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# Plasticity in plant defense and the role of phytochemical dissimilarity in limiting specialist herbivory

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Phytochemical diversity is an effective plant defensive attribute, but much more research has focused on genetic and environmental controls of specific defensive compounds than phytochemical diversity *per se*. Documenting plasticity in phytochemical richness and plant chemical composition as opposed to individual compounds is important for understanding plant defense. This study outlines a multi-site transplant experiment in Cerrado gallery forests in central Brazil, utilizing *Piper arboreum* (Piperaceae), a prevalent and widespread neotropical shrub. Clones from four distinct populations were planted either at their origin site or in a different forest. Secondary metabolite composition varied between populations initially and then changed after transplanting. Interestingly, clones with chemical profiles that were distinct from the populations where they were introduced experienced reduced specialist chrysomelid herbivory compared to clones that were more chemically similar to the existing *P. arboreum* populations where they were planted. Specialist Lepidoptera herbivory also declined in clones transplanted to a new forest, but this change could not be ascribed to chemical profiles. In contrast, generalist herbivory was unaffected by chemical dissimilarity and transplanting. This research adds to the expanding body of evidence suggesting that phytochemical diversity is a dynamic trait exerting unique effects on different herbivore guilds.

## KEYWORDS

generalist herbivore, phytochemistry, metabolomics, *Piper arboreum*, transplant experiment, tropical forest, specialist herbivore

## Introduction

“The world is not colored green, it is colored morphine, caffeine, tannin, phenol, terpene, canavanine, latex ...” (Janzen, 1978). Janzen’s observation indicates that insect herbivores perceive plants as distinct chemical mixtures. These mixtures can be highly diverse, and phytochemical profiles differ substantially within as well as among populations (Arany et al., 2009; Zust et al., 2012; Glassmire et al., 2016; Massad et al., 2017; De-la-Cruz et al., 2020; Massad et al., 2022). Phytochemistry is also quickly evolving (Kessler and Kalske, 2018), particularly in response to specialist herbivory (Endara et al., 2015). Specialist herbivores may be locally adapted to the chemical community they interact with (Glassmire et al., 2017), making host plants of the same species but from different populations potentially less palatable to them. In contrast, generalist herbivory may be less affected by unique chemotypes (Massad et al., 2022).

Understanding how plant chemistry changes across a landscape and how specialist and generalist herbivory differ with host plant chemistry is an important goal for ecology (Hunter, 2016). As herbivore communities turnover across the ranges of their host plants (Salazar and Marquis, 2012; Fine et al., 2013; Salazar et al., 2018; Campos-Moreno et al., 2021; Serejo Rabelo et al., 2021), one may expect phytochemical diversity and similarity to also vary across a species' range. It is therefore informative to quantify genetic and environmental controls of plant antiherbivore defenses (Fajer et al., 1992; Laitinen et al., 2005; Osier and Lindroth, 2006; Arany et al., 2009; Massad et al., 2011; Eisenring et al., 2021).

Different species of herbivores are uniquely affected by different classes of chemical defenses, rendering multiple lines of defense necessary (Koricheva et al., 2004; Agrawal and Fishbein, 2006). For example, alkaloids in *Datura stramonium* effectively limit herbivory by the specialist beetle, *Epitrix parvula*, while a triterpenoid in the same plant reduces damage by a second specialist beetle, *Lema daturaphila* (De-la-Cruz et al., 2020). Other well studied examples include glucosinolates, cardenolides, alkaloids, and iridoid glycosides that often limit generalist but not specialist herbivory (Bowers and Puttick, 1988; Malcolm, 1994; van Dam et al., 1995; Agrawal et al., 2012; Jeschke et al., 2017). In addition, synergistic effects of co-occurring compounds often result in mixtures of defenses that are more effective than isolated compounds (Berenbaum and Neal, 1985; Berenbaum and Zangerl, 1993; Dyer et al., 2003; Richards et al., 2010, 2012). Secondary metabolite diversity is therefore an important plant trait (Wetzel and Whitehead, 2020) that has recently become recognized as an effective anti-herbivore defense both at the level of individual plants ( $\alpha$ -diversity) and at the level of populations ( $\beta$ -diversity; Kessler and Kalske, 2018).  $\alpha$ -diversity can be calculated as Simpson's index (Richards et al., 2015; Wetzel and Whitehead, 2020), and  $\beta$ -diversity can be calculated with similarity indices that measure changes in community (or chemical) composition (Massad et al., 2017; Wetzel and Whitehead, 2020; Massad et al., 2022).

