chemistry at the

University of São Paulo following standard procedures (<http://ca.iq.usp.br/novo/>). C and N were measured as percents; Ca, K, Mg, Na, and P were measured as mg/kg soil.

Immature leaves from *Piper* individuals in the plots were collected in head space vials or 50 mL Eppendorf tubes for volatile analyses. Leaves without herbivory were preferentially selected, but in some cases only leaves with herbivory were available. If the plant did not have expanding leaves, samples were not collected. Volatile samples were frozen at field sites where there were freezers (all except Bulha d'Água where they were stored in sealed vials at ambient temperature) and transferred to the lab in Styrofoam coolers.

Volatile chemistry methods. Leaves were kept frozen at -20° until analysis. Volatiles were analyzed with headspace gas chromatography-mass spectrometry (HS-GC-MS). Samples were run on a Shimadzu Gas Chromatograph coupled to a Mass Spectrometer detector (GCMS-QP2010 Ultra; Shimadzu do Brasil Comércio Ltda, SP) with a Shimadzu AOC-5000 auto-sampler (Shimadzu do Brasil Comércio Ltda, SP). Samples were injected at 250°C and a pressure of 283.8 kPA in split mode with He as the carrier gas. The column was 10 m x 0.1 mm diameter x 0.1 μm (Restek Rxi-5ms, USA), and the flow rate was 0.5 mL/min. The mass spectrometer acquired scans between 1.2 and 12.6 min at an event time of 0.1 sec and a scan speed of 5000. The ion source temperature and the interface temperature were 250°C . The total run time was 12.6 min. See Appendix S1 for a description of peak alignments and Data S1 for the full volatiles dataset.

Data analysis. Complete, aligned volatile signatures were used to calculate multiple community Morisita Similarity Indices (MSI; Chao et al. 2008) describing the similarity of volatile communities for each *Piper* species in a plot where more than one individual per species was represented. Total *Piper* volatile community similarity was also calculated at the plot level. These calculations are analogous to those used for species in a community in that

peak areas were used as abundance data and each peak was treated as a unique species.

Calculations were performed using SPADE (Chao et al. 2008).

The similarity of volatile communities was used in models that also tested the effects of leaf area, leaf area diversity calculated as Simpson's Index (based on leaf area rather than the number of individuals of a species), *Piper* diversity per plot calculated as species equivalents of the Shannon-Wiener Index and Simpson's Index (Jost 2006), canopy openness, and soil properties. Simpson's index of leaf area was calculated to describe resource diversity from an herbivore's perspective. Individuals in a plot varied greatly in their size and leaf area, so this adaptation of a diversity metric was tested as a potentially more informative variable to determine the effect of diversity on herbivory.

Individual level analyses. We used mixed models to examine the effects of volatile and taxonomic communities on specialist and generalist herbivory. Individual plants were treated as replicates in order to determine effects of intraspecific variation in volatiles (MSI of volatiles calculated per species in a plot); plot nested within study site was therefore included as a random factor. Models with the lowest AIC scores are presented.

Plot level analyses. Structural equation models were conducted to uncover causal relationships between volatile community similarity (MSI of all volatiles measured in a plot), *Piper* diversity (in terms of individuals and leaf area), understory community richness, canopy cover, soil properties, and herbivory. Goodness of fit was determined by the χ^2 for the absolute index of the model ($P > 0.05$ indicates the model fits the data). Significant pathways in the model are indicated by t-values > 1.96 , and the model was adjusted to achieve best fit by removing the least predictive pathways. Not significant paths were retained when their inclusion led to the overall best-fit model (the model with the highest P -value). In all analyses, variables were log transformed to ensure normality of residuals as

needed. Models were run with PROC CALIS in SAS/STAT[®] software (Version 9.2, Copyright 2011, SAS Institute Inc, Cary NC).

Results

The minimum number of individuals contributing to the Morisita Similarity Index of species per plot was two, and the maximum number was 37. Within a plot, the number of *Piper* individuals ranged from 7-222 (including many seedlings). The number of *Piper* species in a plot ranged from 3-10. Understory richness (excluding *Piper*) ranged between 17 and 47 species. Fifty-nine *Eois* caterpillars and eight caterpillars of other species were collected from the plots; parasitoids emerged from seven *Eois*. Specialist herbivory ranged from 0-58%, and generalist herbivory ranged from 0-71%; across all individuals, mean specialist herbivory was 4.0%, and generalist herbivory averaged 5.9%.

