

Disentangling dimensions of phytochemical diversity: alpha and beta have contrasting effects on an insect herbivore

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Abstract. Phytochemical diversity is comprised of two main dimensions—the average (alpha) within-plant neighbors or the difference (beta) in the composition of chemicals between plant neighbors. Research, however, has primarily examined the consequences of phytochemical diversity on herbivore performance through a single dimension, even though diversity is multidimensional. Furthermore, the ecological role of phytochemical diversity is not well understood because each of these dimensions exhibits unique biological effects on herbivore performance. Therefore, it has been difficult to tease apart the relative importance of alpha and beta chemical diversities on plant–herbivore interactions. We experimentally manipulated alpha and beta diversities along a chemical gradient to disentangle the relative effects of these dimensions on the performance of a mobile generalist herbivore, *Trichoplusia ni* (Hübner), using 16 genotypes from the *Solanum pennellii* introgression lines. First, we found contrasting effects of alpha and beta diversities on herbivore performance. Second, when comparing diversity across and within chemical classes, herbivore performance was reduced when plant neighbors had greater diversity within chemical classes that are biologically inhibiting at higher quantities (i.e., quantitative defenses such as phenolics and acyl sugars). However, herbivore performance was enhanced when plant neighbors had higher levels of chemical classes that are biologically toxic (i.e., qualitative defenses such as alkaloids). Finally, herbivores performed better on plant dicultures compared to monocultures, and performance was positively associated with plant dicultures only when there were high levels of average alpha diversity within plant neighbors. Our results suggest *T. ni* generalist caterpillars do better when plant neighbors are chemically different because differences provide options for them to choose or to switch between plants to balance chemical uptake. Overall, herbivores interact with a large diversity of plant chemicals at multiple scales, and our results indicate that not all chemical diversity is equal: specific dimensions of phytochemical diversity have unique effects on the dynamics of herbivore performance.

Key words: acyl sugars; alkaloids; alpha diversity; beta diversity; chemical traits; herbivore performance; intraspecific phytochemical diversity; phenolics; qualitative defenses; quantitative defenses.

INTRODUCTION

For over a century, biologists have asked why plants produce such large diversities of chemical compounds and hypothesized about the role of phytochemical diversity in plant–animal interactions (Abbott 1887, Stahl 1888, Fraenkel 1959, Ehrlich and Raven 1964, Jones and Firn 1991, Dyer et al. 2018). Animals that feed on plants likely experience hundreds of unique compounds in each foraging bout and thousands through their lifetimes, depending on their hosts and feeding behavior. Traditionally, chemical ecology examined the role of

phytochemicals by isolating and studying small numbers of compounds at a time (Gershenzon et al. 2012, Richards et al. 2016). Advances in metabolomics and new collaborations with chemists, however, have recently enabled ecologists to measure large numbers of compounds simultaneously and efficiently (Dyer et al. 2018, Kessler and Kalske 2018, Wetzels and Whitehead 2020). Indeed, recent work indicates that the diversity of compounds per se present in plants is strongly correlated with key ecological variables, including herbivory (Johnson et al. 2009, Richards et al. 2015, Glassmire et al. 2019). A major challenge to our understanding of the role of phytochemical diversity, however, is that it is not a simple trait; it is a multidimensional concept encompassing the full complexity of chemical composition varying within and across spatiotemporal and biosynthetic scales (Hare

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and Futuyma 1978, Hunter 2016, Dyer et al. 2018, Wetzel and Whitehead 2020). Resolving the ecological role of phytochemical diversity requires studies that isolate and examine the potentially differing effects of each of these key dimensions of diversity (Jones and Firn 1991, Dyer et al. 2018, Kessler and Kalske 2018).

One key dimension of phytochemical diversity is its spatial scale. Animals interacting with plants experience the richness and evenness of compounds within plants (alpha diversity) and sequential variation in chemical composition as they move among neighboring plants (beta diversity), and each of these scales is likely to have differing ecological consequences. Alpha diversity has been hypothesized to suppress herbivore damage by increasing the number of bioactive compounds and beneficial synergies among compounds within each bite (Table 1; Berenbaum and Zangerl 1996, Gershenzon et al. 2012, Richards et al. 2015). Indeed, *Piper* species with higher levels of alpha chemical diversity experience less herbivory (Richards et al. 2015, Glassmire et al. 2019). Chemical beta diversity, in contrast, has been hypothesized to alter herbivore behavior during host selection (Riolo et al. 2015) and reduce herbivore performance for feeding stages that experience chemical variation by presenting a sequential moving target that hinders herbivore acclimation to chemistry (Table 1; Adler and Karban 1994, Wetzel and Thaler 2016). Indeed, plant species assemblages with higher chemical beta diversity experience lower herbivory (Salazar et al. 2016, Massad et al. 2017). Alternatively, beta diversity could enhance herbivore performance by allowing herbivores the opportunity to choose the best plant or to forage across plants to dilute or balance the intake of any one toxin or nutrient (Bernays et al. 1994, Wetzel and Thaler 2018). The ecological roles of alpha and beta chemical diversities are still poorly understood because these key dimensions have not been fully evaluated in comparison to one another.

A second key dimension of phytochemical diversity is its biosynthetic scale. Phytochemical diversity exists across all the compounds within a plant as well as across compounds within specific biosynthetic classes. Most phytochemicals group by their major structural motifs with different biological effects, and the biological effects of diversity could differ dramatically for herbivores. For example, diversity in alkaloids, acutely toxic qualitative defenses that are effective against herbivores at lower relative concentrations, should have very different ecological consequences from diversity in repellent quantitative defenses, phenolics or acyl sugars, that require higher relative concentrations to inhibit herbivore performance (Feeny 1976, Smilanich et al. 2016). Most studies on the effects of phytochemical diversity on ecological interactions have examined either diversity across all detected compounds (Richards et al. 2015, Sedio 2017, Kessler and Kalske 2018) or the chemical diversity of one focal chemical class (Rasmann and Agrawal 2011, Becerra 2015). Studies that compare the effects of diversity

across and within multiple chemical classes would help reveal the biosynthetic scale at which diversity matters and demonstrate whether the effects of chemical diversity differ for toxins, repellents, and other key biological effects of phytochemicals.