Quantitative changes in plant defenses are influenced by environmental and genetic variation (Massad et al., 2011). In terms of qualitative changes in defenses, differential herbivore pressure and resource availability across sites affect heritable variation in plant chemical profiles and generate chemically distinct populations (Zust et al., 2012; Fine et al., 2013; Hunter, 2016; Glassmire et al., 2017; De-la-Cruz et al., 2020). While hundreds of studies have looked at quantitative changes in defense driven by environmental variation (e.g., Bryant et al., 1983; Herms and Mattson, 1992; Koricheva, 2002; Massad et al., 2011, 2012; Hattas et al., 2017; Chinder et al., 2020), fewer studies have measured environmental effects on phytochemical diversity (Cadena-Zamudio et al., 2022).

Transplant and common garden experiments are very effective for uncovering genetic and environmental controls of plant defense and growth. For example, a common garden experiment replicated across an environmental gradient shows defense traits, but not growth, are under genetic control in *Asclepias speciosa* (Hahn et al., 2019). Similarly, when *Arabidopsis thaliana* individuals from a site with naturally low herbivory were transplanted to a site with high herbivory, they suffered more damage than congeners originating in the high herbivory site. Plasticity in defense also differs between populations, and defense production is less responsive to resource conditions in plants originating from a nutrient poor, high herbivory environment (Arany et al., 2009), where there may be greater selection for high

levels of defense (Coley et al., 1985). Transplant experiments with tree species in the Amazon have also demonstrated herbivory is lower in individuals originating from resource poor areas (Fine et al., 2004) where plant defense is greater (Fine et al., 2006, 2013).

To understand environmental versus genetic variation in phytochemical profiles as well as the effects of phytochemical similarity and diversity on specialist and generalist herbivores, we examined changes in phytochemistry across populations in a reciprocal transplant experiment. In contrast to the transplant experiments cited above, we focus on phytochemical diversity and phytochemical similarity as potential antiherbivore defenses. The effects of genotype, transplanting to novel sites, and plant chemistry were evaluated separately for specialist and generalist herbivory. Plant growth was also analyzed in response to genotype, transplanting, and herbivory. This work focused on a chemically rich, widespread neotropical understory species, *Piper arboreum* Aubl. (Piperaceae), and it combined a classic ecological design with modern methods in LC-MS metabolomics to test the following hypotheses. (1) Populations may be phytochemically distinct, and genotype may have stronger control on phytochemical profiles than the transplant environment. (2) Transplanting may limit specialist herbivory as herbivores may not be adapted to the chemistry of novel genotypes. (3) Chemical dissimilarity between transplanted individuals and naturally occurring conspecifics may limit herbivory, particularly by specialists. (4) Phytochemical diversity may limit generalist herbivory. (5) Growth may be limited by herbivory but be unaffected by genotype.

## Methods

### Focal species and study sites

*Piper arboreum* Aubl. is a widespread understory shrub occurring in tropical forests from Mexico through Paraguay (Kew Royal Botanic Gardens, <https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:680435-1>; Yuncker, 1972). The possibility that populations of *P. arboreum* are cryptic species is currently under investigation. The genus *Piper* is highly abundant in neotropical forest understories, including cerrado gallery forests, and it is also extremely species rich, with about 2,600 described species, mostly in the Neotropics (Callejas-Posada, 2020). The chemistry of many species of *Piper* has been studied (Dyer et al., 2003; Dyer and Palmer, 2004; Kato and Furlan, 2007; Uckele et al., 2021; Philbin et al., 2022). The genus is known for its phytochemical diversity, both within and between species (Richards et al., 2015; Massad et al., 2017; Glassmire et al., 2019; Massad et al., 2022), so further study of the ecological effects of its chemical diversity may help explain plant-herbivore interactions. *Piper arboreum* in particular produces at least five antifungal amides, cinnamoyl derivatives (Vasques da Silva et al. 2002) and several terpenoids (Navickiene et al., 2006). Plant-insect herbivore interactions on *Piper* are also well studied, and patterns of herbivory are known for specialist Lepidoptera, Coleoptera, and generalist Acrididae as well as other taxa (Dyer and Letourneau, 1999; Connahs et al., 2009; Dyer et al., 2010; Serejo Rabelo et al., 2021). Because of its wide distribution, likely intraspecific phytochemical variation (Richards et al., 2015; Massad et al., 2017, 2022; Glassmire et al., 2019; Uckele et al., 2021), and knowledge of its herbivore community, *P. arboreum* was chosen as the focal species for the present study.