Individual level patterns. Herbivory was significantly associated with volatile community similarity (Table 1). Mixed models showed greater intraspecific similarity in volatile communities was associated with increased damage by both specialists and generalists. Specialist herbivory was also positively correlated with *Piper* diversity as measured by the Shannon-Wiener index, but it was negatively correlated with Simpson's index of *Piper* leaf area, which is a more accurate measure of the diversity of resources in a plot. Lastly, specialist damage was positively related to Ca and Mg and negatively correlated with K in the soil. Generalist herbivory decreased slightly with canopy openness, increased with the total amount of *Piper* leaf area in a plot, and decreased with soil P (Table 1). Some parameter estimates exceeded ± 1 , indicating potential colinearity, but the best fit models included the predictors presented.

Plot level patterns. Structural equation modelling revealed that generalist herbivory on *Piper* had strong positive effects on understory richness; 1 SD of change in generalist damage (a 2.4% change in herbivory) led to an increase of 4.6 species in a plot. In contrast, specialist herbivory had negative effects on *Piper* richness (1 SD of change in herbivory decreased *Piper* richness by 0.6 species in a plot; Fig. 1). Generalist herbivory increased as the volatile community in a plot became more similar (1 SD of change in volatile community similarity led to a 1.1% increase in herbivory), and where less light reached the understory (herbivory increased by 0.8% with each SD of decrease in canopy openness). Greater resource diversity, measured as Simpson's index of leaf area also led to an increase in generalist herbivory, but it reduced specialist damage. Specialist herbivory was higher in soils richer in Mg (model goodness of fit = 0.84, $X^2 = 23.9$, $df = 17$, $P = 0.12$). A model including direct and indirect effects of Simpson's index for *Piper* individuals fits slightly less well but demonstrates a significant correlation between Simpson's index of individuals and Simpson's index of leaf area (Fig. S1).

Discussion

The results reported here demonstrate a unique aspect of how plant volatile diversity affects terrestrial communities. Local *Piper* volatile similarity was associated with higher specialist and generalist herbivory, and increased generalist herbivory on *Piper* is in turn associated with higher understory plant diversity. This result is consistent with studies highlighting the importance of functional diversity as a community parameter, and in this case the diversity of plant volatiles within a community helps clarify relationships between biodiversity and plant-herbivore interactions. Associational resistance and the J-C hypothesis predict that herbivory, particularly by specialists, should be greater in patches with a high density of conspecifics (Janzen 1970; Connell 1971; Tahvanainen and Root 1972). Empirical

Accepted Article

data provide substantive support for the predicted negative density dependence outcome of these hypothesized interactions (Carson et al. 2008; Terborgh 2012; Comita et al. 2014), but fewer studies examine the mechanism behind this pattern by measuring herbivory (Mangan et al. 2010; Dyer et al. 2010; Alvarez-Loayza and Terborgh 2011). Most research on the J-C hypothesis focuses on specialist herbivores, but generalist herbivores can also contribute to maintaining tropical forest diversity (Dyer et al. 2010). Our work supports this mechanistic link between increased generalist herbivory and greater plant diversity, revealing that generalist damage on a dominant genus has positive effects on local plant richness, possibly by preventing competitive exclusion (Viola et al. 2010).

Herbivores experience greater phytochemical than taxonomic diversity in their host-searching, as evidenced by the high level of intraspecific diversity in *Piper* volatile bouquets we report here. The relationships between herbivory and taxonomic diversity predicted by Janzen and Connell may therefore be obscured by effects of volatile diversity. Our results support Langenheim and Stubblebine's adaptation of the J-C hypothesis (1983) by demonstrating that generalist herbivory is reduced within chemically distinct volatile communities, contrary to our original hypothesis. Of course, other factors are responsible for variation in herbivory, and we quantified substantive effects of plant resources (soil nutrients and light) and leaf area diversity on herbivory that are consistent with theoretical predictions; the mechanisms of those relationships are explored more carefully in other studies (Strong et al. 1984; Massad and Dyer 2010).

