Short title: Effects of *Capsicum* genotypic diversity

**EFFECTS OF PEPPER (*Capsicum chinense*) GENOTYPIC DIVERSITY ON INSECT HERBIVORES**

Luis Abdala-Roberts\(^1\)*, Jorge C. Berny-Mier y Terán\(^2,3\), Xoaquín Moreira\(^4\), Antonio Durán-Yáñez\(^1\), and Felipe Tut-Pech\(^2\)

\(^1\)Departamento de Ecología Tropical, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Apartado Postal 4-116, Itzimná. 97000. Mérida, Yucatán, México.

\(^2\)Campo Experimental Mocochá, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Km. 25 Carretera Mérida-Motul, Mocochá, Yucatán, México 97454.

\(^3\)Present address: Department of Plant Sciences, University of California-Davis, One Shields Avenue, Davis, California, USA 95616.

\(^4\)Misión Biológica de Galicia (MBG-CSIC), Apdo. 28, 36080 Pontevedra, Spain.

*Corresponding author:

Luis Abdala-Roberts

Email: abdala.luis@yahoo.com.

Tel: +52 (999) 9423206.

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Abstract

1. There is growing interest on the effects of plant genotypic diversity on higher trophic levels. Here we asked whether genotypic diversity in *Capsicum chinense* peppers influenced attack by leaf-mining fly larvae (*Lyriomyza trifolii*) and fruit-eating weevils (*Anthonomus eugenii*), as well as parasitoid attack associated with weevils.

2. We established genotypic monocultures (N=10, two plots/each of five inbred lines), and polycultures (N=5, random mixtures of three out of the five lines) and conducted weekly surveys of fruit number, leafmines, and weevil fruit attack over a five-month period. In addition, we collected fruits to rear weevils and estimated parasitism associated with this herbivore.

3. There was a tendency for a negative effect of diversity on leafminer attack, with polycultures exhibiting a 24% reduction in leafminer attack relative to monocultures. In contrast, diversity did not influence weevil fruit attack or the level of parasitism associated with the weevil.

4. Our findings show that plant genotypic diversity effects vary among herbivore species, presumably due to differences in herbivore traits (e.g. diet breadth, mobility). We also emphasize that manipulating plant genotypic diversity can be an important consideration for pest management in this commercially important crop.

**Keywords:** *Capsicum*, diversity, genetic variation, herbivore, parasitoid.
Introduction

A fundamental goal in ecology is to understand the effects of plant diversity on ecosystem function (Hooper et al., 2005; Cardinale et al., 2011). Although a great deal of work has focused on manipulating plant species diversity and measuring effects on plant growth (Hopper, 1998; Loreau & Hector, 2001; Tilman et al., 2002) and higher trophic levels (Koricheva et al., 2000; Haddad et al., 2009; Scherber et al., 2010), there is also increasing evidence for parallel effects of within-species plant genotypic diversity on ecosystem function and consumers (reviewed by Hughes et al., 2008; Bailey et al., 2009; McArt & Thaler, 2013). Both forms of plant diversity, intra- and interspecific, have been shown to influence consumer abundance and diversity through increased producer biomass via plant-plant facilitation or reduced plant competition (Vandermeer, 1989; Hooper, 1998), as well as through increased habitat or resource heterogeneity (due to genotype or species trait variation) influencing consumer foraging behavior (Hämback et al., 2014).