*Piper arboreum* clones were created from four populations in gallery forests in four protected areas of the cerrado in the Distrito Federal and Goiás in central Brazil (Supplementary Table S1). The cerrado is the second largest biome in South America, and evergreen gallery forests originally accounted for 5% of the cerrado's area (Dias, 1996). Distances between sites varied from 7 km to 235 km. Caterpillar communities differ between focal sites (Serejo Rabelo et al., 2021), and mean *P. arboreum* density (as measured in a minimum of 38 10 m diameter plots per site) varied from 730 to 1,243 plants per ha.

## Experimental design

In each of the four gallery forests (populations), we selected three plants of *P. arboreum*, resulting in a total of 12 mother plants. We cut each mother plant into six clones with one node and three leaves, yielding 72 cuttings. We then planted the clones from each mother plant in one of the four forests, with each forest receiving cuttings from one mother plant from that forest and cuttings from a mother from each of the other three forests. While this method does not allow us to quantify potential confounding factors, such as maternal effects, it provides valuable insights into the relative contributions of genetic and environmental factors (including epigenetic effects) to trait variation. Although we cannot examine specific genotypes, our experimental design enables us to investigate questions related to genotype-environment interactions.

We transplanted 24 cuttings in three of the four gallery forests from which we collected genotypes, namely Estação Ecológica de Águas Emendadas (ESECAE), Reserva Ecológica do Roncador (RECOR), and Parque Nacional de Brasília (PNB). We did not use the fourth site of origin, Parque Nacional da Chapada dos Veadeiros (PNCV), for transplanting due to its distance from the other sites and the logistical challenges of regularly monitoring the clones. Each transplant location was planted with six clones originating from the same site (i.e., from the same mother plant) and 18 clones from the other two sites (six from the same mother plant at one site and six from the same mother plant at the second site). We planted the clones in 10 L buckets using commercial potting soil, and randomly placed the buckets within the experimental sites, with a minimum distance of 2 m between them. The total experimental footprint in each forest was 600 m<sup>2</sup>. We planted the clones in April 2015, and the experiment ended in April 2016. We measured the initial height of all clones.

During the course of a year, the clones were searched for lepidopteran herbivores, and all leaves were photographed to measure herbivory each month. Percent herbivory was measured using ImageJ.<sup>1</sup> Herbivory was separated based on the type of herbivore (classifications included families of Lepidoptera, Chrysomelidae, Acrididae, and other herbivores). Analyses of data from the final month of measurements are presented. Eggs, caterpillars, and pupae of Lepidoptera found on the experimental plants were photographed monthly; herbivores were left on the experimental plants to avoid interrupting herbivory. Plant height and the dry biomass of roots, stems, and leaves were measured at the end of the experiment. Relative growth in height was calculated as the final height minus the initial height divided by the initial height.

## Chemical analyses

Leaf samples from each mother plant were collected for chemical analysis at the time the clones were prepared, and leaf samples were collected from plants at the end of the experiment as well. In remote field sites, flash freezing samples is not possible. As our objective was not to quantify or identify all metabolites present at a given point in time but rather to compare phytochemical similarity and richness between samples, all samples were collected following the same sampling protocol at roughly the same time of day to minimize variation associated with sample collection. These methods, although not perfect, have been used effectively in multiple studies in chemical ecology throughout the tropics (Richards et al., 2015; Salazar et al., 2018; Massad et al., 2022). Leaves were dried at ambient temperature in paper bags. Samples were ground, and 100 mg of each leaf were extracted in 800  $\mu$ L of MeOH. Extracts were centrifuged at 10,000 RPM for 20 min, and the supernatant was collected and dried under N<sub>2</sub> flow. Methanol is an effective solvent for extraction compounds of varying polarities (Huang et al., 2010; Mollik et al., 2022).