The multidimensional nature of phytochemical diversity suggests it may have a variety of important ecological roles, but this complexity also challenges our ability to isolate each dimension and resolve their effects experimentally. Studies that have examined only one of these scales rather than comparing effects between scales may have overlooked testable predictions (Wetzel and Whitehead 2020). This work has led to the discovery of important patterns, but it has made it difficult to link specific scales and dimensions of phytochemical diversity to ecological outcomes. Recent experimental studies have begun manipulating spatiotemporal variation in chemistry—chemical beta diversity—using biosynthetic knockouts that suppress or overexpress plant defense (Schuman et al. 2015), wild genotypes with a polymorphism in the concentration of a single class of defensive compounds (Bustos-Segura et al. 2017), and artificial diets with differing concentrations of a key toxin (Pearse et al. 2018). These studies have shown conclusively that chemical beta diversity plays a major role in plants' ability to defend against herbivores. Regardless, it remains unclear how the effects of beta diversity compare to the effects of alpha diversity and how these results might change for different biosynthetic compound classes. Resolving these issues requires studies that manipulate phytochemical diversity using systems of plant genotypes that represent differences among multiple chemical class axes in both alpha and beta diversity.

In this study, we experimentally measured the independent and interactive effects of alpha and beta chemical diversities across and within three key biosynthetic compound classes (alkaloids, acyl sugars, and phenolics) on the survival, growth, and development of a mobile generalist caterpillar (*Trichoplusia ni* Hübner). We manipulated the chemical diversity experienced by the caterpillars by rearing them in monocultures or polycultures using 16 plant genotypes from the *Solanum lycopersicum* × *pennellii* backcrossed introgression lines (Eshed and Zamir 1994, 1995), an emerging model system in molecular biology and plant breeding for mapping phenotypes to genotypes. These introgression lines are ideal for manipulating phytochemical diversity because collectively they represent substantial phytochemical variation, but any two genotypes are genetically and phenotypically similar except for relatively subtle phenotypic differences across multiple trait classes. This variation enabled us to explore multiple scales and types of chemical diversity while maintaining the power to link herbivore response variables back to specific differences. We did this by forming 25 plant treatment combinations representing a chemical gradient of alpha and beta chemical diversities (Fig. 1). Our experiment addressed three questions: (1) How do alpha

TABLE 1. The framework of hypotheses used to test the consequences of phytochemical diversity on herbivore performance. These hypotheses are described in context of alpha and beta diversities. The outcomes are in the context of a single herbivore species, *Trichoplusia ni*, which is a diet generalist and known to be highly mobile. By *alpha* chemical diversity we mean the number of unique chemicals and the evenness of their abundances within plants. By *beta* chemical diversity we mean the chemical compositional dissimilarity between plants. Relevant path coefficients are from the best path model (Fig. 3).

| Predictions | Citations | Outcomes from our experiment | Relevant path coefficients |
|--|--|--|----------------------------|
| The screening hypothesis predicts that higher levels of alpha diversity will have a negative association with herbivore performance because more kinds of chemicals available increase the probability of a deterrent effect against herbivores. Similarly, the synergy hypothesis predicts that higher levels of alpha diversity increase the probability of interactions between chemicals that have a greater effect than expected based on additive effects of individual chemicals. | Screening hypothesis: Jones and Firn (1991), Berenbaum and Zangerl (1996); synergy hypothesis: Berenbaum and Neal (1985), Gershenson et al. (2012), Richards et al. (2016) | Our results supported the screening and synergy hypotheses and suggest that higher levels of alpha diversity of quantitative chemical defenses (phenolics and acyl sugars) inhibit herbivore performance, either through additive or interactive effects between chemicals. | II, III, IV |
| The diet mixing hypothesis predicts that herbivores will perform better with greater beta diversity in the kinds of chemicals available between plant neighbors. Plants that are chemically different from one another may provide options for herbivores to select the least toxic one, dilute abundant chemicals, and/or balance nutritional demands. | Diet mixing hypothesis: Bernays et al. (1994), Singer et al. (2002) | Our results supported the diet mixing hypothesis and indicate that greater beta diversity of qualitative defenses (alkaloids) between plants and greater chemical richness are associated with enhanced herbivore performance. Higher levels of beta diversity between plant neighbors may provide options for herbivores to choose between plant quality or switch between plants to dilute toxicity. | V, VI, VII |
| The moving target hypothesis predicts that herbivores will have a difficult time feeding between plant neighbors that have greater beta diversity in chemicals. Similarly, the acclimation hypothesis predicts that high chemical dissimilarity between plants would be physiologically difficult for herbivores to acclimate to. | Moving target hypothesis: Karban and Myers (1989); acclimation hypothesis: Wetzel and Thaler (2016) | Our results rejected the moving target and acclimation hypotheses and instead indicate that greater beta diversity of qualitative defenses between plants are associated with enhanced herbivore performance. Plants that are chemically different may provide options of toxic and less toxic plants for herbivores to choose or switch between. | None |

and beta chemical diversities influence herbivore performance? (2) How do the effects of diversity across all biosynthetic classes differ from the effects of diversity within specific biosynthetic classes? (3) How do alpha and beta diversities influence the effects of plant dicultures on herbivore performance? We predicted that treating phytochemical diversity as multidimensional will improve our understanding of the ecological role that plant defense traits have on plant–insect interactions.

