Received: 16 December 2022 | Accepted: 29 April 2023

DOI: 10.1111/afe.12574

ORIGINAL ARTICLE

Agricultural and Forest Entomology

Royal Entomological Society

Effects of seedling conspecific density and heterospecific frequency on insect herbivory in a tropical dry forest

Luis Abdala-Roberts[1] | Jorge Carlos Berny-Mier y Terán[2] | Carla Vázquez-González[3,4] | Ariel Cohuo[2] | Jorge León[2] | Limbenr Valle[2] | Kailen A. Mooney[3] | Enrique Reyes-Novelo[5] | Xoaquín Moreira[4]

[1]Departamento de Ecología Tropical, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, Mexico

[2]Centro de Educación Ambiental de la Península de Yucatán, Mérida, Mexico

[3]Department of Ecology and Evolutionary Biology, University of California, Irvine, Irvine, California, USA

[4]Misión Biológica de Galicia (MBG-CSIC), Pontevedra, Spain

[5]Centro Regional de Investigaciones Dr. Hideyo Noguchi, Universidad Autónoma de Yucatán, Mérida, Mexico

Correspondence
Luis Abdala-Roberts, Departamento de Ecología Tropical, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, apartado postal 4-116, Itzimná, 97000 Mérida, Mexico.
Email: abdala.luis@yahoo.com

Funding information
Centro de Educación Ambiental de la Península de Yucatán

Abstract

1. Conspecific plant density and heterospecific frequency are key drivers of herbivore damage. However, most studies have investigated their effects separately and for single (rather than multiple) focal plant species.
2. We conducted an experiment involving three tree species, namely: *Cordia dodecandra* (Boraginaceae), *Manilkara zapota* (Zapotaceae), and *Piscidia piscipula* (Fabaceae). We manipulated understory densities of *M. zapota* and *C. dodecandra* (focal species) and their frequency relative to *P. piscipula*. Three months after planting, we surveyed insect leaf chewer and sucking damage on the former two. Because these species are attacked by different herbivores, we predicted a negative effect of heterospecific frequency on herbivory.
3. Density and frequency varied in the direction and function of their effects on herbivory depending on the plant species and attacking herbivore. As expected, *Piscidia piscipula* frequency had a negative linear effect on *M. zapota* leaf-chewer damage, whereas conspecific density did not affect chewer damage on this species. In contrast, density and frequency had non-linear effects on *C. dodecandra* chewer damage, namely positive (hump-shaped) and negative (U-shaped) relationships, respectively. In addition, density and frequency had positive linear effects on *C. dondecandra* damage by leafhoppers.
4. These findings call for more work jointly assessing plant inter-specific variation in density- and frequency-dependent variation in herbivory and its underlying drivers.

KEYWORDS
associational effect, density-dependence, leaf-chewer, sap-sucking insect, seedlings, tropical dry forest

INTRODUCTION

Plant populations and communities vary in numerous features that have important ecological impacts on herbivores and, thus, on the outcome of plant-herbivore interactions. Population-level factors involving conspecific plants include patch size (e.g., Bach, 1988; Hambäck, Björkman, & Hopkins, 2010), abundance or density (e.g., Rausher & Feeny, 1980; Underwood & Halpern, 2012), genotypic diversity (e.g., Abdala-Roberts & Mooney, 2014; Hughes, Inouye, Johnson, Underwood, & Vellend, 2008), and patch spatial distribution and connectivity (e.g., Tack & Roslin, 2010). Among these, plant conspecific density, i.e., number of individuals of a species per unit of area, has

been one of the most studied (Bach, 1980; Hambäck et al., 2010; Shea, Smyth, Sheppard, Morton, & Chalimbaud, 2000; Underwood & Halpern, 2012) and lies at the center of theoretical and empirical work on topics such as herbivore functional responses (Abdala-Roberts & Mooney, 2015) and plant population regulation mediated by herbivory (Wright, 2002). On the other hand, community-level characteristics involve interactions among heterospecific plants and include features such as plant species richness (e.g., Andow, 1991; Loreau et al., 2001), functional diversity (e.g., Schuldt et al., 2014), and species composition (Vehviläinen, Koricheva, & Ruohomäki, 2007). This line of research has shed insight into how habitat complexity, mediated by variables such as species relative frequencies or commonness, affects plant-herbivore interactions and the role plant trait inter-specific variation plays in shaping such interaction outcomes (Agrawal, Lau, & Hambäck, 2006; Moreira, Abdala-Roberts, Rasmann, Castagneyrol, & Mooney, 2016). Combined, research on population- and community-level effects has deepened our understanding of plant bottom-up controls on herbivory and how interaction outcomes affect ecological communities and ecosystems.

Studies on the effects of conspecific plant density on herbivores have found contrasting results. For example, a number of investigations have reported that herbivore attack rates decrease with host plant density indicative of a negative density-dependent response (e.g., Type II, i.e., non-linear saturating, functional response depictive of herbivore satiation; Holling, 1966; Abdala-Roberts & Mooney, 2013), producing a so-called resource dilution effect (Kim & Underwood, 2015; Otway, Hector, & Lawton, 2005), whereas others have found increasing attack with host plant density, that is, a resource concentration effect (Kim & Underwood, 2015; Root, 1973) (Table S1). The latter pattern, termed positive density-dependence, is expected under the Janzen-Connell Model (Connell, 1971; Janzen, 1970), which predicts that herbivory decreases with distance from parental trees mediated by decreasing density of seeds or seedlings away from fruiting adults (reviewed by Hyatt et al., 2003; Carson, Anderson, Leigh, & Schnitzer, 2008). Resource concentration effects may occur, for example, through higher herbivore recruitment or greater residence time on high-density host plants or patches (Hambäck, Inouye, Andersson, & Underwood, 2014; Kim & Underwood, 2015). In contrast, dilution effects can arise, for example, if herbivores avoid high densities when competition results in reduced plant growth (and nutritional status) and/or higher defences (Halpern, Bednar, Chisholm, & Underwood, 2014). In addition, both processes can be also influenced by how herbivore dispersal rates relate to patch size or distance between patches, where different scaling relationships as a function of herbivore movement lead to one or the other outcome (Hambäck & Englund, 2005; Hambäck, Vogt, Tscharntke, Thies, & Englund, 2007). Despite abundant evidence for herbivore (positive or negative) density-dependent responses, relatively few studies have experimentally manipulated conspecific plant density (see studies in Abdala-Roberts, Parra-Tabla, Moreira, & Ramos-Zapata, 2017), and this includes Janzen-Connell work (but see Bagchi et al., 2010; Germany, Bruelheide, & Erfmeier, 2019). Efforts to manipulate multiple levels of conspecific plant density are required to establish a causal link between plant density and herbivory as well as to robustly assess the strength and type of density-dependent responses and their ecological significance.