Samples were redissolved in 1 mL MeOH, and the solution (10  $\mu$ L) was injected into an HPLC-HRESMS system. The HPLC (Shimadzu, Kyoto, Japan) equipped with an autosampler, binary pump, column oven, and a single-wavelength UV-Vis detector. The analyses were performed using a Kinetex C-18 column (Phenomenex, 100 Å, 100  $\times$  1 mm, 2.6  $\mu$ m PFP) at 40°C. A linear gradient elution was performed using a flow rate of 200  $\mu$ L/min, with solvent A (water/formic acid, 99.9:0.1 v/v) and solvent B (MeOH:formic acid, 99.9:0.1 v/v). Conditions were held at 85% A from 0 to 2 min, 70% A from 2 to 10 min, and 100% B from 35 to 38 min. MicroQToF II (Bruker) were performed in positive-ion scan mode (100–1,000 Da), using N<sub>2</sub> as the nebulizer gas at 4 Bar and the drying gas at 8 L/min and 4.5 kV capillary voltage at 200°C. The collision and the quadrupole energy were set to 12 and 6 eV, respectively. RF1 and RF2 funnels were programmed to 400 and 200 Vpp, respectively. The HPLC-HRESMS raw data were converted to .netCDF using Data Analysis 4.3 software (Bruker). The data were analyzed with XCMS online software (version 03.03; Tautenhahn et al., 2012; Gowda et al., 2014) for feature detection (min peak width 10; max peak width 60; mz deviation 10), retention time correction (obwarp method; profStep 0.5) and alignment (bw 5 s; minfrac 0.7; mzwid 0.025). A multigroup analysis with netCDF converted data was used to align and extract the data.<sup>2</sup> Peak area data were scaled prior to analysis by dividing the absorbance of a given peak by the total absorbance across all peaks.

The metabolites extracted and quantified using this approach are not all assumed to be defensive, but they are likely to be part of metabolic pathways that yield defensive compounds. Overall measures of metabolite complexity or diversity should capture the defensive efficacies of different mixtures without a need for annotation or a requirement to attribute defensive attributes to any one peak (Richards et al., 2015; Dyer et al., 2018).

## Data analyses

Chemical similarity between samples from different genotypes and transplant locations was visualized using principal component

<sup>1</sup> <https://imagej.net/>

<sup>2</sup> [https://xcmsonline.scripps.edu/landing\\_page.php?pgcontent=mainPage](https://xcmsonline.scripps.edu/landing_page.php?pgcontent=mainPage)



analysis (PCA) with the `prcomp` function in R version 4.0.3 (R Core Team, 2020). There were a total of 733 peaks detected across samples, and the area of each peak was used in the PCA. Data were scaled for the analysis. In order to quantify potential differences between groups, an analysis of similarity (ANOSIM) was also conducted using the Bray–Curtis distance and 9,999 permutations. The analysis was run with the `vegan` package (Oksanen et al., 2022). In addition, a principal coordinate analysis was conducted with the same data (see methods and results in the [Supplementary material](#)).

Phytochemical dissimilarity between planted clones at the end of the experiment and the natural population in the transplant location was calculated as the Euclidean distance between samples. The resulting variable was used in analyses of herbivory (see below). First, average peak values were calculated for each genotype-transplant location combination and the original mother plants (to represent the natural population in the planting location). Peak values were then normalized with a *z*-transformation and the Euclidean distance was calculated using the `dist` function. Phytochemical diversity was also calculated for each genotype  $\times$  transplant combination at the end of the experiment and for each original mother plant (to represent the average phytochemical diversity in a given location) with Simpson's index (Jost, 2006). Peaks were included as species, and their areas were used as abundances.

The effects of genotype (the identity of the mother plant), transplanting (being transplanted to a new site or planted in the site of origin), the transplant location, phytochemical dissimilarity, and phytochemical diversity were tested on different types of herbivory with Bayesian ANCOVA. Herbivory was analyzed separately for Lepidoptera, Chrysomelidae, and Acrididae. Lepidoptera damage came from the families Hesperidae and Nymphalidae. Members of these families specialize on *Piper*. Chrysomelid species also specialize on *Piper*. Acrididae are generalist herbivores (Dyer et al., 2010). Models were run with the `R2jags` package (Su and Yajima, 2020) using four chains, a burn-in period of 1,000 iterations per chain, and 1,000,000 model iterations per chain. Continuous variables were *z*-transformed prior to analyses. Model performance was evaluated with trace and density plots and Geweke, Heidelberger–Welch, and Raftery–Lewis convergence diagnostics in the `superdiag` package (Tsai et al., 2012). Non-predictive covariates were removed until just categorical treatments remained in the models.