Greater similarity in volatile communities was related to both higher specialist and generalist damage (parameter estimates from the mixed models for individuals were large, 0.7 and 0.4, respectively; Table 1). Remarkably, this pattern has been reported elsewhere in

the Neotropics - data from Costa Rican *Piper* communities also show that increased diversity of low molecular weight compounds leads to reduced specialist damage (Salazar et al. 2016). Generalist damage increased with greater volatile similarity both within and between species (individual and plot level results, respectively), while specialist herbivory increased only with greater intraspecific volatile similarity (individual level results). This indicates the two feeding groups may use different cues to locate host plants. According to Bernays' Neural Limitation hypothesis (2001), specialist herbivores have evolved neural sensitivity that allows them to key in on host specific volatiles, whereas generalists may be responsive to more broadly occurring signals. Our data support this, demonstrating that even in the presence of the diverse stimuli of tropical rainforests, specialists and generalists have different sensitivities to volatile bouquets.

Although manipulations of simpler systems provide similar results regarding the role of volatiles in specialist herbivore host-searching (Yoneya et al. 2010; Magalhães et al. 2012; Wäsche et al. 2014), this study demonstrates the importance of natural levels of volatile signaling in a highly diverse, tropical rainforest. In conclusion, phytochemical diversity is an underexplored, but informative variable for studies of plant-herbivore interactions (Hilker 2014), and generalist herbivores responding to phytochemical diversity on an abundant genus can increase tropical forest alpha-diversity.

Acknowledgments

We are grateful for the assistance of dedicated students and colleagues in our fieldwork. We would also like to thank the managers of the reserves where we worked for providing us access and logistical support. Thank you very much to Eric Tepe for his patient

assistance with *Piper* identification. Lastly, we gratefully acknowledge the support of grants from CNPq (401673/2012-0) and FAPESP (2009/51850-9 and 2014/23417-7).

Literature cited

- Alvarez-Loayza, P., and J. Terborgh. 2011. Fates of seedling carpets in an Amazonian floodplain forest: Intra-cohort competition or attack by enemies? *Journal of Ecology* 99:1045–1054.
- Andrade, E. H. A., L. M. M. Carreira, M. H. L. Silva, J. D. Silva, C. N. Bastos, P. J. C. Sousa, E. F. Guimarães, J. G. S. Maia. 2008. Variability in essential oil composition of *Piper marginatum sensu lato*. *Chemical Biodiversity* 5:197–208.
- 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.
- Bernays, E. A. 2001. Neural limitations in phytophagous insects: Implications for diet breadth and evolution of host affiliation. *Annual Review of Entomology* 46:703–727.
- Bodner, F., G. Brehm, J. Homeier, P. Strutzenberger, and K. Fiedler. 2010. Caterpillars and Host Plant Records for 59 Species of Geometridae (Lepidoptera) from a Montane Rainforest in Southern Ecuador. *Journal of Insect Science* 10:67.
10.1673/031.010.6701
- Bustos-Segura, C., E. H. Poelman, M. Reichelt, J. Gershenzon, and R. Gols. 2017. Intraspecific chemical diversity among neighbouring plants correlates positively with plant size and herbivore load but negatively with herbivore damage. *Ecology Letters* 20:87-97.
- Carroll, M. J., E. A. Schmelz, and P. E. A. Teal. 2008. The attraction of *Spodoptera frugiperda* neonates to cowpea seedlings is mediated by volatiles induced by conspecific herbivory and the elicitor inceptin. *Journal of Chemical Ecology* 34:291–300.
- Carson, W. P., J. T. Anderson, E. G. Leigh Jr., and S. A. Schnitzer. 2008. Challenges associated with testing and falsifying the Janzen-Connell Hypothesis: A review and critique. Pages 210-241 *in* W.

P. Carson and S. A. Schnitzer, editors. *Tropical Forest Community Ecology*. Blackwell Publishing, Malden, USA.

Chao, A., L. Jost, S. C. Chiang, Y.-H. Jiang, and R. L. Chazdon. 2008. A two-stage probabilistic approach to multiple-community similarity indices. *Biometrics* 64:1178–1186.

Coley, P., and J. Barone. 1996. Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics* 27:305–335.