Host plant frequency or relative density, that is, how abundant a species is relative to other co-occurring species ("heterospecific frequency "hereafter), is an important community-level driver of herbivory which often varies with plant diversity or composition (reviewed by Hambäck et al., 2014; Underwood, Inouye, & Hambäck, 2014). Here, increasing heterospecific frequency can drive reductions in herbivory on focal species by interfering with the detection of host plants through mechanisms such as chemical masking or physical interference (e.g., Abdala-Roberts et al., 2015; Castagneyrol, Giffard, Péré, & Jactel, 2013; Hambäck, Ågren, & Ericson, 2000), resulting in so-called associational resistance (reviewed by Barbosa et al., 2009; Underwood et al., 2014) (Table S1). In other cases, however, a higher frequency of heterospecifics may increase damage on host plants when plant species (e.g., closely related taxa) share herbivores which spill over from neighboring heterospecifics to focal hosts, resulting in associational susceptibility or apparent competition (Barbosa et al., 2009; Holt & Bonsall, 2017) (Table S1). This body of research has contributed to understand and predict herbivore responses to plant community complexity (Moreira, Pérez-Ramos, Abdala-Roberts, & Mooney, 2017), and how herbivory in turn mediates plant diversity and species composition (Wright, 2002).

Conspecific density and heterospecific frequency effects on herbivory have historically been studied separately (see Kim & Underwood, 2015). For example, Janzen-Connell studies testing for seedling conspecific density effects usually do not experimentally account for heterospecific frequency effects, whereas work testing for associational effects has not accounted for changes in focal plant densities (but see Bach, 1980). Addressing these factors separately can lead to erroneous conclusions regarding their influence on herbivory, one reason being that they are likely to be correlated. For example, all else being equal, increasing host plant density is directly related to decreasing heterospecific frequency if the density of neighbours remains constant. Accordingly, if herbivory increases with conspecific density, it is unclear whether this is evidence of density-dependent foraging (as predicted by the Janzen-Connell Model) or if this pattern is due to greater apparency mediated by lower heterospecific frequency. At the same time, however, these factors can also vary independently; for example, if both host plant density and heterospecific density increase, thenheterospecific frequency would remain constant. In addition, another important consideration is that each driver may influence herbivory differently owing to different underlying mechanisms, which can only be teased a part by experimentally manipulating both factors. For instance, herbivores rely on different chemical and physical cues for locating host plants in conspecific versus heterospecific patches (Hambäck et al., 2014); the former involves selecting host plants in response to intra-specific variation whereas the latter involves the detection of host plants within a matrix of heterospecific neighbouring plants. Overall, studies testing both factors for multiple co-occurring plant species are needed

to address patterns of inter-specific variation and its underlying drivers, as well as increase inference on variability or similarity in the outcomes of plant-herbivore interactions.

We conducted a field experiment in a tropical dry forest site in Yucatan (Mexico) to test for conspecific tree seedling density and heterospecific frequency effects on insect leaf herbivory on *Cordia dodecandra* and *Manilkara zapota* ("focal species" hereafter), co-occurring species that are rare at the study site. These species are generally rare in tropical dry forests in Yucatan (Pennington & Sarukhán, 2005), including the study site (L. Abdala-Roberts pers. obs.), and have undergone population reductions due to anthropogenic pressures such as logging (Cámara-Romero, Jiménez-Osornio, Estrada-Medina, & Hernández García, 2021; Weterings, Weterings-Schonck, Vester, & Calmé, 2008). A third more common species, *Piscidia piscipula*, was used to test for heterospecific effects on these focal species and is highly common in tropical dry forests in the northern Yucatan Peninsula (often as a dominant species; Pennington & Sarukhán, 2005) and is therefore likely to be a neighbour of the other two in understory patches across sites in the region (Pennington & Sarukhán, 2005). Based on this, understanding conspecific and heterospecific seedling interactions associated with *C. dodecandra* and *M. zapota* has important implications for conservation and restoration practices involving these species. To this end, we measured leaf damage by chewing insects on both focal species as well as leafhopper damage on one of them (*C. dondecandra*). Specifically, we asked: (i) Does conspecific seedling density of the focal species influence insect herbivory? (ii) Does the frequency of *P. piscipula* influence herbivory on the focal species? And (iii) Are density and frequency effects similarly common and do their effects vary in strength or function depending on the plant or herbivore species? Higher heterospecific frequency was predicted to negatively influence herbivory on the focal species (i.e., associational resistance) as the tree species seedlings did not share insect herbivores at the study site. We did not have predictions for conspecific density effects given lack of information on behaviour and other traits influencing functional responses of the observed herbivores attacking focal species. Resulting patterns can inform further work on causes behind any such plant-based bottom-up controls on herbivory.

MATERIALS AND METHODS

Study system

The study was conducted at the San Nicolás Preserve (20°55′53.1″N, 88°33′3″W), a c. 500-ha protected site managed by the Centro de Educación Ambiental de la Peninsula de Yucatan (CEAPY) located nearby the town of Cenotillo, Yucatán (Mexico). This study area consists mainly of secondary tropical dry forests protected since 2007. *Piscidia piscipula* (Fabaceae) is highly common at several sites (L. Abdala-Roberts, pers. obs.), and is likewise highly common in tropical dry forests of the northern Yucatan Peninsula (Pennington & Sarukhán, 2005). There are also a number of species which are rare at the study site, including focal species *C. dodecandra* (Boraginaceae) and *M. zapota* (Sapotaceae), but are important food sources for local vertebrate fauna, including birds and mammals (Cancino, 2017) and highly valued timber species (Canché-Collí, Rodríguez, & Canto, 2020). Both focal species have been strongly negatively impacted by illegal logging and land use change in the Yucatan Peninsula (Cámara-Romero et al., 2021; Weterings et al., 2008) as well as at the study site (L. Abdala-Roberts, pers. obs.).