A Bayesian structural equation model (BSEM) was constructed to test causal relationships between relative growth rate in height, herbivory from the three distinct groups of herbivores, and the effects of phytochemical diversity (of original mother plants and individuals at the end of the experiment), phytochemical dissimilarity, genotype, and transplanting on herbivory. The direct effects of genotype and transplanting on growth were also tested. Models were built with the `lavaan` (Rosseel, 2012) package. Bayesian posterior probabilities for the modeled parameter estimates were calculated using the `blavaan` package (Merkle and Rosseel, 2016). Bayesian analyses were run with two chains of 10,000 samples, a burn-in of 20 iterations, and an adaptation of 100 iterations. Bayesian posterior probabilities are presented with marginal log-likelihood and posterior predictive *p*-values (PPP values). Non-predictive relationships were successively removed from models, and AIC scores and  $X^2$  goodness of fit statistics were used to determine the best fit model. All analyses were run using R version 4.0.3 (R Core Team, 2020).

## Results

Peak richness (corresponding to unique chemical compounds) of transplanted clones ranged from 680 to 731. In addition, phytochemical diversity (quantified with Simpson's index) ranged from 53.8 to 84.8, although there were no patterns in diversity between genotypes or transplant locations. Chemotypes could be distinguished based on their population of origin (ANOSIM  $R=0.24$ ,  $p=0.0001$ ) and their transplant location (ANOSIM  $R=0.27$ ,  $p=0.0001$ ), although there was much overlap between plants based on their genotype and transplant location. The first principal component of the PCA explained 24.2% of the variation in the data, and the second explained 15.8% of the variation (Figure 1; see [Supplementary Figure S1](#) for the PCA plot with 95% confidence ellipses). The third PC explained 9.5% of the variation. The retention times and masses of the 10 peaks with the highest loadings on PC1, 2 and 3 are shown in [Supplementary Figure S2](#). Similar results were obtained with the principal coordinates analysis (see [Supplementary material](#)).

No covariates included in the Bayesian ANCOVA were predictive, so the models were simplified to Bayesian ANOVA. Parameter estimates from the Bayesian ANOVA support the hypothesis that transplanting decreased both specialist lepidopteran and chrysomelid herbivory, and Chrysomelidae damage was higher in the Reserva Ecológica do Roncador relative to the other sites (Table 1; Figure 2). No variables were predictive of generalist acridid damage. Based on path coefficients from the Bayesian SEM, specialist lepidopteran herbivory was influenced by transplanting; lepidopteran damage was over 13 times higher on clones planted in their site of origin as opposed to those that were transplanted. Both chrysomelid herbivory and relative growth in height differed between genotypes according to the SEM. Specialist chrysomelid damage also decreased when transplanted clones were less chemically similar to the natural populations surrounding them. Again, in contrast to the specialist herbivores, nothing was predictive of generalist acridid herbivory. Lastly, herbivory did not affect growth in height ( $X^2=12.05$ ,  $df=13$ ,  $p=0.52$ ; marginal log-likelihood =  $-646.4$ , PPP = 0.56; Figure 3).

## Discussion

Phytochemical diversity can exert community wide effects on terrestrial insects through mediating plant–herbivore interactions and can be highly variable both between and within species (Kursar et al., 2009; Endara et al., 2015; Richards et al., 2015; Massad et al., 2017; Salazar et al., 2018; Glassmire et al., 2019; Massad et al., 2022). In the case of *P. arboreum* in Cerrado gallery forests, phytochemical diversity was high, differed between isolated populations, and changed with transplanting. Growth of *P. arboreum* was affected by genotype, which was not hypothesized, although this finding is consistent with other studies (Siemann and Rogers, 2001; Osier and Lindroth, 2006; Zhao et al., 2011; Holeski et al., 2013; Paudel et al., 2019).

As hypothesized for this system, there were population level differences in plant chemistry, changes in phytochemistry after transplanting, and greater effects of novel chemistry on specialist versus generalist herbivores. Despite the antiherbivore effects of phytochemical diversity in other *Piper* species (Richards et al., 2015), however, chemical diversity did not affect herbivory in our experiment. Interestingly, variation in *P. arboreum* phytochemistry arose post-transplanting. Although the ANOSIM distinguished between chemotypes based on