There are important ecological implications stemming from research on plant diversity effects on higher trophic levels. One of these is that studies have frequently found that plant diversity reduces herbivore damage (reviewed by Andow, 1991; Barbosa et al., 2009). According to the “Resource Concentration Hypothesis” (RCH; Root, 1973), herbivore foraging behavior is density-dependent and increasing plant diversity at a constant plant density reduces the chance of herbivores finding a specific host plant species (or genotype), which in turn lowers herbivore abundance and damage (reviewed by Hambäck et al., 2014). Alternatively, the “Enemies Hypothesis” (EH; Root, 1973) holds that greater habitat complexity at high plant diversity which results in higher predator recruitment (e.g. because of greater availability of shelters or prey) and thus stronger suppression of herbivores (reviewed by Russell, 1989). While both hypotheses have
received much attention, not all studies are supportive (Loranger et al., 2014), and these inconsistencies have been attributed at least partly to unaccounted variation in herbivore traits (Vehviläinen et al., 2007; Plath et al., 2012; Abdala-Roberts et al., 2015). For instance, the dynamics predicted by the RCH depend on herbivore dietary specialization (Root, 1973): specialist herbivores should be negatively influenced by diversity whereas generalist herbivores should exhibit weak (or variable) responses since they are not limited to feeding on a specific host plant (Jactel & Brockerhoff, 2007; Castagneyrol et al., 2013; Abdala-Roberts et al., 2015). Similarly, the dynamics predicted by the EH are more likely to occur for generalist herbivores because these are more susceptible to natural enemies and frequently lack defense mechanisms present in specialist herbivores (e.g. crypsis, sequestration of plant toxic compounds; Singer et al., 2014). Finally, other traits such as mobility and feeding mode are also thought to be important predictors of plant diversity effects on herbivores (Bommarco & Banks, 2003).

In this study, we present results from a field experiment testing for the effects of genotypic diversity (“diversity” hereafter) in Capsicum chinense Jacq. peppers, a widely cultivated species in southern Mexico, on herbivory by two insect herbivores, the leaf-mining fly Liriomyza trifolii (Burgess) and the fruit-eating weevil Anthonomus eugenii Cano. Specifically, we asked the following: i) Does C. chinense diversity influence herbivore attack and do these herbivores vary in their responses to pepper diversity? Liriomyza trifolii is a broad dietary generalist feeding on species across more than 20 plant families, whereas A. eugenii has a narrower diet as it only feeds on a few species of Capsicum and Solanum (Elmore et al., 1934; Tun-Dzul, 2001). Thus, following the RCH, we predicted that A. eugenii would be more strongly (negatively) influenced by diversity than L. trifolii, as dietary specialists are more sensitive to changes in density of a preferred
pepper genotype across levels of diversity. Alternatively, following the EH, the generalist herbivore should be more strongly affected by diversity because generalists are more susceptible to enhanced effects by natural enemies at high diversity. Accordingly, to evaluate the EH we also tested for diversity effects on parasitism rates associated with one of these herbivores, *A. eugenii*. In considering these predictions, it is important to note that these herbivores also vary in other traits such as mobility and feeding mode which could influence herbivore responses to diversity. Overall, this study builds towards a better understanding of plant genotypic diversity effects on different herbivore species coexisting on the same host plant, in this case a commercially important crop in southern Mexico.

**Methods**

**Study system**

*Capsicum chinense* (Solanaceae), commonly known as “Habanero” peppers, are a major horticultural crop in southeast Mexico. In Yucatán, the state in México with the second greatest production of *C. chinense*, the total area planted with this crop has progressively increased during the last decade (SIAP, 2014). However, yields remain low and this is largely due to the negative impact of insect pests and pathogens. Two important insect herbivores attacking this crop in southern Mexico are the pepper weevil *Anthonomus eugenii* (Coleoptera: Curculionidae) and *Liriomyza trifolii* leafminers (Diptera: Agromyzidae). *Anthonomus eugeneii* is native to southern Mexico and Central America, but has extended its distribution throughout the southern United States, South America and the Caribbean Islands (Bartlett *et al.*, 1978; Burke & Woodruff, 1980). This weevil has a narrower diet breadth as it feeds primarily on species of *Capsicum* and *Solanum* within Solanaceae (Elmore *et al.*, 1934). Adult females deposit their eggs on flower buds or
immature fruits and larvae feed on seeds and placental tissues (Burke & Woodruff, 1980).