Observations during the study indicated that *M. zapota* was only attacked by chewing insects, mainly leaf cutter ants (*Acromyrmex octospinosus*), whereas *C. dodecandra* was heavily attacked by sap-feeding insects (order Heteroptera), mainly a leafhopper, *Idona* sp. (Hemiptera, Cicadellidae), and in the case of chewing insects by chrysomelid beetles (Coleoptera, Chrysomelidae). On the other hand, *P. piscipula* (i.e., the heterospecific neighbour) does not share herbivores with the former two, as it was attacked mainly by a leaf miner (Lepidoptera, Gelechioidea) and by grasshoppers (Orthoptera, Acrididae), and to a lesser extent by beetles (Coleoptera, Curculionidae). The most common herbivores attacking each plant species were therefore different, suggesting little to no overlap among tree species in associated herbivore communities.

Experimental design and response variables

Seeds were obtained from 6 to 7 adult trees of each species sourced from tropical dry forest remnants or edges near the town of Bacalar, in southern Quintana Roo (Mexico), ca. 250 km south of San Nicolás. In early July 2021, we pooled seeds across parental trees and germinated them in a greenhouse at the Preserve. In late August 2021, once seedlings were 1–1.5 months old, we planted them in 1 by 1 m understory quadrats (units of replication) of varying densities of each focal species and frequencies of *P. piscipula*. For each focal species, we established 12 density by frequency combinations (sensu Kim & Underwood, 2015), involving eight levels of focal species density (1, 2, 3, 4, 6, 9, 12 and 18 individuals per 1-m^2 quadrat) and six levels (0, 0.25, 0.33, 0.50, 0.67, and 0.75 per m^2 quadrat) of *P. piscipula* frequency (see Table S2; Figures S1 and S2; in the case of heterospecific frequency, values were obtained from dividing the number of seedlings of *P. piscipula* by the total number of seedlings in the plot). This design was replicated across six nearby sites which were similar in forest physiognomy and understory conditions (L. Abdala-Roberts, pers. obs.). Overall, study sites were composed of successional tropical dry forest vegetation (c. 20- to 30-year-old) with some elements of older vegetation (larger trees), and the understory was partially shaded with some canopy openings (Figure S2). For quadrats with *M. zapota*, each level of density or frequency was replicated 3–6 times and 3–12 times, respectively, whereas for quadrats with *C. dodecandra*, 4–10 and 4–15 times, respectively. We established at total of 96 quadrats ($N = 949$ plants), 53 of which tested for effects of conspecific plant density and heterospecific frequency on *C. dodecandra* (568 plants: 369 of *C. dodecandra* and 199 of *P. piscipula*) and 36 used to test for density and

TABLE 1 Results from general linear models selected based on AIC and likelihood ratio test comparisons (see Table S3) testing for the effects of conspecific density of the focal plant species and heterospecific (*Piscidia piscipula*) frequency on percent of leaf area removed by chewing insects (chewer damage) on *Manilkara zapota* (model conditional $R^2 = 0.19$) and *Cordia dodecandra* (conditional $R^2 = 0.34$) seedlings, and on chlorosis severity due to leaf hopper feeding (leafhopper damage) on *C. dodecandra* seedlings (conditional $R^2 = 0.58$).

	Manilkara zapota			*Cordia dodecandra*					
	Chewer damage			Chewer damage			Leafhopper damage		
	$DF_{num,den}$	*F*	*p*	$DF_{num,den}$	*F*	*p*	$DF_{num,den}$	*F*	*p*
Plant Height	1, 29	1.12	0.299	1, 47	8.16	**0.006**	1, 47	0.16	0.694
Density	1, 29	2.07	0.161	1, 45	7.84	**0.007**	1, 47	**4.33**	**0.043**
$Density^2$	–	–	–	1, 45	11.34	**0.002**	–	–	–
Frequency	1, 29	4.26	**0.048**	1, 46	4.65	**0.036**	1, 47	**5.56**	**0.023**
$Frequency^2$	–	–	–	1, 46	4.72	**0.035**	–	–	–

Note: *F*-values, degrees of freedom ($DF_{num, den}$) and associated *p*-values for each factor are shown. Significant *p*-values (<0.05) are shown in bold. Models also included plant height as a covariate, as well as the random effect of site and its interactions with density and frequency (see Section 2).

frequency effects on *M. zapota* (381 plants: 255 of *M. zapota* and 126 of *P. piscipula*). Seedling height ranged from c. 7 to 20 cm depending on the species (see ahead). Distance between quadrats at each site was ca. 2 m, and plants were spaced by ca. 25 cm within each quadrat. Past studies testing for density and/or frequency effects using seedlings or herbs have used similar inter-plot distances (e.g., Hambäck et al., 2000; Kim & Underwood, 2015; Moreira, Mooney, Zas, & Sampedro, 2012), whereby adjacent plots may not always be independent (e.g., depending on herbivore mobility). Quadrats was weeded 1 month prior to transplantation. Distance between sites was at least 20 m.