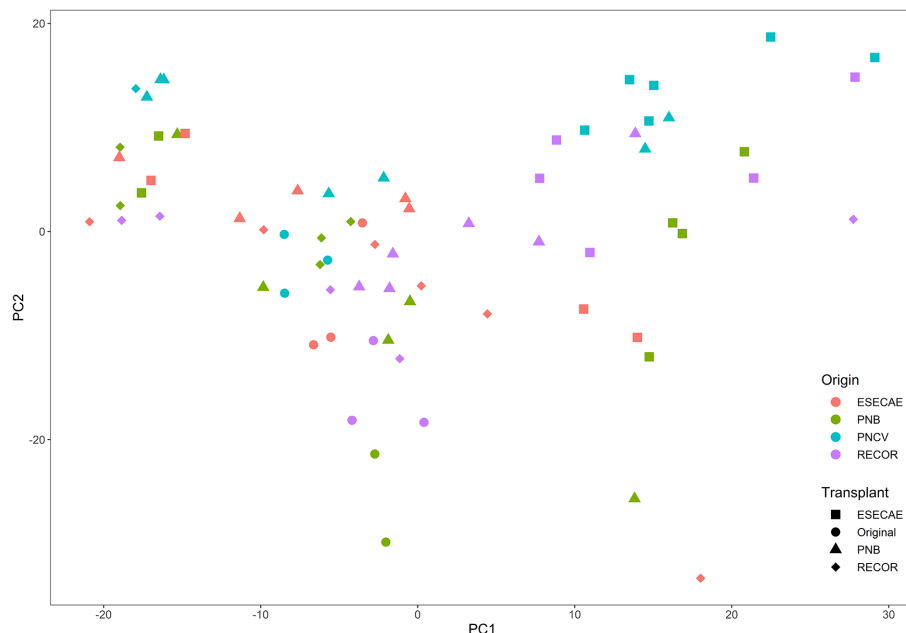


FIGURE 1

PCA of chemotypes from different populations transplanted in different forests. 'Origin' refers to the location of the mother plants that generated experimental clones. 'Transplant' refers to where the clones were planted, except for the 'original' plants which represent the mother plants from which clones were derived. The chemical profiles of all plants except the 'originals' were measured at the end of the experiment. Colors indicate sites of origin, and shapes indicate transplant locations..

**TABLE 1** Posterior probabilities with 90% credibility intervals from Bayesian ANOVA describing specialist Lepidoptera and chrysomelid herbivory and generalist acridid herbivory on *P. arboreum* from four populations transplanted across three sites. Bold values have 90% credibility intervals that do not cross zero.

	Lepidoptera herbivory	Chrysomelidae herbivory	Acrididae herbivory
Intercept	0.75 (−0.09, 1.58)	1.82 (1.00, 2.63)	−0.16 (−1.02, 0.70)
Genotype	0.91 (−0.06, 0.24)	−0.08 (−0.23, 0.06)	−0.30 (−1.03, 0.22)
Transplanted (yes/no)	<b>−0.65 (−1.03, −0.28)</b>	<b>−0.62 (−0.98, −0.25)</b>	0.24 (−0.15, 0.63)
Planting location	0.08 (−0.11, 0.28)	<b>−0.26 (−0.45, −0.07)</b>	−0.06 (−0.20, 0.20)

their origin and transplant location, there was a great deal of overlap between chemotypes at the end of the experiment. These results indicate phytochemical expression in *P. arboreum* is plastic, both quantitatively and qualitatively. Other work with *Piper* also shows there is measurable variation in defense between subpopulations; furthermore, this variation is associated with changes in lepidopteran herbivore communities (Glassmire et al., 2016). Changes in plant chemistry in our experiment may have resulted from differential herbivory in addition to influencing herbivory. Work with another *Piper* species actually shows declines in imides rather than induction with herbivory (Glassmire et al., 2023).

A clear pattern emerged regarding chemical similarity and specialist herbivory on *P. arboreum*. Specialist chrysomelid damage was lower on clones that differed chemically from plants of the population where they were transplanted. In contrast, chemical similarity was shown to limit specialist herbivory across *Piper* species in the Mata Atlântica. However, those data combined chrysomelid and lepidoptera damage (Massad et al., 2022), and lepidopteran herbivory was not affected by chemistry in the present study. Nonetheless, lepidopteran herbivory was reduced more than tenfold on transplanted clones relative to clones planted in their location of origin ( $1.82 \pm 1.3\%$  (S.E.) vs.  $0.14 \pm 0.07\%$ ). This suggests that unquantified plant traits affect host selection and consumption by Lepidoptera on *P. arboreum*.