Larvae pupate inside fruits and recently emerged adults make an exit cavity to then start feeding on floral buds and immature fruits (Mau & Martin, 1994). In contrast, *L. trifolii* is a broad generalist that feeds on plant species from more than 20 families, including Solanaceae (Tun-Dzul, 2001). It is originally a Neartic and Neotropical species, but now has a cosmopolitan distribution (Minkengerg & van Lenteren, 1986). Late instar larvae drop on the ground and normally pupate in the soil (Minkengerg & van Lenteren, 1986).

Adults feed and oviposit on young leaves, and larvae feed on the leaf mesophyll and produce serpentine, irregular mines (Parrella, 1987). It is important to note that these two herbivores varied not only in diet breadth, but also in traits such as mobility and feeding mode which could also influence herbivore responses to diversity. Thus, any claim on the effects of diet breadth *per se* on herbivore responses should be made with caution.

**Pepper genotypes and experimental design**

We used five inbred lines (hereafter “genotypes”) of *Capsicum chinense* selected *a priori* to include a wide range of phenotypic variation (Berny-Mier y Terán *et al.*, 2013), namely: a typical orange habanero, a Belizean red habanero, an Antillean yellow habanero, a South American habanero of small fruits, and the Cuban habanero. These genotypes differ in vegetative (e.g., plant size, architecture) and reproductive traits (flowering phenology, fruit size and yield) (Trujillo-Aguirre & Pérez-Llanes, 2004), and data collected from plants of the present study confirmed this by showing significant variation among the selected genotypes in fruit output, fruit size, and resistance to insect herbivores (Berny-Mier y Terán *et al.*, 2013; Table S1 in the Supplementary material).
The field experiment was conducted at the Mococha Research Station of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), in Yucatan, Mexico (21°6’40”N, 89°26’35”W). In July 2010, we established 15, 5×5-m plots of *C. chinense*, of which 10 were genotypic monocultures (two plots per genotype) and five were genotypic polycultures composed of mixtures of three out of the five genotypes. In each polyculture plot, we randomly assigned positions to plants of each genotype and each genotype was planted in the soil in equal proportions. In addition, genotypes were equally represented across polyculture plots (each one planted in three plots of this treatment level). Distance between plots was 2.5 m, and within each plot the planting design was 30 cm between plants within rows, and 1.25 m between rows. Planting density was 2.64 plants/m² (66 plants/plot), for a total of 990 plants. Of these, eight plants per genotype, per plot were randomly selected and monitored from July to November 2010, which represented the entire growing season. As a result, statistical analyses were based upon a sample size of 40 plants per genotype (N = 200 plants). The fertilization and irrigation regime was the same across all plots and followed standard agricultural practices used for *C. chinense* in southern Mexico (see Tun-Dzul, 2001), with the exception that insecticides were not used. Plots were randomly located throughout the site, and plots as well as rows between plots were weeded periodically.

For each experimental plant, we recorded data on leaf miner and fruit weevil attack, as well as parasitoid attack rates on weevils. For leaf miners, we did this by counting the number of leaf mines of *L. trifolii* from a sample of two to six randomly chosen leaves, depending on availability of new leaves per plant; surveys were conducted every two weeks, from July 2010 to September 2010. We recorded leaf mines exclusively on young, fully-expanded leaves, and during each survey we sampled a different set of leaves. In most
cases only one leafminer was found per leaf (L. Abdala-Roberts, personal observation). For fruit weevils, from late September 2010 to mid-November 2010, we collected all the fruits produced per plant on a weekly basis and recorded how many of these were attacked by *A. eugenii*. Attacked fruits were identified based on the presence of yellowing of the fruit petiole which is a reliable sign of weevil infestation (data from this study). Finally, to estimate levels of parasitism associated with *A. eugenii*, at the end of the sampling season (early to mid-November) we collected a random subsample of fruits from the last two fruit harvests (2-6 plants sampled per genotype, per plot depending on availability; N = 92 plants), and placed each fruit individually in 0.5-l plastic containers with moistened cotton to rear weevils and record parasitoid emergence based upon observations conducted every two days throughout a two-week period. After two weeks, all fruits were dissected for further examination. We calculated the proportion of mined leaves (# leaves with mines / total leaves sampled), the proportion of attacked fruits (# of attacked fruits / total fruits sampled), and the proportion of parasitized weevils (# parasitoids / [# parasitoids + # weevils]) per plant for statistical analyses. For leafminer and weevil attack, we calculated proportions separately for each survey and then used the average across surveys for statistical analyses. For fruit output, we analyzed the total (cumulative) number of fruits sampled across surveys.