In late November 2021, 2 months after transplantation, we selected a random subsample of one to three seedlings for quadrats with focal species densities of 1–9 individuals, and five seedlings for quadrats with focal species densities of 12 and 18 ($N = 107$ *M. zapota* seedlings and 168 *C. dodecandra* seedlings). For both *M. zapota* and *C. dodecandra*, we visually estimated the percent of leaf area removed by chewing insect herbivores (chewer damage hereafter) for all leaves per plant (in all cases by the same experienced observer, LAR). In addition, for *C. dodecandra* we visually scored the whole-plant level of chlorosis (scale: 1–4, ranging from a small amount of chlorosis on some leaves to extensive chlorosis on all leaves) caused by leafhopper feeding (leafhopper damage hereafter), which fed exclusively on this species. All visual estimates of leaf damage were based on exact estimates which have been shown to be equally (or more) accurate than binned estimates (i.e., based on a scale; see Johnson, Bertrand, & Turcotte, 2016). In addition, we ran a regression on actual vs. visually estimated values of percent leaf area loss for *C. dodecandra* using leaves from saplings which were not part of the experiment, and found a strong relationship ($R^2 = 0.94$, $N = 40$). Actual percent leaf area loss for this subsample was estimated using BioLeaf – Foliar Analysis™ a professional mobile application (Brandoli Machado et al., 2016). It was not possible to estimate visual estimates precision for *M. zapota* since there were no available seedlings or saplings for leaf collection. We did not collect leaves from experimental seedlings of either species as this would likely affect future growth or survival (particularly for *M. zapota* which had 2–3 leaves), thus compromising data collection planned for the following seasons. For both focal species, we measured plant height at the beginning of the experiment (*M. zapota*: 7.59 ± 0.24 cm [mean ± SE]; *C. dodecandra*: 27.95 ± 0.53 cm; *P. piscipula*: 21.55 ± 0.59 cm). Seedling mortality throughout the experiment was negligible (<5%) and therefore did not influence treatment effects.

Statistical analyses

We ran general linear mixed models jointly testing for the effects of conspecific plant density and heterospecific frequency (number of *P. piscipula* individuals divided by the total plant number per quadrat), both treated as continuous variables, on chewer damage separately for each focal species, and in the case of *C. dodecandra* also on leafhopper damage. Accordingly, by simultaneously testing for both factors, analyses test for each driver while holding the other constant. In all cases, we used mean values across plants per quadrat. Models also included site (random) as a spatial block (dropped for the *M. zapota* model as it did not explain any variance) as well as plant height as a covariate to control for effects of focal plant size on herbivory. Chewer damage was square-root transformed to normalize model residuals (Figure S3). We tested for quadratic terms and an interaction between linear terms for density and frequency on all responses. Specifically, we compared quadratic models and those with interactions against the simplified model with linear terms for frequency and density using likelihood ratio tests and selected those with the lowest AIC (see Table S3 for model structures and comparisons). In the case of chewer damage on *C. dodecandra*, the model with quadratic terms for both density and frequency outperformed the simplified model with linear terms (LRT $\chi^2 = 11.88$, $p = 0.003$, AIC = 127.62; see Table S3) and was thus chosen. Models including the density by frequency interaction never outperformed the simplified linear model (Table S3) and were thus rejected.

We visualized the effects of density and frequency on chewer and leafhopper damage on each focal species by plotting partial residuals (i.e., model estimates after removing the effects of covariates and fixed