METHODS

The *Solanum pennellii* backcrossed introgression lines (PILs) comprise 83 genotypes, each of which is genetically identical to a homozygous genotype of domesticated tomato (*Solanum lycopersicum* cv. M82) except

each introgression line genotype has a single, unique homozygous chromosomal substitution from *S. pennellii*, representing a few hundred genes (Eshed and Zamir 1995). *Solanum pennellii* is a wild, Andean tomato resistant to multiple pests (Gentile et al. 1968, Gentile and Stoner 1968). The PILs are an excellent system to manipulate chemical traits because in addition to genetic control, these varieties have only subtle phenotypic differences in flowering phenology and leaf expansion rates. In this study, we focused on foliar chemistry, because chemistry on and within trichomes and epidermal cells is an important aspect of tomato defense against herbivores (Glas et al. 2012, Luu et al. 2017, Weinhold et al. 2017) and is well characterized in these genotypes (Schillmiller et al. 2010). In particular, we examined all compounds detected in foliar chemistry and focused on

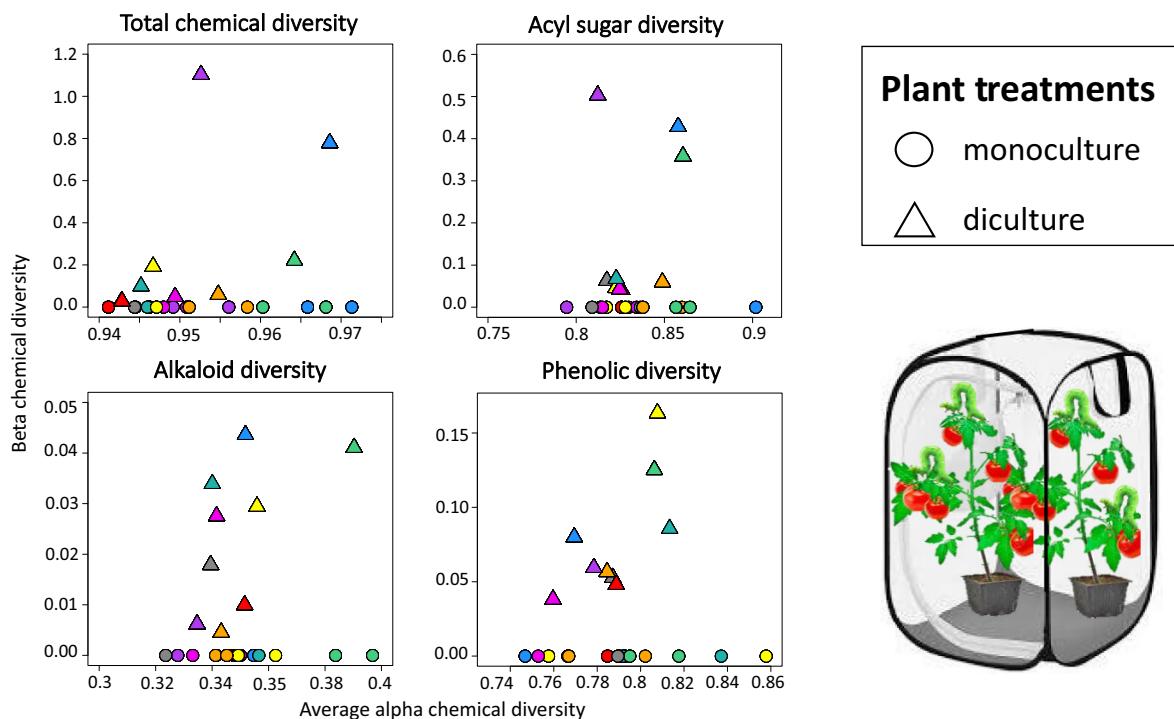


FIG. 1. The alpha and beta chemical diversities in each of the 25 plant treatment combinations in our experiment (9 dicultures and 16 corresponding monocultures). We examined alpha and beta diversities at four dimensions: total chemical diversity, acyl sugar diversity, alkaloid diversity, and phenolic diversity. Each point represents a single treatment combination with circles representing monocultures and triangles representing dicultures. Each color groups a diculture with its corresponding two monocultures (see Fig. 4B for specific plant genotype pairings). The average alpha diversity refers to the average chemistry (i.e., compound richness relative to abundance) of the two plants in a cage that a caterpillar would experience if it were feeding randomly between plants within each treatment. Multicollinearity did not inhibit model estimation performance because alpha and beta correlations were below 0.6. The illustration shows one replicate with two potted plants and four cabbage looper caterpillars (*Trichoplusia ni*).

compounds in three biosynthetic classes that are important for tomato defense: acyl sugars and phenolics, which are quantitative defenses that are repellents and digestibility reducers, and alkaloids, which are acutely toxic qualitative defenses (Friedman 2002, Slimestad et al. 2008, Luu et al. 2017).

We selected 16 of the 83 genotypes (15 introgression genotypes and the *S. lycopersicum* parent) and used them to establish 25 plant treatment combinations representing a range of alpha and beta chemical diversities across phenolics, acyl sugars, and alkaloids. Specifically, there were two potted plants in each treatment combination—16 monocultures of each of our 16 genotypes and nine dicultures of different combinations of the 16 genotypes. We selected the 16 genotypes and their diculture combinations based on their chemical diversity, such that monocultures had a chemical gradient ranging from low to high alpha diversity and dicultures represented all combinations of low–high alpha and low–high pairwise beta diversity (see Fig. 1 for experimental design and Table 1 for an enumeration of the hypotheses tested; see Appendix S1: Table S1 for genotypes, treatments, and their chemical diversity levels). The strength of this plant

system is the high genetic similarity of these genotypes allowing for the partitioning of chemical diversity by individual biosynthetic classes, which is in contrast to experiments using wild-type plants exhibiting variation across multiple biosynthetic chemical classes simultaneously. Most of our dicultures had intermediate levels of beta diversity and a few had extremely high beta diversity. When selecting genotypes and diculture combinations, we quantified alpha and beta diversities using chemical data from Schillmiller et al. (2010). These authors extracted leaves of each genotype by dipping standardized whole leaflets in *tert*-butyl methyl ether, analyzing extracts using liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS), and integrating peak areas normalized to an internal standard (propyl-4-hydroxybenzoate) and sample dry mass (Schillmiller et al. 2010). This data set and our study were focused on genetically based phenotypic variation, which is a primary source of phenotypic variation in this system (Eshed and Zamir 1995, Schillmiller et al. 2010); moreover, we minimized plasticity by growing plants in a common greenhouse.