**Statistical analyses**

**Effects of diversity on fruit number, herbivores, and parasitoids.** - We performed general linear models with PROC GLM in SAS 9.2 (SAS Institute, Cary N.C.) to test for an effect of *C. chinense* genotypic diversity (fixed, two levels) on the number of fruits produced per plant, the proportion of leaves with mines per plant, and the proportion of fruits attacked by
the weevil per plant. In all three cases, data exhibited normally distributed residuals. In
addition, we ran a generalized linear model in PROC GENMOD (SAS 9.2) to test for a
diversity effect on the proportion of parasitized weevils per plant, using a binomial
distribution (logit link), as data were not normally distributed after transformation. There
was no evidence of overdispersion for this latter model. In all four cases, we used plot-level
data.

Whenever a diversity effect was significant (P < 0.05) or marginally significant
(0.05 < P < 0.10) in the above models, we determined if such effect was additive, i.e. due to
the presence of one or more resistant or susceptible genotypes in mixtures (i.e. the
“sampling” effect; Houston, 1997), or non-additive where diversity results in higher or
lower values as a consequence of emergent, patch-level properties due to interactive effects
among genotypes (Johnson et al., 2006). Following Johnson et al. (2006), we calculated
pepper genotype means for each variable in monoculture (i.e. expected values), and
compared these values to the mean of each genotype in polyculture (i.e. observed values).
For each response variable, we compared observed and expected values with a one-way
general linear model using PROC MIXED in SAS 9.2. These models also included the
effects of plot and genotype nested within plot, making them similar to a paired test
comparing observed vs. expected values for each genotype (Johnson et al., 2006). A
significant difference between observed and expected values is necessarily due to non-
additivity as the comparison is performed by specifying the monoculture values of each
genotype (i.e. sampling effects are accounted for by including genotype-specific expected
values).
Results

Effects of plant diversity on leafminer attack
There was a tendency for a negative effect of *C. chinense* diversity on the number of mined leaves by *L. trifolii*, though this effect was marginally significant (*P* = 0.07; Table 1). Genotypic polycultures exhibited a 1.3-fold reduction in the percent of leaves with mines (22.98 ± 2.26%) relative to monocultures (30.21 ± 2.34%) (Fig 1A). This decrease in leafminer attack was lower than would be expected based upon sampling effects alone (*F*$_{1,14}$ = 13.28, *P* = 0.003), showing that the negative effect of diversity on leafminers was non-additive.

Effects of plant diversity on fruit output and weevil attack
Plant diversity did not influence fruit output as indicated by no significant difference in total fruit number between *C. chinense* genotypic monocultures (98.48 ± 32.49 fruits) and polycultures (70.36 ± 19.42 fruits) (Table 1; Fig. 1B). In addition, we found no significant difference in the proportion of fruits attacked by *A. eugenii* between monocultures (24.97 ± 5.38%) and polycultures (28.61 ± 7.69%) (Table 1; Fig. 1C).

Effect of plant diversity on parasitism associated with the fruit weevil
Overall, 18.6% of the sampled weevils were parasitized. Plant diversity did not influence weevil parasitism as the generalized linear model indicated no significant difference in the
proportion of parasitized weevils between *C. chinense* genotypic monocultures (9.81 ±
3.81%) and polycultures (7.51 ± 3.99%) (Table 1; Fig. 1D).