Variation in plant nutrition may be responsible for changes in specialist damage, as specialist herbivores often respond positively to plant nutritional quality (Prudic et al., 2005; Coley et al., 2006; Kurze et al., 2017). Generalists also respond to nutrient quality (Coley et al., 2006), but specialist herbivores, particularly Lepidoptera, are often less negatively affected than generalists by specific chemical compounds (Rothwell and Holeski, 2020), supporting our results.

Generalist herbivory, both on *P. arboreum* and on other *Piper* species, is not affected by the diversity or similarity of intrafoliar chemistry (Massad et al., 2022), although generalist damage is reduced by the diversity and similarity of volatile organic compounds, which likely influence host-searching (Massad et al., 2017; Salazar et al., 2018). Overall, the pattern that generalist herbivores are more negatively affected than specialists by specific chemical compounds (Massad et al., 2011; Rothwell and Holeski, 2020) and the lack of effect of intrafoliar phytochemical similarity on generalists suggests phytochemical dissimilarity may be more relevant to specialists, such as Chrysomelidae, adapted to specific compounds in their host plants. In contrast, broadly feeding generalists may be more responsive to specific defenses than the diversity of defenses *per se*.

Overall, our study shows that *P. arboreum* exhibits high levels of phytochemical richness and plasticity. The chemical dissimilarity