Comita, L. S., S. A. Queenborough, S. J. Murphy, J. L. Eck, K. Xu, M. Krishnadas, N. Beckman, and Y. Zhu. 2014. Testing predictions of the Janzen-Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. *Journal of Ecology* 102:845–856.

Connell, J. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. Pages 298–312 *in* den Boer, P. J. and G. Gradwell, editors. *Dynamics of Populations*. Pudoc, Wageningen.

Courtois, E. A., C. E. T. Paine, P.-A. Blandinieres, D. Stien, J.-M. Bessiere, E. Houel, C. Baraloto, and J. Chave. 2009. Diversity of the Volatile Organic Compounds Emitted by 55 Species of Tropical Trees: a Survey in French Guiana. *Journal of Chemical Ecology* 35:1349–1362.

Courtois, E. A., K. G. Dexter, C. E. T. Paine, D. Stien, J. Engel, C. Baraloto, and J. Chave. 2016. Evolutionary patterns of volatile terpene emissions across 202 tropical tree species. *Ecology and Evolution* 6:2854–2864.

Dyer, L. A., and A. D. N. Palmer, editors. 2004. *Piper: A Model Genus for Studies of Phytochemistry, Ecology, and Evolution*. Springer US, Boston, MA.

Dyer, L.A., D. K. Letourneau, C. D. Dodson, M. A. Tobler, J. O. Stireman, and A. Hsu. 2004. Ecological causes and consequences of variation in defensive chemistry of a Neotropical shrub. *Ecology* 85:2795-2803.

- Dyer, L. A., D. K. Letourneau, G. V. Chavarria, and D. S. Amoretti. 2010. Herbivores on a dominant understory shrub increase local plant diversity in rain forest communities. *Ecology* 91:3707–3718.
- García-Robledo, C., and C. C. Horvitz. 2009. Host plant scents attract rolled-leaf beetles to Neotropical gingers in a Central American tropical rain forest. *Entomologia Experimentalis et Applicata* 131:115–120.
- Glassmire, A. E., C. S. Jeffrey, M. L. Forister, T. L. Parchman, C. C. Nice, J. P. Jahner, J. S. Wilson, T. R. Walla, L. A. Richards, A. M. Smilanich, M. D. Leonard, C. R. Morrison, W. Simbaña, L. A. Salagaje, C. D. Dodson, J. S. Miller, E. J. Tepe, S. Villamarin-Cortez, and L. A. Dyer. 2016. Intraspecific phytochemical variation shapes community and population structure for specialist caterpillars. *New Phytologist* 212:208–219.
- Halitschke, R., J. A. Stenberg, D. Kessler, A. Kessler, and I. T. Baldwin. 2008. Shared signals - “alarm calls” from plants increase apparency to herbivores and their enemies in nature. *Ecology Letters* 11:24–34.
- Hilker, M. 2014. New Synthesis: Parallels Between Biodiversity and Chemodiversity. *Journal of Chemical Ecology* 40:225–226.
- Janzen, D. 1970. Herbivores and the number of tree species in tropical forests. *American Naturalist* 104:501-528.
- Jost L. 2006. Entropy and Diversity. *Oikos* 113:363-375.
- Kato, M. J., and M. Furlan. 2007. Chemistry and evolution of the Piperaceae. *Pure and Applied Chemistry* 79:529-538.
- Kleine, S., and C. Mueller. 2011. Intraspecific plant chemical diversity and its relation to herbivory. *Oecologia* 166:175–186.
- Langenheim, J., and W. Stubblebine. 1983. Variation in leaf resin composition between parent tree and progeny in *Hymenea* - Implications for herbivory in the humid tropics. *Biochemical Systematics and Ecology* 11:97–106.