We used the Schillmiller et al. (2010) LC-TOF-MS chemical data to calculate alpha diversity with

Simpson's diversity index and beta diversity with dissimilarity in multivariate chemical composition. We square-root transformed all peaks (peak area $\text{IS}^{-1} \text{gdw}^{-1}$) prior to calculating alpha and beta diversities across all biosynthetic classes and for alkaloids, phenolics, or acyl sugars to obtain diversities within biosynthetic classes (as identified by Schillmiller et al. 2010). We used Simpson's alpha diversity [$1 - D$; where $D = \sum(n/N)^2$], because the *S. pennellii* introgression lines are similar in chemical composition, and Simpson's attributes more weight to abundant chemicals (Oksanen et al. 2017). The values of the alpha diversity metric, in other words, emphasize variation in the evenness of abundant compounds, rather than the presence or absence of low-abundance compounds. We calculated average alpha diversities for each treatment by averaging alpha for all compounds, just acyl sugars, just alkaloids, or just phenolics across the two plants in each treatment. We defined the average alpha as the average chemical diversity that an herbivore would experience if it were moving randomly between plants within each treatment. Alpha ranged from 0.94 to 0.97 (Appendix S1: Table S1). For comparison, a value of 0.94 indicated high chemical diversity in the tropical plant genus *Piper* (Richards et al. 2015), and plants that differed by only 0.03 led to significantly different herbivore performance (Glassmire et al. 2019).

We quantified multivariate chemical dissimilarity (i.e., beta chemical diversity) by arranging genotypes into a multivariate chemical space using nonmetric multidimensional scaling (NMDS) with Bray–Curtis dissimilarities and calculating the Euclidian distances between genotypes in the ‘vegan’ package in R (Marion et al. 2017, Oksanen et al. 2017). We set the NMDS to a single dimension ($k = 1$) to collapse all the variation onto a single axis. The NMDS stress was less than 0.15 for total diversity, acyl sugars, and alkaloids, indicating strong representation, but greater than 0.3 for phenolics, indicating weaker representation (Clarke and Warwick 1994). We chose to proceed regardless, to yield a single axis of dissimilarity for each biosynthetic class. This approach is similar to factor analysis, which is used for latent variables in structure equation modeling (Shipley 2016).

We obtained the selected genotypes (Appendix S1: Table S1) from the C. M. Rick Tomato Genetics Resource Center at the University of California, Davis, and grew all plants from seed in 10-cm pots (Suremix potting soil, Michigan Grower Products, Inc.) in a climate-controlled greenhouse at Michigan State University with supplemental lighting (16-h day). When the plants were 7 weeks old, we established each treatment by placing plants into mesh cages ($34 \times 34 \times 61$ cm) with canopies touching throughout the duration of the experiment. There were 6 replicates for each of the 16 monocultures, except for two that had 3 and 9, and 12 replicates for each diculture except for two that had 3 and 9 (see Appendix S1: Table S1 for full sample sizes). About 24 h after placing plants into cages, we added

two third-stage *T. ni* caterpillars to the base of each plant, for a total of four caterpillars per cage, and ensured that caterpillars were moving between plants. We selected *T. ni* for their broad diet breadth and mobility. *Trichoplusia ni* is a diet generalist that feeds across plant families, including Brassicaceae, Solanaceae, and Malvaceae. Furthermore, *T. ni* caterpillars are highly mobile and can easily move between neighboring plants. An ongoing study quantifying movement behavior in *T. ni* while feeding on the same tomato dicultures demonstrated that 20% of individuals moved to the neighboring plant after 48 h and 80% moved as far as three plants away from the initial plant after 1 week (Hauri et al., unpublished). *Trichoplusia ni* colonies came from Benzon Research and were reared on artificial diet (Southland Products Inc.) at room temperature. We recorded the individual mass of four caterpillars before placing them in cages and checked them for survival every 48 h until pupation, when we recorded their plant position and measured individual pupal mass. The 48-h checks over a span of 2 weeks validated that caterpillars were moving between overlapping plant canopies using twist ties to denote previous location. Our response variables were developmental rate ($\log(1/\text{development time})$), log pupal mass, and survival. We also scored the total feeding damage on each plant in four categories (0 = no damage, 1 = 0–10%, 2 = 11–50%, 3 = >50%). We spread the replicates across three temporal blocks, which were included as a random effect in all statistical models.

Statistical analyses

We tested hypothesized relationships between chemical diversity and herbivore developmental rate and pupal mass using path analysis (Shipley 2016) in the ‘piecewise’ structural equation modeling (SEM) and ‘lme4’ R packages (Bates et al. 2014, Lefcheck 2016). We determined the top model using the following decision rules about adding and dropping specific variables (summary statistics for candidate models in Appendix S1: Tables S2, S3): (1) Links had to be biologically feasible based on our hypothesized relationships (Table 1); (2) the initial model was a saturated model that included every biologically relevant variable; (3) variables were dropped or added one at time; (4) models were excluded if they had a singular fit; (5) Akaike's information criteria (AICs) were assessed among all potential models that were not saturated and a top model was selected if its AIC was two units lower than other models; saturated models were not considered because they are overparameterized and predictors may potentially have high variance (Shipley 2016), as evident by the zero degrees of freedom and chi square (refer to the supplement Appendix S1: Tables S2, S3, S4 for the summary statistics from all candidate models). In all models, we used caterpillar sex as a fixed effect because *T. ni* males are larger than females (Appendix S1: Fig. S1). We used double-headed arrows to represent covariance between

development rate and pupal mass, so as not to assume causal relationships between these variables. All path models had cage and temporal block as random effects.

For our a priori specified path models for diversity across biosynthetic classes, we used average alpha and beta diversities of all compounds as predictors for herbivore development rate and herbivore pupal mass. For our a priori specified path models for diversity within biosynthetic classes, we included causal relationships between alpha and beta diversity within biosynthetic classes of acyl sugars, alkaloids, and phenolics to the response variables herbivore pupal mass and development rate (Table 1). Chemical richness of compounds was included to tease apart effects from richness and evenness.