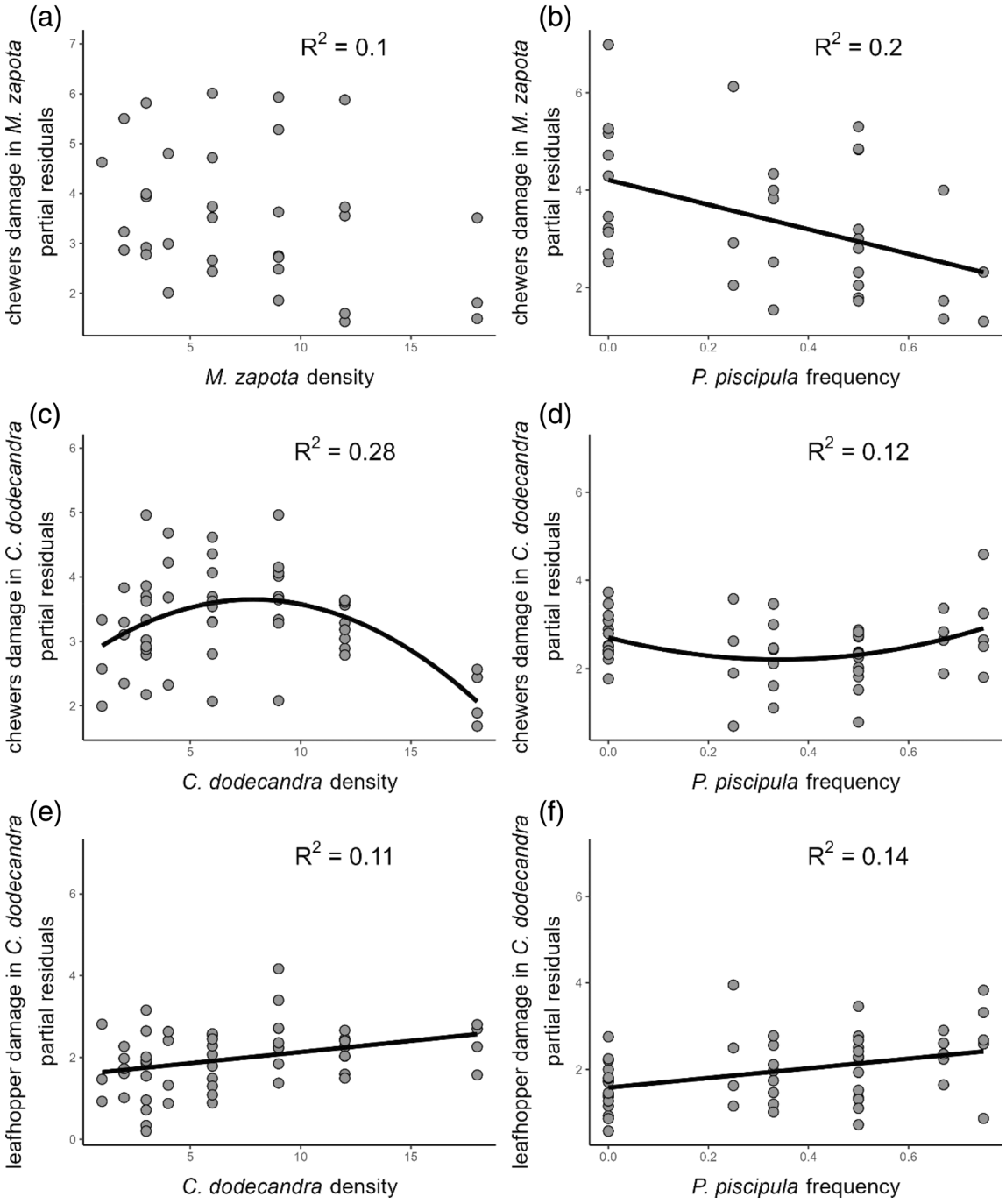

FIGURE 1 Partial residuals graphs (estimates after removing the effects of additional covariates and fixed and random effects in selected models – See Table 1) showing the effects of conspecific density of focal plant species and frequency of heterospecific (*Piscidia piscipula*) on percent of leaf area removed by chewing insects ("chewer damage") for *Manilkara zapota* and *Cordia dodecandra* seedlings, and on damage by leaf hoppers ("leafhopper damage") for *C. dodecandra* seedlings. (a) Density effects (non-significant) on chewer damage on *M. zapota*. (b) Frequency effects on chewer damage on *M. zapota*. (c) Density effects on chewer damage on *C. dodecandra*. (d) Frequency effects on chewer damage on *C. dodecandra*. (e) Density effects on leaf hopper damage on *C. dodecandra*. (f) Frequency on leafhopper damage on *C. dodecandra*. Densities are the number of individuals of each focal species m^{-2} and frequencies the proportion of individuals represented by *P. piscipula* m^{-2}.

and random effects) against each predictor. All models were ran in R software version 3.06.0 (R Core Team, 2013) using the *lmer* function from the *lmerTest* package (Kuznetsova, Brockhoff, & Christensen, 2017).

RESULTS

Leaf damage by chewing insects on *M. zapota* was on average 4.53 ± 1.11% and ranged from 0 and 22.67% across quadrats, whereas

mean chewer damage on *C. dodecandra* was 2.65 ± 0.39% and ranged from 0 to 15.36%. In addition, the mean score of leafhopper damage, which was exclusive to *C. dodecandra*, was 2.66 ± 0.15 and ranged from 1.00 to 4.00.

Results from the linear mixed models showed that both conspecific plant density and heterospecific frequency significantly affected insect herbivory on *M. zapota* and *C. dodecandra*, but effects were contingent on the plant species and herbivore type. In the case of *M. zapota*, we found no effect of conspecific plant density on chewer damage (Table 1; Figure 1a). However, as predicted, the frequency of heterospecifics had a significant negative effect on damage by chewing insects (Table 1; Figure 1b). For *C. dodecandra*, however, conspecific density and heterospecific frequency had contrasting non-linear effects on chewer damage (Table 1; significant quadratic effects of density and frequency; $\Delta AIC = -7.88$ relative to simplified model with no quadratic terms, Table S3). Specifically, conspecific density exhibited a hump-shaped relationship with chewer damage on *C. dodecandra* whereby damage was highest at intermediate densities (Figure 1c). In contrast, the *P. piscipula* frequency effect, albeit weaker, depicted a U-shaped relationship whereby chewer damage on *C. dodecandra* was highest at both low and high of the former species (Figure 1d). Finally, conspecific density and heterospecific frequency both had positive linear effects leafhopper damage on *C. dodecandra* (Table 1; Figure 1e,f).

DISCUSSION

Our study allowed to jointly assess and separate the independent influences of conspecific density and heterospecific frequency on herbivory. In short, results indicated contrasting effects of plant conspecific density and heterospecific frequency on insect leaf herbivory for seedlings of the studied focal tree species. Specifically, and as predicted, chewer damage on *M. zapota* decreased with increasing frequency of *P. piscipula* indicative of associational resistance, but was not affected by conspecific density. In contrast, chewer herbivory on *C. dodecandra* showed non-linear patterns, whereby damage was highest or lowest at intermediate values of density and frequency (respectively), suggesting that multiple mechanisms could be simultaneously at work in each case. In addition, leaf hopper damage increased with *C. dodecandra* density consistent with a resource concentration effect as well as increased with *P. piscipula* frequency suggesting associational susceptibility. Together, these findings depict variable outcomes whereby density and frequency effects on chewers varied in strength, direction, and function both within and between focal tree species, as well as also varied between herbivore types (chewers vs. leafhoppers) within *C. dodecandra*.

We observed contrasting effects of seedling conspecific density and heterospecific frequency on chewer damage within each focal plant species as well as for each predictor across species. On the one hand, conspecific density of *M. zapota* seedlings had no effect on chewer damage, largely leaf cutter ants (not strictly herbivores), suggesting attack by these insects behaved in a density-independent manner. This result contrasts with abundant evidence for density-dependent herbivory, often resulting in resource concentration effects (i.e., positive density-dependent herbivory; Kim & Underwood, 2015; Forrister, Endara, Younkin, Coley, & Kursar, 2019), or, perhaps more commonly, dilution effects (negative density-dependence; Abdala-Roberts & Mooney, 2013, 2015). The small size of *M. zapota* seedlings (e.g., 7.5 cm high, on average) might explain this finding, resulting in low detectability (through plant traits that mediate attraction; Hambäck et al., 2000) and thus herbivory rates not being high enough (4.53% on average) to produce density-dependence (see Ctvrtecka, Sam, Brus, Weiblen, & Novotny, 2014). Further work testing exclusively for density effects which includes a greater range of density levels could provide a more robust assessment of this factor. By contrast, *P. piscipula* frequency drove, as predicted, a decrease in chewer damage on *M. zapota*, indicating an associational resistance effect. The mechanism behind this pattern requires further investigation but could involve lowered apparency or interference (physical or chemical) for chewers (e.g., leaf cutter ants or beetles) feeding on *M. zapota* (e.g., Castagneyrol et al., 2013; Hambäck et al., 2000), particularly given the size difference and growth rate compared to considerably larger *P. piscipula* seedlings.