- Letourneau, D. K. 2004. Mutualism, Antiherbivore Defense, and Trophic Cascades: *Piper* Ant-Plants as a Mesocosm for Experimentation. Pages 5–32 in L. A. Dyer and A. D. N. Palmer, editors. *Piper: A Model Genus for Studies of Phytochemistry, Ecology, and Evolution*. Springer US.
- Magalhaes, D. M., M. Borges, R. A. Laumann, E. R. Sujii, P. Mayon, J. C. Caulfield, C. A. O. Midega, Z. R. Khan, J. A. Pickett, M. A. Birkett, and M. C. Blassioli-Moraes. 2012. Semiochemicals from herbivory induced cotton plants enhance the foraging behavior of the cotton boll weevil, *Anthonomus grandis*. *Journal of Chemical Ecology* 38:1528–1538.
- Mangan, S. A., S. A. Schnitzer, E. A. Herre, K. M. Mack, M. C. Valencia, E. I. Sanchez, and J. D. Bever. 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature* 466:752-755.
- Marques, A. M., and M. A. C. Kaplan. 2011. Seasonal evaluation and chemical composition of volatile fractions from *Piper claussonianum* by hydrodistillation and SPME. *Journal of Essential Oil Research* 23:15–19.
- Massad, T. J., and D. A. Dyer. 2010. A meta-analysis of the effects of global environmental change on plant-herbivore interactions. *Arthropod-Plant Interactions* 4:181-188.
- Massad, T. J., R. M. Fincher, A. M. Smilanich, and L. Dyer. 2011. A quantitative evaluation of major plant defense hypotheses, nature versus nurture, and chemistry versus ants. *Arthropod-Plant Interactions* 5:125–139.
- Mayer, V., D. Schaber, and F. Hadacek. 2008. Volatiles of myrmecophytic *Piper* plants signal stem tissue damage to inhabiting *Pheidole* ant-partners. *Journal of Ecology* 96:962–970.
- Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O’Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2013. *Vegan: Community Ecology Package*. R Package Version. 2.0-10.
- Parmar, V. S., S. C. Jain, K. S. Bisht, R. Jain, P. Taneja, A. Jha, O. D. Tyagi, A. K. Prasad, J. Wengel, C. E. Olsen, and P. M. Boll. 1997. Phytochemistry of the genus *Piper*, *Phytochemistry* 46:597–673.

- Richards, L. A., L. A. Dyer, A. M. Smilanich, and C. D. Dodson. 2010. Synergistic effects of amides from two *Piper* species on generalist and specialist herbivores. *Journal of Chemical Ecology* 36:1105–1113.
- Richards, L. A., L. A. Dyer, M. L. Forister, A. M. Smilanich, C. D. Dodson, M. D. Leonard, and C. S. Jeffrey. 2015. Phytochemical diversity drives plant–insect community diversity. *Proceedings of the National Academy of Sciences* 112:10973–10978.
- Salazar, D., A. Jaramillo, and R. J. Marquis. 2016. The impact of plant chemical diversity on plant–herbivore interactions at the community level. *Oecologia* 181:1199–208.
- Sanchez-Hidalgo, M. E., M. Martinez-Ramos, and F. J. Espinosa-Garcia. 1999. Chemical differentiation between leaves of seedlings and spatially close adult trees from the tropical rainforest species *Nectandra ambigens* (Lauraceae): an alternative test of the Janzen-Connell model. *Functional Ecology* 13:725–732.
- Strong, D. R., J. H. Lawton, and R. Southwood. 1984. *Insects on Plants: Community Patterns and Mechanisms*. Harvard University Press: Boston.
- 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.
- Terborgh, J. 2012. Enemies Maintain Hyperdiverse Tropical Forests. *American Naturalist* 179:303–314.
- D. V. Viola, D. V., E. A. Mordecai, A. G. Jaramillo, S. A. Sistla, L. K. Albertson, J. Stephen Gosnell, B. J. Cardinale, and J. M. Levine. 2010. Competition–defense tradeoffs and the maintenance of plant diversity. *Proceedings of the National Academy of Sciences* 107: 17217–17222.
- Wäschke N., K. Hardge, C. Hancock, M. Hilker, E. Obermaier, and T. Meiners. 2014. Habitats as complex odour environments: How does plant diversity affect herbivore and parasitoid orientation? *PLoS ONE* 9: e85152. doi:10.1371/journal.pone.0085152

Xiao, Y., Q. Wang, M. Erb, T. C. J. Turlings, L. Ge, L. Hu, J. Li, X. Han, T. Zhang, J. Lu, G. Zhang, and Y.

Lou. 2012. Specific herbivore-induced volatiles defend plants and determine insect community composition in the field. *Ecology Letters* 15:1130–1139.