Finally, we isolated the effect of beta diversity on herbivore performance and searched for predictors of this effect by calculating effect sizes for each of the nine dicultures. We calculated a single metric of herbivore performance with a principal components analysis (PCA) of developmental rate and pupal mass. The first component (PC1) accounted for and removed the negative correlation between those variables. The second component (PC2) accounted for 30% of the total variation and represented the positive correlation between development rate relative to pupal mass. Thus, PC2, our performance variable, described whether caterpillars were large (positive values) or small (negative values) given their developmental rate (Appendix S1: Fig. S2). Finally, we calculated diculture effect sizes using one linear model for each diculture to estimate the difference between herbivore performance in the diculture versus the mean of the two monocultures of the corresponding genotypes. Positive diculture effect sizes indicate herbivores had higher performance in diculture relative to the mean of the monocultures. Third, we used model selection to search for predictors of the diculture effect size. For a set of models focused on diversity across all biosynthetic classes, we used alpha and beta diversities as predictors. For a set of models focused on diversity within biosynthetic classes, we used alpha and beta diversity in acyl sugars, phenolics, alkaloids, and total chemical richness as predictors. All models had sex as a fixed effect and cage and temporal block as random effects. We compared models using likelihood ratio tests (Bolker et al. 2009).

We examined the effects of chemical diversity on caterpillar survival using binomial generalized linear mixed models in 'lme4' in R (Bates et al. 2014). For diversity across all biosynthetic classes, predictors were alpha and beta diversities. For diversity within biosynthetic classes, predictors were alpha and beta diversities in acyl sugars, alkaloids, and phenolics. Finally, we estimated the overall effect of beta diversity on survival using a model with a predictor for monoculture/diculture. All models included sex as a fixed effect and cage and temporal block as random effects. All analyses were conducted in R version 3.6.2 (R Core Team 2018).

RESULTS

Herbivore growth and development

Relationships between diversity across all biosynthetic classes and herbivore performance.—The top path model based on lowest delta AIC linking diversity across all biosynthetic classes to herbivore development rate and pupal mass was a good fit (Fig. 2; path analysis model summary statistics: Fisher's $C = 3.09$; $df = 2$; $P = 0.2$). These results suggest that alpha diversity was a significant predictor reducing development time but had a positive effect on pupal mass, suggesting that there could be chemicals that increase pupal mass while slowing down their feeding. Conversely, beta diversity had little influence on herbivore performance. The summary stats for all models are found in Appendix S1: Table S2.

Relationships between diversity within biosynthetic class and herbivore performance.—Diversity within biosynthetic classes provided a better fit to the data (Fig. 3; Appendix S1: Table S3, S4; path analysis model summary statistics: Fisher's $C = 3.4$; $df = 4$; $P > 0.4$; $R^2_{\log\text{Mass}} = 0.31$; $R^2_{\text{DevRate}} = 0.9$), suggesting that diversity within chemical biosynthetic classes is more biologically relevant than diversity across all chemical classes. For alpha chemical diversity, caterpillars had reduced

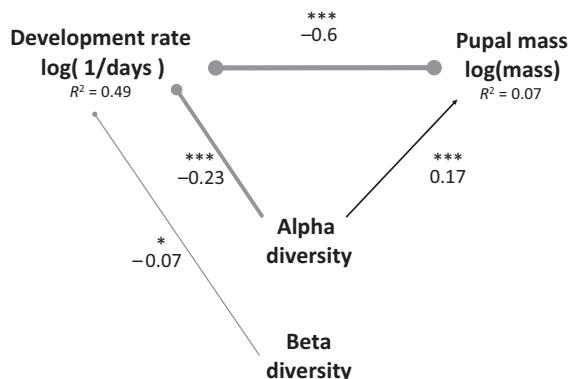


FIG. 2. Path model testing the effect of diversity across biosynthetic classes. This was our best candidate model demonstrating that higher levels of alpha chemical diversity significantly reduced development rate relative to increased pupal mass in *Trichoplusia ni* caterpillars (Fisher's $C = 3.09$; $df = 2$; $P > 0.2$). This suggests that caterpillars are feeding on chemicals that slow their development rate relative to their pupal mass. The direct positive effects of chemical diversity are depicted as arrows, and the direct negative effects are gray blunt-ended lines. The numbers beside the lines are the standardized path coefficients, representing the relative effect of variation explained by the predictor variable. The asterisks beside the standardized path coefficients represent significant relationships. Missing arrows represent causal pathways that were not in the best model but were not themselves significant. Please refer to Appendix S1: Table S2 for summary statistics of all candidate models.

development rates in association with higher levels of alpha diversity within the chemical classes of acyl sugars (spc = -0.23) and phenolics (spc = -0.14). These results suggest that diversity in these quantitative defenses slows the growth of caterpillars. For beta chemical diversity, caterpillars grew faster when there was greater turnover in alkaloid diversity between tomato neighbors (spc = 0.13), suggesting that caterpillars benefited from either choosing between plants or switching between plants that differ in the toxicity of qualitative defenses. Furthermore, we found that caterpillars were consuming both plants fairly equally because both plants sustained roughly equal damage within all dicultures (Appendix S1: Fig. S3). Overall, our results indicated that herbivore performance changed for better or for worse depending on the type of chemical defense exhibited within and between plant neighbors. Specifically, quantitative defenses reduced performance if there were on average higher amounts of inhibiting chemicals within plant neighbors, and qualitative defenses enhanced performance when there was a high turnover in acutely toxic chemicals, that is, alkaloids, between plant neighbors.