We found non-linear and yet contrasting effects of each factor on *C. dodecandra* chewer damage. Non-linear saturating herbivore responses to conspecific plant density are common and indicate herbivore satiation (Abdala-Roberts & Mooney, 2013; Holling, 1966). However, in our case the relationship was hump-shaped, with a peak in damage at intermediate densities and a decrease at high *C. dodecandra* densities. This pattern suggests an additional mechanism (besides satiation) by which damage decreases at high densities, that is, a resource dilution effect. For example, the observed shift from resource concentration at low densities to dilution at high densities could respond to an herbivore-related mechanism leading to chewer satiation (mobility, feeding time, etc.) or to reductions in leaf quality (lower nutrients or higher defences) due to increased seedling competition at high density (see Halpern et al., 2014). In addition, this hump-shaped pattern could have also arisen from temporal dynamics in herbivore recruitment to plant patches whereby herbivore abundances equilibrate faster at low host plant densities than at high host plant densities (Hambäck et al., 2007). On the other hand, we also found a non-linear effect of *P. piscipula* frequency on chewer damage on *C. dodecandra*, though in this case an inverse pattern depicting a U-shaped relationship suggests a different underlying process. By comparison, Kim and Underwood (2015) found a hump-shaped relationship between heterospecific frequency and leaf herbivory on *Solanum carolinense*. They attributed this pattern to concomitant effects of inter-specific competition causing reductions in plant defence allocation at low to intermediate frequencies and an indirect negative effect of heterospecifics on herbivores due to spillover of a plant pathogen at high frequencies. In our case, and albeit a rather weak effect ($R^2 = 0.12$), the observed U-shaped pattern suggests instead associational resistance at low *P. piscipula* frequencies (as predicted) which then shifts to associational susceptibility at moderate to high frequencies. It is possible that some form of interference by heterospecifics rather than inter-specific

competition drove reductions in *C. dodecandra* (associational resistance) chewer damage from low to intermediate heterospecific frequencies, but that inter-specific competition turned important at high heterospecific frequencies leading to reductions in *C. dodecandra* seedling defences and thus associational susceptibility. Alternatively, *P. piscipula* is a nitrogen-fixing species and high frequencies may have increased *M. zapota* nitrogen leaf content and thus nutritional quality to chewing insects. Further work is needed to test and tease apart these mechanisms. Together, the observed patterns nonetheless highlight that non-linear effects of conspecific density and heterospecific frequency are potentially common as well as governed by potentially different mechanisms.

In contrast to the non-linear responses of chewers feeding on *C. dodecandra*, there were positive linear effects of both conspecific density and *P. piscipula* frequency on leaf hopper damage, indicating a resource concentration effect (positive density dependence) and associational susceptibility (respectively). This difference in leaf hopper response to host plant density relative to chewers suggests different underlying traits and drivers within this tree species. Relative to chewers, leafhoppers reached outbreak levels during the study and given high dispersal were possibly better able to track host plant abundance resulting in a resource concentration effect across the range of host plant densities. On the other hand, the observed effect of associational susceptibility by *P. piscipula* is interesting considering little to no overlap in herbivore communities between this species and *C. dodecandra*. As discussed above for chewers feeding on *C. dodecandra*, it is possible that *P. piscipula* increased leaf nitrogen content in this species, leading to greater preference and damage by leaf hoppers. In this case, leaf hoppers could have been especially sensitive to any such trait changes in *C. dodecandra* (Hubberty & Denno, 2006), leading to a consistent (linear) increase in damage across the range of *P. piscipula* frequencies. Follow-up controlled experiments testing for this and other plant-trait-based mechanisms are needed to test this.

We note that one limitation of our study is we lack information on the spatial scale at which seedling conspecific density and frequency effects on insect herbivores are strongest. Previous work has shown that the strength or function of conspecific density and heterospecific frequency effects may vary across scales (Hambäck, Björkman, Ramert, & Hopkins, 2009; Xiao et al., 2017). Therefore, testing for density and frequency effects at different scales (e.g., replicating different quadrat sizes as well as distance between quadrats) can help understand which insect host finding or selection processes as well as plant-based features are most important at each scale for different herbivore species (Hambäck et al., 2014; Kim & Underwood, 2015), and in turn result in observed patterns of herbivory. Studies that include multiple plant and herbivore species or guilds can provide further insights into how traits at each trophic level shape scale-dependent responses.

Conclusions and applied prespective

The present study deepens our understanding of plant-based resource heterogeneity effects on herbivory by teasing a part the effects conspecific density and heterospecific frequency, as well as by comparing responses across tree species and for different herbivore with contrasting traits. Results point out a range of possible outcomes for the strength and function of seedling conspecific density and heterospecific frequency on insect herbivory, suggesting different underlying mechanisms depending on the plant species and type of herbivore. Further work assessing the role of plant (e.g., defences) and herbivore (e.g., diet breadth) species traits and their relation to spatial scale, is needed to shed mechanistic insight on the drivers of interaction outcomes.

It is important to also stress that the focal species studied, *C. dodecandra* and *M. zapota* are of conservation concern given their dwindling populations in substantial portions of the Yucatan Peninsula, including the study region. Proper management and recovery of their populations requires information on the predictors of seedling recruitment and establishment success, among which negative impacts of herbivory can be decisive especially during early stages of plant development. In this sense, an understanding of herbivore responses to seedling density and heterospecific neighbours can provide a useful framework for understanding how plant-based bottom-up drivers naturally contribute to seedling recruitment success. Knowledge gained can in turn inform and optimize reforestation practices in recovering tropical dry forests from a perspective of herbivory mitigation, e.g., in enrichment planting designs using differing densities customized based on the main herbivores present and the type of responses to plant density they exhibit, or the use of heterospecific neighbours (e.g., common or likely neighbours in regenerating forests) to reduce herbivory via associational resistance mechanisms.

AUTHOR CONTRIBUTIONS

Luis Abdala-Roberts: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; writing – original draft; writing – review and editing. **Jorge Berny-Mier y Teran:** Funding acquisition; investigation; methodology; project administration; resources; writing – review and editing. **Carla Vázquez-González:** Conceptualization; formal analysis; investigation; methodology; resources; writing – original draft; writing – review and editing. **Ariel Cohuo:** Methodology; project administration; resources. **Jorge León:** Methodology; project administration. **Linbenr Valle:** Methodology; project administration. **Kailen Mooney:** Conceptualization; investigation; resources; writing – review and editing. **Enrique Reyes-Novelo:** Investigation; methodology; resources; writing – review and editing. **Xoaquín Moreira:** Conceptualization; investigation; methodology; resources; writing – original draft; writing – review and editing.