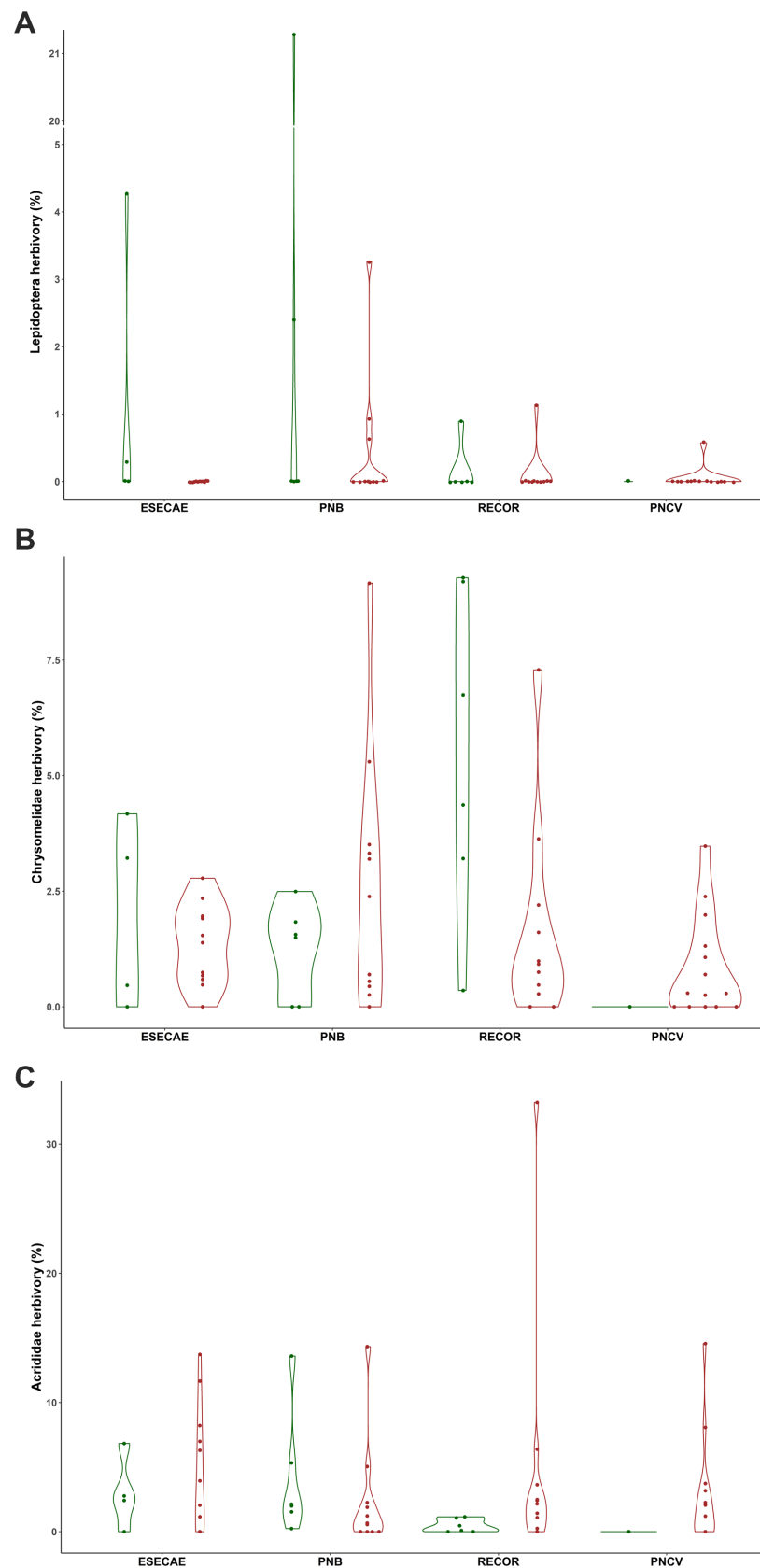


FIGURE 2

Violin plots and data points of (A) Lepidoptera, (B) Chrysomelidae, and (C) Acrididae herbivory as affected by transplanting and the planting location. Transplanting reduced specialist Lepidoptera and chrysomelid herbivory, and the planting location also influenced chrysomelid damage. Nothing was predictive of generalist acridid herbivory.

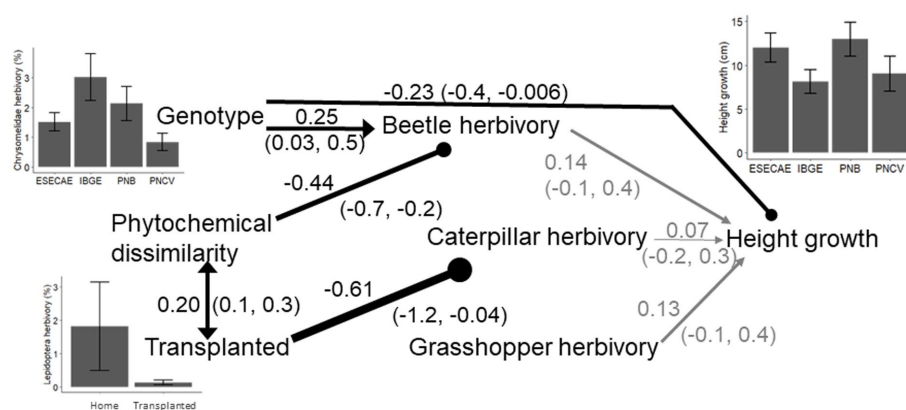


FIGURE 3

Bayesian structural equation model showing causal relationships (single headed arrows) and correlations (double headed arrow) between genotype, transplanting, phytochemical dissimilarity between experimental clones and native populations of *Piper arboreum*, herbivory and relative growth in height. Height was only affected by the original genotype. Genotype also affected beetle (chrysomelid) herbivory. Beetle damage was reduced when clones were less chemically similar to the native population they were planted into, while transplanting decreased caterpillar (Lepidoptera) herbivory. Generalist grasshopper (acridid) herbivory was not affected by transplanting, phytochemical dissimilarity, or genotype. Values are Bayesian posterior probabilities and 90% credibility intervals; the thickness of the arrows indicates the relative strength of the interactions, and relationships in grey had 90% credibility intervals that crossed zero. Bar charts show the means and SE of relationships between genotype and transplanting and herbivory and growth.

among plants limited specialist chrysomelid herbivory, whereas specialist lepidoptera herbivory was lower on transplanted individuals and not related to phytochemical diversity and similarity. Generalist herbivory is harder to predict, but there are documented relationships between generalists and phytochemical diversity (e.g., Salazar et al., 2018). The high degree of variation in plant chemistry observed between populations and transplanted clones suggests that phytochemical complexity is an important plant trait that may be maintained by the pressure of diverse herbivores that are differentially adapted to unique defense compounds (Kessler and Kalske, 2018; Wetzel and Whitehead, 2020). Our data on this widespread species demonstrate the potential of plants to produce a diversity of chemical compounds and the existence of plasticity in the production of specialized metabolites. Future work should seek to identify chemical compounds in metabolic extracts and continue to test the role of phytochemical diversity and chemical similarity in plant-herbivore interactions.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

RR designed the study, collected the field data and chemistry samples, and edited the manuscript. LD designed the study, assisted in the field, advised on analyses, helped write the manuscript, and provided funding. LY analyzed the chemistry samples and helped write the manuscript. ID designed the study and provided funding. WS measured the herbivory data. AK extracted the chemistry samples. MK provide laboratory support and funding and edited the manuscript. TM analyzed the data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2023.1175590/full#supplementary-material>

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