Effects of beta diversity on herbivore performance.—We examined the chemical effects underlying plant genetic

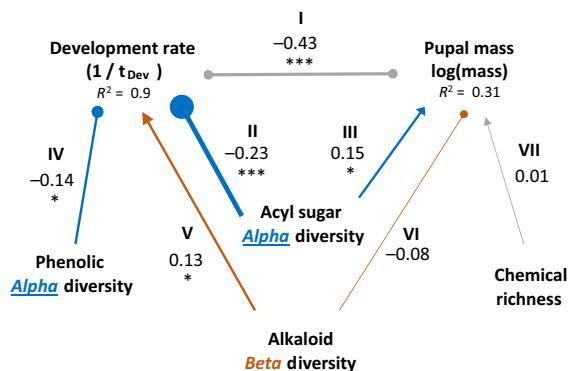


FIG. 3. The top path model testing the effect of diversity within biosynthetic classes suggests that alpha diversity of quantitative chemical defenses reduced herbivore performance, whereas beta diversity of qualitative defenses enhanced performance (Fisher's $C = 3.4$; $df = 4$; $P > 0.4$). The direct positive effects of chemical diversity are depicted as arrows, and the direct negative effects are blunt-ended lines. Blue arrows represent quantitative defenses, whereas orange arrows represent qualitative defenses. Roman numerals denote specific relationships between variables that are relevant to Table 1. The numbers beside the arrows are the standardized path coefficients and describe the variation explained by the direct predictor variable, while accounting for the variation explained by the other indirect predictor variables interacting with the response variable. The asterisks beside the standardized path coefficients represent significant relationships. Missing arrows represent causal pathways that were not in the best model. Nonsignificant pathways were included in the best model but were not themselves significant. Please refer to Appendix S1: Tables S3, S4 for summary statistics of all candidate models.

diversity inherent between dicultures and corresponding monocultures. We did this by isolating the effects of chemical traits on beta diversity of dicultures to determine the influence of switching between hosts of different plant varieties on the performance of herbivores. We found that caterpillars feeding on diculture diets had higher pupal mass relative to their development time compared to caterpillars feeding on corresponding monoculture diets (Fig. 4; slope = -0.24 ; t value = -2.73 ; P value < 0.007). Although there was an overall positive effect of diculture treatment on herbivore performance, results differed depending on which grouping of diculture and corresponding monoculture was compared (Fig. 4B). These results indicate that traits other than genetic diversity are influencing herbivore performance.

Effects of beta diversity across chemical classes.—Caterpillar performance was enhanced when there were higher levels of average alpha diversity in plant dicultures (Fig. 5A; linear mixed-model likelihood ratio tests: $\chi^2 < 0.002$, $df = 1$, $P > 0.8$). Conversely, beta chemical diversity did not significantly influence differences in herbivore performance between di- and monocultures, indicating that not all plant genetic dicultures have high levels of beta chemical diversity (linear mixed-model likelihood ratio tests: $\chi^2 < 0.002$, $df = 1$, $P > 0.8$). These results demonstrate that *T. ni* caterpillars generally benefit from genotypic dicultures, but that the strength of this benefit increases with the level of alpha chemical diversity.

Effects of beta diversity within chemical classes.—Caterpillar performance was enhanced when there were higher levels of average alpha diversity of acyl sugars (Fig. 5b; linear mixed-model likelihood ratio tests: $\chi^2 < 0.11$, $df = 1$, $P = 0.04$) and alkaloids (linear mixed-model likelihood ratio tests: $\chi^2 < 0.1$, $df = 1$, $P = 0.07$). Beta diversity of acyl sugars, alkaloids, and phenolics, however, did not significantly influence herbivore performance (linear mixed-model likelihood ratio tests: $\chi^2 > 0.28$, $df = 1$, $P > 0.5$). The combined results suggest that caterpillars develop faster and are larger in dicultures and that the size of this effect is positively related with the chemical diversity within plants (alpha diversity).

Herbivore survival

For diversity across all chemical classes, the mixed-effects logistic regression found no difference in caterpillar survival (binomial survival models: slope = -12.3 ; z value = -0.9 ; $df = 424$; $P > 0.3$) in regard to alpha and beta diversities. For diversity within chemical classes, caterpillar survival was not affected by any combination of diversity within chemical classes and was not significantly different from the null model (likelihood ratio tests: $\chi^2 = 0.4$; deviance = 828.5 ; $df = 3$; $P > 0.9$).

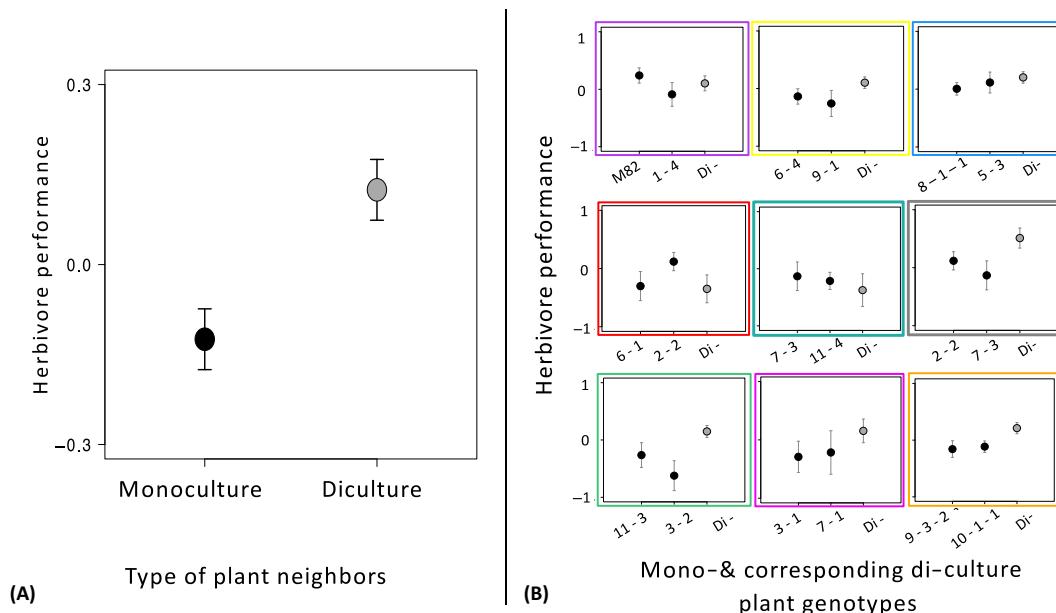


FIG. 4. Herbivore performance was positively associated with diets consisting of dicultures compared to corresponding monocultures, though this varied depending on the plant pairing. Herbivore performance on the y-axis was calculated from the second component of a principal components analysis examining individual caterpillar development rate relative to pupal mass. Panel (A) depicts the overall performance means across all monocultures and dicultures. Caterpillars performed better on average in dicultures than in monocultures (Slope = -0.24 ; t value = -2.73 ; $P < 0.01$). Panel (B) shows herbivore performance in each of the nine dicultures and their corresponding two monocultures. Herbivore performance is calculated from a principal component analysis of developmental rate and pupal mass (see methods). Colors correspond to Fig. 1B.