ACKNOWLEDGEMENTS

The authors thank Cynthia Wilber and María M. Cab for logistic support. Funding and infrastructure for plant propagation and transplantation was provided by the Centro de Educacion Ambiental de la Peninsula de Yucatan (CEAPY).

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data will be submitted to DRYAD upon acceptance.

ORCID

Xoaquín Moreira https://orcid.org/0000-0003-0166-838X

REFERENCES

Abdala-Roberts, L., Mooney, K., Quijano-Medina, T., Campos-Navarrete, M.J., González-Moreno, A. & Parra-Tabla, V. (2015) Comparison of tree genotypic diversity and species diversity effects on different guilds of insect herbivores. *Oikos*, 124, 1527–1535.

Abdala-Roberts, L. & Mooney, K.A. (2013) Environmental and plant genetic effects on tri-trophic interactions. *Oikos*, 122, 1157–1166.

Abdala-Roberts, L. & Mooney, K.A. (2014) Ecological and evolutionary consequences of plant genotype diversity in a tri-trophic system. *Ecology*, 95, 2879–2893.

Abdala-Roberts, L. & Mooney, K.A. (2015) Plant and herbivore evolution within the trophic sandwich. In: Hanley, T. & La Pierre, K. (Eds.) *Trophic interactions: bottom-up and top-down interactions in aquatic and terrestrial ecosystems*. Cambridge, UK: Cambridge University Press, pp. 340–364.

Abdala-Roberts, L., Parra-Tabla, V., Moreira, X. & Ramos-Zapata, J. (2017) Ecological and evolutionary consequences of tri-trophic interactions: spatial variation and effects of plant density. *American Journal of Botany*, 104, 241–251.

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

Andow, D.A. (1991) Vegetational diversity and arthropod population response. *Annual Review of Entomology*, 36, 561–586.

Bach, C.E. (1980) Effects of plant density and diversity on the population dynamics of a specialist herbivore, the striped cucumber beetle, *Acalymma vittata* (fab). *Ecology*, 61, 1515–1530.

Bach, C.E. (1988) Effects of host plant patch size on herbivore density: underlying mechanisms. *Ecology*, 69, 1090–1102.

Bagchi, R., Swinfield, T., Gallery, R.E., Lewis, O.T., Gripenberg, S., Narayan, L. et al. (2010) Testing the Janzen-Connell mechanism: pathogens cause overcompensating density dependence in a tropical tree. *Ecology Letters*, 13, 1262–1269.

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

Brandoli Machado, B., Orue, J.P.M., Arruda, M.S., Santos, C.V., Sarath, D.S., Goncalves, W.N. et al. (2016) BioLeaf: a professional mobile application to measure foliar damage caused by insect herbivory. *Computers and Electronics in Agriculture*, 129, 44–55.

Cámara-Romero, J.L., Jiménez-Osornio, J.J., Estrada-Medina, H. & Hernández García, F. (2021) Potential distribution of *Cordia dodecandra* and its variation related to the change of land use on the Yucatan peninsula, Mexico. *Tropical and Subtropical Agroecosystems*, 24, 14.

Canché-Collí, C., Rodríguez, R. & Canto, A. (2020) Cuando la separación rinde frutos en el amor: el árbol de siricote, *Cordia dodecandra* (Boraginaceae). *Desde el Herbario CICY*, 12, 133–138.

Cancino, L. (2017) *Diásporas consumidas o dispersadas por aves y mamíferos de la Península de Yucatán*. México: Tesis de Licenciatura, Universidad Autónoma de Yucatán.

Carson, W.P., Anderson, J.T., Leigh, E.G. & Schnitzer, S.A. (2008) Challenges associated with testing and falsifying the Janzen-Connell hypothesis: a review and critique. In: Carson, W.P. & Schnitzer, S.A. (Eds.) *Tropical Forest Community Ecology*. Oxford, UK: Wiley-Blackwell, pp. 210–242.

Castagneyrol, B., Giffard, B., Péré, C. & Jactel, H. (2013) Plant apparency, and overlooked driver of associational resistance to insect herbivory. *Journal of Ecology*, 101, 418–429.

Connell, J. (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: Den Boer, P.J. & Gradwell, G.R. (Eds.) *Dynamics of populations*. Wageningen, Netherlands: Centre for Agricultural Publishing and Documentation, pp. 298–312.

Ctvrtecka, R., Sam, K., Brus, E., Weiblen, G.D. & Novotny, V. (2014) Frugivorous weevils are too rare to cause Janzen-Connell effects in New Guinea lowland rain forest. *Journal of Tropical Ecology*, 30, 521–535.

Forrister, D.L., Endara, M.J., Younkin, G.C., Coley, P.D. & Kursar, T.A. (2019) Herbivores as drivers of negative density dependence in tropical forest saplings. *Science*, 363, 1213–1216.

Germany, M.S., Bruelheide, H. & Erfmeier, A. (2019) Janzen-Connell effects in a forest BEF experiment: strong distance-dependent seedling establishment of multiple species. *Ecology*, 100, e02736.

Halpern, S.L., Bednar, D., Chisholm, A. & Underwood, N. (2014) Plant-mediated effects of host plant density on a specialist herbivore of *Solanum carolinense*. *Ecological Entomology*, 39, 217–225.

Hambäck, P.A., Ågren, J. & Ericson, L. (2000) Associational resistance: insect damage to purple loosestrife reduced in thickets of sweet gale. *Ecology*, 81, 1784–1794.

Hambäck, P.A., Björkman, C., Ramert, B. & Hopkins, R.J. (2009) Scale-dependent responses in cabbage herbivores affect attack rates in spatially heterogeneous systems. *Basic and Applied Ecology*, 10, 228–236.

Hambäck, P.A., Björkman, M. & Hopkins, R.J. (2010) Patch size effects are more important than genetic diversity for plant–herbivore interactions in *brassica* crops. *Ecological Entomology*, 35, 299–306.