**Discussion**

We found contrasting effects of diversity on each herbivore; there tended to be a negative
effect of diversity on *L. trifolii* leafminer attack, but no effect whatsoever on *A. eugenii* fruit
attack. In addition, diversity did not influence the amount of parasitism associated with *A.
eugenii*, suggesting that natural enemies did not mediate effects of plant genotypic diversity
on this herbivore’s recruitment patterns.

Our results showed a 24% reduction in leaf miner attack for *C. chinense* genotypic
polycultures relative to monocultures. Although this result was not statistically significant
(P = 0.07), we consider that the effect size observed is biologically meaningful and that it
was likely burdened by low statistical power. While sampling effects cannot be entirely
ruled out (e.g. higher incidence of resistant pepper genotypes in mixtures), our analyses
indicated that this result was driven by non-additive dynamics from interactions among
genotypes in mixtures (Johnson *et al*., 2006). Similarly, a number previous studies have
also reported negative effects of plant diversity on insect herbivores (reviewed by Andow,
1991; Barbosa *et al*., 2009) driven by habitat heterogeneity interfering with herbivore
foraging (Björkman *et al*., 2010; reviewed by Hambäck *et al*., 2014) or changes in plant
traits reducing herbivore recruitment or damage (e.g. higher leaf defenses; McArt & Thaler,
2013; Moreira *et al*., 2014). However, the fact that *L. trifolii* was negatively influenced by
*C. chinense* genotypic diversity, despite being a broad dietary generalist herbivore, runs
counter to the RCH which predicts that specialist (rather than generalist) herbivores should
be negatively influenced by diversity (Jactel & Brockerhoff, 2007). This suggests that diet
breadth did not mediate diversity effects on this herbivore, and that instead other traits such as dispersal ability or feeding mode were responsible for this finding (Bommarco & Banks, 2003). For instance, adult *Liriomyza* females exhibit high dispersal rates (Jones & Parrella, 1986) and highly mobile herbivores are thought to respond more strongly to plant diversity than sedentary herbivores because they can disperse more readily and choose among plant patches of varying diversity (Bommarco & Banks, 2003). Finally, although speculative because we did not measure parasitism associated with the leafminer, it is worth noting that our finding agrees with the EH which predicts negative effects of diversity on generalist herbivores such as *L. trifolii* because these are highly susceptible to enhanced effects of natural enemies in species or genotype mixtures. Nonetheless, further work is necessary to test this hypothesis and determine if natural enemies mediate *C. chinense* genotypic diversity effects on *L. trifolii*.

Pepper genotypic diversity had no overall effect on fruit attack by the relatively more diet-specialized herbivore *A. eugenii*, which is unsupportive of the RCH which predicts negative effects of plant diversity on specialist herbivores. Thus, it appears that this herbivore it is not strongly influenced by changes in density or traits of specific genotypes across levels of genotypic diversity, at least in the case of *C. chinense*. However, we caution that predictions of plant genotypic diversity based upon herbivore diet breadth alone may not be straightforward in this case as *A. eugenii* is not a strict specialist on *C. chinense* or even the genus *Capsicum*. In addition, it is also possible that other traits such as dispersal ability could have limited the response of this herbivore to genotypic diversity in *C. chinense*. For example, previous studies have shown that *A. eugenii* exhibits low dispersal and highly clumped distributions (Riley *et al.*, 1992), and herbivores with low mobility may exhibit weaker responses to plant diversity (Bommarco & Banks, 2003).
Thus, to test for effects of diet breadth, we should ideally examine responses to diversity by multiple herbivore species located at different points along a continuum of dietary specialization, while controlling for other traits (e.g. dispersal ability, feeding guild; Ali & Agrawal, 2012). At the same time, however, our result could be interpreted as supportive of the EH which predicts weaker effects of diversity on specialist (relative to generalist) herbivores as these are better defended against natural enemies. Accordingly, there was no effect of diversity on parasitism associated with this specialist weevil, which supports the idea that natural enemies did not mediate diversity effects on this herbivore (for similar findings see Langer, 1996; Björkman et al., 2010).