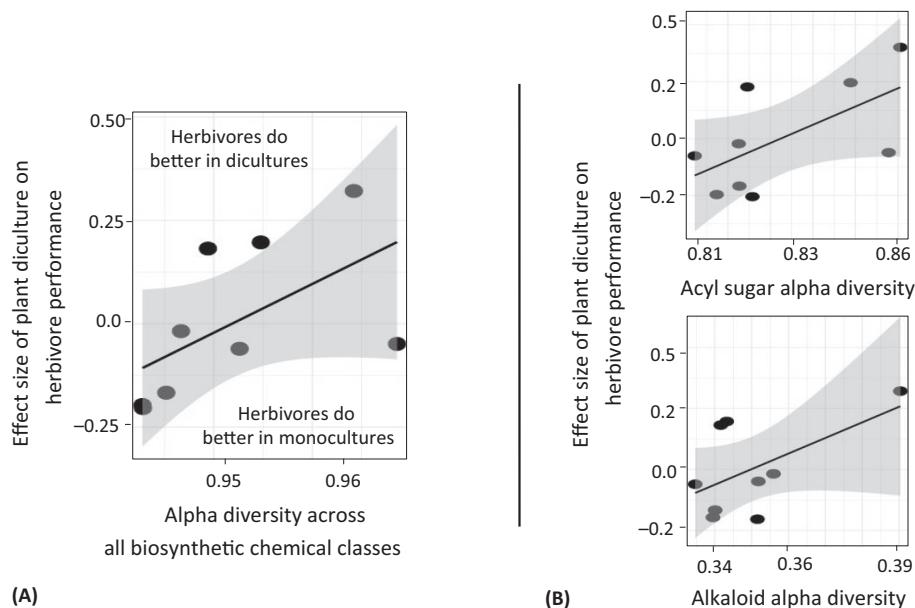


FIG. 5. The effect of dicultures on herbivore performance more positive with increasing alpha chemical diversity. The effect size of dicultures on herbivore performance (y-axis) is the model-predicted difference in herbivore performance between each diculture and the mean of each of the corresponding two monocultures. Positive values indicate higher herbivore performance in a diculture relative to the mean of the two corresponding monocultures. Panel A examines the consequences of total diversity on herbivore performance. Herbivores performed significantly better on dicultures compared to the associated monocultures when levels of alpha diversity were high ($\chi^2 < 0.2$, $df = 1.9$, $P < 0.001$). Panel B examines the consequences of diversity within chemical classes on herbivore performance. Herbivores performed significantly better in dicultures than in monocultures when there was high alpha diversity in acyl sugars (top; $\chi^2 < 0.11$, $df = 1$, $P = 0.04$) and alkaloids (bottom; $\chi^2 < 0.1$, $df = 1$, $P = 0.07$).

For the effects of plant dicultures on herbivore performance, caterpillar survival was similar between monocultures and dicultures (Appendix S1: Fig. S4; t -test: $t = 0.93$, $df = 421.99$, $P > 0.3$).

DISCUSSION

Phytochemical diversity is hypothesized to be a major component of plant–insect interactions (Hunter 2016, Glassmire et al. 2016, 2019, Bustos-Segura et al. 2017, Kessler and Kalske 2018). It is not well understood, however, which dimensions and scales of phytochemical diversity mediate these ecological effects because of the lack of manipulative experiments teasing apart the effects of alpha and beta diversity on herbivore performance for multiple biosynthetic compound classes (Schuman et al. 2016). We addressed this gap by experimentally manipulating plant chemical diversity using a genetically controlled plant system, the *S. pennellii* introgression lines, and quantified plant chemical effects on the performance of a mobile generalist herbivore, *T. ni*. We found, first, that alpha and beta diversities demonstrated contrasting effects on herbivore performance in that alpha chemical diversity reduced herbivore performance while beta diversity enhanced herbivore performance (Fig. 3). Second, diversity within biosynthetic classes was a stronger predictor of herbivore performance than diversity across all biosynthetic classes, and the effects of diversity in phenolics and acyl sugars were opposite in sign from the effects of alkaloids (Fig. 3). These findings indicate that generalist herbivores may not respond the same way to different kinds of chemical classes, and specific diversity effects on performance can be difficult to distinguish when diversity of all chemical classes are combined. Third, the effect of plant dicultures on herbivore performance depended upon the amount of alpha chemical diversity in the diculture, suggesting that there is an interactive effect between alpha and beta chemical diversities (Figs. 4 and 5). Overall, our results supported the screening and synergy hypotheses when examining alpha diversity, and we found support for the diet mixing hypothesis when examining beta diversity, suggesting that identifying relevant dimensions and scales of diversity is essential to testing major hypotheses about the ecological role of phytochemical diversity.