Yoneya, K., R.Ozawa, J. Takabayashi. 2010. Specialist leaf beetle larvae use volatiles from willow leaves infested by conspecifics for reaggregation in a tree. *Journal of Chemical Ecology* 36:671-679.

Table 1. Mixed model results describing specialist and generalist herbivory at the individual level.

Specialist herbivory	F	d.f.	P	PE
Shannon-Wiener index of <i>Piper</i> in a plot	15.2	1/1126	0.0001	2.4
Simpson's index of leaf area	11.2	1/1126	0.0008	-1.6
K (mg/kg)	12.3	1/1126	0.0005	-0.9
Mg (mg/kg)	8.2	1/1126	0.004	0.5
Ca (mg/kg)	6.4	1/1126	0.01	0.5
Volatile community similarity (species within a plot)	5.6	1/1126	0.02	0.6

Generalist herbivory				
P (mg/kg)	13.9	1/1104	0.0002	-0.001
Canopy openness	10.7	1/1104	0.001	-0.04
Volatile community similarity (species within a plot)	10.6	1/1104	0.001	0.4
<i>Piper</i> leaf area in a plot	5.5	1/1104	0.02	0.1

Notes: Replication was at the level of the individual to look for species level effects, and plot nested within study site was included as a random factor. Specialist herbivory AIC = 4326.8; generalist herbivory AIC = 3426.6. PE is parameter estimate.

Figure 1. Structural equation model diagram of causal relationships (single headed arrows) between chemical and taxonomic communities and total herbivory on *Piper* at the plot level. The round tipped arrows indicate a negative relationship. Values beside arrows are standardized parameter estimates. Grey parameter estimates are not significant, but their inclusion generates the best model. Volatile community similarity encompasses the volatile community for all *Piper* individuals measured in a plot. One standard deviation of change in volatile similarity resulted in a 0.46 SD change in generalist herbivory, and 1 SD of change in generalist herbivory caused 0.47 SD of change in understory richness. Resource availability and canopy openness were also important for generalist herbivory.

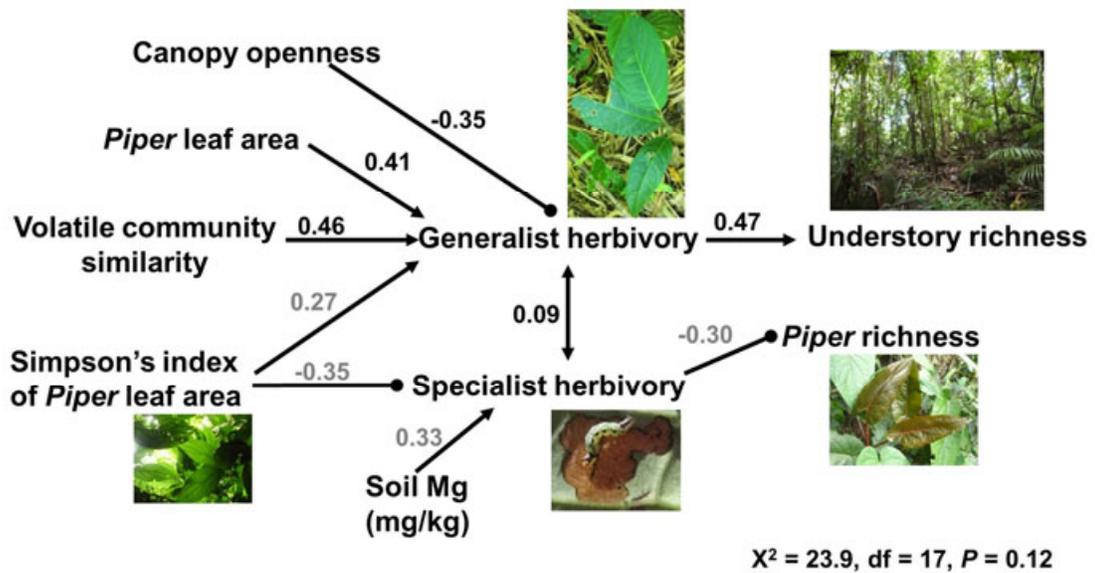


Fig. 1.