Taken together, results from this study reveal contrasting effects of plant genotypic diversity on different species of insect herbivores. Such effects are likely dependent upon herbivore species-specific traits (e.g. diet breadth, mobility, feeding mode), as well as plant genetically-based variation in key traits influencing consumers. Accordingly, a predictive understanding of plant genotypic diversity effects on higher trophic levels will be achieved only to the extent that we identify which genetically-based plant traits underlie such dynamics (Mooney and Singer 2012, Barbour et al., 2015), and how such traits interact with herbivore traits (Hambäck et al., 2014). Finally, our findings suggest that by reducing the abundance of generalist herbivores (i.e. leafminers), genotypic mixtures can serve as a pest control strategy in *C. chinense*, a commercially important crop which is usually planted in genotypic monocultures throughout southern Mexico.
Acknowledgements

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Author contributions

LAR and JBMyT designed the experiment, LAR, JBMyT, ADY, and FTP conducted the experiment and collected the data, LAR analyzed the data, and LAR, JBMyT and XM wrote the article.

Literature cited


Table 1. Results (F- or $\chi^2$ statistics, and P-values in parenthesis) from general (or generalized in the case of parasitism) linear models testing for the effects of Capsicum chinense genotypic diversity on fruit number, the proportion of leaves mined by the fly Liriomyza trifolii, the proportion of fruits attacked by the weevil Anthonomus eugenii, and the proportion of parasitized weevils (weevil parasitism). Marginally significant (0.05 < P < 0.10) effects are in italics.

<table>
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<tr>
<th>Response</th>
<th>Diversity effect</th>
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<tbody>
<tr>
<td>Number of fruits</td>
<td>$F_{1,13} = 0.33$ (0.571)</td>
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<tr>
<td>Leaf miner attack</td>
<td>$F_{1,13} = 3.80$ (0.073)</td>
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<tr>
<td>Fruit weevil attack</td>
<td>$F_{1,13} = 0.15$ (0.703)</td>
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<tr>
<td>Weevil parasitism</td>
<td>$\chi^2_{1,7} = 0.01$ (0.950)</td>
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Figure legends

Figure 1. Effects of Capsicum chinense genotypic diversity on fruit number (A), the proportion of leaves with mines by Liriomyza trifolii (B), the proportion of fruits attacked by the pepper weevil Anthonomus eugenii (C), and the proportion of parasitized A. eugenii weevils (D) from a field experiment in southern Mexico (Yucatan). Bars are means (± S.E.); n.s. = non-significant difference (P > 0.05); the diversity effect on leafminer attack was marginally significant (0.05 < P < 0.10).
Figure 1.

A) Number of fruits

B) Percent of mined leaves

C) Percent of attacked fruits

D) Percent of parasitized weevils

Diversity treatment

Monoculture

Polyculture

n.s.

P = 0.073

n.s.
Table S1. Variation in reproductive traits and resistance to insect herbivores and virus infection among habanero pepper (*Capsicum chinense*) inbred lines used in the present study (from Berny-Mier y Terán *et al.*, 2013). We present the magnitude of variation (X-fold variation among the two most extreme lines for each variable) and test of genetic variation among lines for fruit weight, fruit output, attack by *Liriomyza trifolii* leaf miners (proportion of attacked leaves), attack by *Anthonomus eugenii* fruit weevils (proportion of attacked fruits), and severity of symptoms of co-infection by viruses transmitted by the whitefly *Bemisia tabaci* (scale of 1 to 9). Data were collected from plants of the same diversity experiment reported in the present study, and tests of genotype effects are based upon general linear models including pepper line (fixed) and plot (random). Line effect: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

<table>
<thead>
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<th>Variable</th>
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<td>Leaf miner attack</td>
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<td>Weevil fruit attack</td>
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<tr>
<td>Severity of virosis</td>
<td>2-fold</td>
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