Hambäck, P.A. & Englund, G. (2005) Patch area, populations density and the scaling of migration rates: the resource concentration hypothesis revisited. *Ecology Letters*, 8, 1057–1065.

Hambäck, P.A., Inouye, B.D., Andersson, P. & Underwood, N. (2014) Searching for food in complex environments: a theory for relative attraction and associational effects. *Ecology*, 95, 1370–1383.

Hambäck, P.A., Vogt, M., Tscharntke, T., Thies, C. & Englund, G. (2007) Top-down and bottom-up effects on the spatiotemporal dynamics of cereal aphids: testing scaling theory for local density. *Oikos*, 116, 1995–2006.

Holling, C.S. (1966) The functional response of invertebrate predators to prey density. *Memoirs of the Entomological Society of Canada*, 98, 5–86.

Holt, R.D. & Bonsall, M.B. (2017) Apparent competition. *Annual Review of Ecology, Evolution and Systemtics*, 48, 447–471.

Hubberty, A.F. & Denno, R.F. (2006) Consequences of nitrogen and phosphorus limitation for the performance of two planthoppers with divergent life-history strategies. *Oecologia*, 149, 444–455.

Hughes, R.A., Inouye, B.T., Johnson, M.T., Underwood, N. & Vellend, M. (2008) Ecological consequences of genetic diversity. *Ecology Letters*, 11, 609–623.

Hyatt, L.A., Rosenberg, M.S., Howard, T.G., Bole, G., Fang, W., Anastasia, J. et al. (2003) The distance dependence prediction of the Janzen-Connell hypothesis: a meta-analysis. *Oikos*, 103, 590–602.

Janzen, D.H. (1970) Herbivores and the number of tree species in tropical forests. *American Naturalist*, 104, 501–528.

Johnson, M.T.J., Bertrand, J.A. & Turcotte, M. (2016) Precision and accuracy in quantifying herbivory. *Ecological Entomology*, 41, 112–121.

Kim, T.N. & Underwood, N. (2015) Plant neighborhood effects on herbivory: damage is both density and frequency dependent. *Ecology*, 96, 1431–1437.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2017) lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software*, 82, 1–26.

Loreau, M., Naeem, S., Inchausti, P., Bengtsoon, J., Grime, J.P., Hector, A. et al. (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science*, 294, 804–808.

Moreira, X., Abdala-Roberts, L., Rasmann, S., Castagneyrol, B. & Mooney, K.A. (2016) Plant diversity effects on insect herbivores and their natural enemies: current thinking, recent findings, and future directions. *Current Opinion in Insect Science*, 14, 1–7.

Moreira, X., Mooney, K.A., Zas, R. & Sampedro, L. (2012) Bottom-up effects of host-plant species diversity and top-down effects of ants interactively increase plant performance. *Proceedings of the Royal Society B*, 279, 4464–4472.

Moreira, X., Pérez-Ramos, I., Abdala-Roberts, L. & Mooney, K.A. (2017) Functional responses for contrasting seed predator guilds to masting in two Mediterranean oak species. *Oikos*, 126, 1042–1050.

Otway, S.J., Hector, A. & Lawton, J.H. (2005) Resource dilution effects on specialist insect herbivores in a grassland biodiversity experiment. *Journal of Animal Ecology*, 74, 234–240.

Pennington, T.D. & Sarukhán, J. (2005) *Árboles Tropicales de México: manual para la identificación de las principales especies*. México: UNAM, Fondo de Cultura Económica.

R Core Team. (2013) *R: a language and environment for statistical computing*. Vienna, Austria: R Core Team.

Rausher, M.D. & Feeny, P. (1980) Herbivory, plant density, and plant reproductive success: the effect of *Battus philenor* on *Aristolochia reticulata*. *Ecology*, 61, 905–917.

Root, R.B. (1973) Organization of a plant – arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs*, 43, 95–124.

Schuldt, A., Assman, T., Bruelheide, H., Durka, W., Eichenberg, D., Härdtle, W. et al. (2014) Functional and phylogenetic diversity of woody plants drive herbivory in a highly diverse forest. *New Phtyologist*, 202, 864–873.

Shea, K., Smyth, M., Sheppard, A., Morton, R. & Chalimbaud, J. (2000) Effect of patch size and plant density of Paterson's curse (*Echium plantagineum*) on the oviposition of a specialist weevil, *Mogulones larvatus*. *Oecologia*, 124, 615–621.

Tack, A.J.M. & Roslin, T. (2010) Overrun by the neighbors: landscape context affects strength and sign of local adaptation. *Ecology*, 91, 2253–2260.

Underwood, N. & Halpern, S.L. (2012) Insect herbivores, density dependence, and the performance of the perennial herb *Solanum carolinense*. *Ecology*, 93, 1026–1035.

Underwood, N., Inouye, B.D. & Hambäck, P.A. (2014) A conceptual framework for associational effects: when do neighbors matter and how would we know? *Quarterly Review of Biology*, 89, 1–19.

Vehviläinen, H., Koricheva, J. & Ruohomäki, K. (2007) Tree species diversity influences herbivore abundance and damage: meta-analysis of long-term forest experiments. *Oecologia*, 152, 287–298.

Weterings, M.J.A., Weterings-Schonck, S.M., Vester, H.F.M. & Calmé, S. (2008) Senescence of *Manilkara zapota* trees and implications for large frugivorous birds in the southern Yucatan peninsula, Mexico. *Forest Ecology and Management*, 256, 1604–1611.

Wright, J.S. (2002) Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia*, 130, 1–14.

Xiao, Z., Mi, X., Holyoak, M., Xie, W., Cao, K., Yant, X. et al. (2017) Seed–predator satiation and Janzen–Connell effects vary with spatial scales for seed-feeding insects. *Annals of Botany*, 119, 109–116.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting Information.

How to cite this article: Abdala-Roberts, L., Berny-Mier y Terán, J.C., Vázquez-González, C., Cohuo, A., León, J., Valle, L. et al. (2023) Effects of seedling conspecific density and heterospecific frequency on insect herbivory in a tropical dry forest. *Agricultural and Forest Entomology*, 1–9. Available from: https://doi.org/10.1111/afe.12574