Alpha and beta diversities had contrasting effects on herbivore performance

Trichoplusia ni performance was reduced with alpha diversity and enhanced with beta diversity. This result suggests that plant diversity reduces herbivore performance mainly through mechanisms posited by the screening and synergy hypotheses (Jones and Firn 1991, Berenbaum and Zangerl 1996, Richards et al. 2016). Our results are consistent with these hypotheses, suggesting that the huge diversity of many kinds of chemicals

that comprise plants is an adaptive response to defend against *T. ni* caterpillars. In contrast, plant defense mechanisms posited by the moving target and acclimation hypotheses that rely on chemical differences among neighboring plants appear to be less important in our system (Adler and Karban 1994, Wetzel and Thaler 2016). This result is consistent with the diet-breadth phytochemical diversity hypothesis, which posits that generalists, like the one in this study, that adapted to use a diversity of hosts with differing chemical phenotypes, may be well adapted to high levels of beta chemical diversity (Wetzel and Whitehead 2020). Studies of beta diversity across larger spatial and phylogenetic scales, however, have found that beta diversity can reduce feeding damage (Salazar et al. 2016, Bustos-Segura et al. 2017, Massad et al. 2017), suggesting that the effects of beta diversity are scale dependent. Diversity across chemical classes, structural scaffoldings, and biological modes of action may pose a challenge to herbivores, whereas mere variation in the abundance of common compounds among closely related plant genotypes may benefit herbivores, at least for mobile generalists. From the perspective of the plant, our findings suggest pressure from a mobile generalist herbivore may evolutionarily select for higher levels of alpha chemical diversity as a defensive strategy rather than select for chemical differences from conspecific neighbors. The biological effect on herbivores, however, may depend on herbivore mobility because beta chemical diversity between plants would not be as important for sedentary insects. Furthermore, the fact that we found effects of intraspecific beta diversity on *T. ni*, an extreme diet generalist feeding across plant families, suggests that the influence of beta diversity may change across herbivore diet breadth.

The biological effect of individual chemical classes matters

Diversity within biosynthetic classes was more predictive of herbivore performance than diversity across all chemical classes. This result may have occurred because the effects of diversity differed among our three focal chemical classes. Lumping diversity across classes to examine total chemical diversity therefore may have masked the specific effects of diversity within each biosynthetic class. Overall, phytochemical diversity is comprised of many components and combining all biosynthetic classes can be misleading, because of missed interactive, contrasting, or additive effects, such as with studies that focus on single compounds (Richards et al. 2016).

When we examined results at the scale of diversity within our three focal chemical classes, we found that acyl sugar and phenolic alpha diversity reduced herbivore performance. Acyl sugars and phenolics putatively function to inhibit feeding and are quantitative defenses that are more effective at reducing herbivore performance at high concentrations (Feeny 1976). Our results

suggest that quantitative defenses may also be more effective at high chemical diversities by effectively overwhelming herbivores with many different kinds of mildly detrimental compounds. This result suggests that the screening and synergy hypotheses, which predict that alpha chemical diversity is an effective herbivore defense, may be most relevant for quantitative defenses (Jones and Firn 1991, Berenbaum and Zangerl 1996). In contrast, beta diversity—variation between plants—in acyl sugars and phenolics had no effect on herbivores, suggesting that evenness of chemicals is more inhibiting within plant neighbors, compared to turnover of chemicals between plant neighbors for quantitative defenses.

For alkaloids, conversely, beta diversity enhanced herbivore performance, whereas alpha diversity had no effect. Alkaloids, such as glycoalkaloid compounds in tomatoes, are acutely toxic qualitative defenses that are effective at relatively low concentrations. The toxic effects of qualitative defenses on herbivore performance was mitigated only when plant neighbors were chemically dissimilar, perhaps because herbivores were able to balance nutrient deficiencies and dilute toxic chemicals as predicted by the diet mixing hypothesis (Bernays et al. 1994, Bernays and Minkenberg 1997). We found that enhanced herbivore performance was contingent on chemicals that are biologically toxic rather than acyl sugars and phenolics that biologically inhibit feeding. For that reason, the diet mixing hypothesis seemed to apply only towards qualitative defenses, but not for quantitative defenses. Overall, our results suggest that it may take only one good toxic compound to defend against herbivores when it comes to qualitative defenses for plants, but this does not apply for quantitative defenses.

The effect of beta diversity became more positive with alpha diversity

When we isolated the effects of beta diversity on herbivore performance by comparing performance between dicultures to the mean of their corresponding monocultures, we found that herbivore performance became more enhanced as alpha diversity increased (Figs. 4A and 5). That is, when chemical turnover between plant neighbors had higher evenness rather than being dominated by only a few chemicals then caterpillars performed better. Thus, we found an interaction between alpha and beta diversities, and this was true in both quantitative (Fig. 5B, acyl sugars) and qualitative (Fig. 5B, alkaloids) defenses. If plant dicultures benefited caterpillars by allowing them to switch between plant genotypes to dilute the ingestion of any one toxin, then alpha diversity may have accentuated this benefit by presenting caterpillars with more toxins to be diluted or nutrients to balance. This idea is an extension of the diet mixing hypothesis (Bernays et al. 1994, Bernays and Minkenberg 1997), by suggesting that nutrient balancing and toxin dilution matters more when plants have high

levels of alpha chemical diversity. Alternatively, dicultures could have benefited herbivores not through diet mixing but simply by allowing them to choose the highest-quality plant. If so, then our results would suggest that having the ability to choose becomes more beneficial when plants have higher levels of average alpha chemical diversity. One line of evidence demonstrating that caterpillars were moving between plants in dicultures is the total plant damage estimated (Appendix S1: Fig. S3). The damage shows that most caterpillars always fed at least some from both host plants, and some fed more from one of the two plants in the diculture treatments, suggesting that some caterpillars were potentially choosing or avoiding certain genotypes. Regardless, our results imply that the effects of different dimensions of phytochemical diversity on insect herbivores are not independent—the ecological consequences of diversity among plants may depend on the level of diversity within plants.

CONCLUSION

This work indicates that phytochemical diversity plays a multidimensional role in ecological interactions between plants and other organisms, with each scale and type of diversity having potentially unique, contrasting, or interactive ecological effects. Phytochemical diversity is multidimensional and should be examined from all directions because each dimension provides insights into why chemical variation is maintained from the perspectives of plants and herbivores. Determining the key dimensions of phytochemical diversity would provide answers as to why plants produce a diversity of compounds and have evolutionarily maintained such an extensive suite of chemical diversity. This means for the fields of intraspecific trait variability, plant–animal interactions, and chemical ecology that we cannot just look at total diversity or look at diversity within one biosynthetic class, but rather it is important to compare herbivore performance among biosynthetic classes.

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