

Comparison of tree genotypic diversity and species diversity effects on different guilds of insect herbivores

Luis Abdala-Roberts, Kailen A. Mooney, Teresa Quijano-Medina, María José Campos-Navarrete, Alejandra González-Moreno and Víctor Parra-Tabla

L. Abdala-Roberts (*labdala@uci.edu*) and K. A. Mooney, Dept of Ecology and Evolutionary Biology, Univ. of California, Irvine, 321 Steinhaus Hall, Irvine, CA 92697-2525, USA. – LAR, M. J. Campos-Navarrete and V. Parra-Tabla, Depto de Ecología Tropical, Campus de Ciencias Biológicas y Agropecuarias, Univ. Autónoma de Yucatán, Apartado Postal 4-116, Itziminá, MX-97000 Mérida, Yucatán, México. – T. Quijano-Medina, Wageningen Univ., Droevendaalsesteeg 2, NL-6708 PB Wageningen, the Netherlands. – A. González-Moreno, Inst. Tecnológico de Conkal, Km. 16.3 Antigua Carretera Mérida-Motul, MX-97345 Conkal, Yucatán, México.

Although the effects of plant diversity on herbivores are contingent upon herbivore traits and the source of plant diversity (e.g. intra- and interspecific), most studies have analyzed these effects separately. We compared the effects of genotypic diversity of big-leaf mahogany *Swietenia macrophylla* with that of tree species diversity on two specialist caterpillars (*Hypsipyla grandella* stem borers and *Phyllocnistis meliacella* leaf miners) and three generalist leafhoppers (Cicadellidae) feeding on mahogany in a large-scale (7.2 ha) forest diversity experiment in southern Mexico. The experiment consisted of fifty-nine 21 × 21-m plots, with 64 tree saplings each (3-m spacing between plants). Plots were either mahogany monocultures or species polycultures of four species (including mahogany) and – within each of these two plot types – mahogany was represented by either one or four genotypes. Throughout a five-month period, beginning six months after planting, we measured mahogany growth and monitored herbivore and predator (spider) abundance. We found no effect of mahogany genotypic diversity on either specialist caterpillars or generalist leafhoppers, and this result was consistent across levels of tree species diversity. In contrast, species diversity had significant effects on both specialists but neither of the generalist herbivores. Specifically, species diversity lowered *H. grandella* attack at the middle of the sampling season, but increased attack at the end of the season, whereas *P. meliacella* abundance was consistently reduced. Such effects were not mediated by effects of species diversity on plant growth (of which there were none), but rather through resource heterogeneity. Diversity did not influence spider abundance. This study is one of few to directly compare sources of plant diversity, and uniquely compares such effects among herbivores with contrasting life histories (e.g. diet breadths). Overall, we demonstrate that plant species diversity effects outweigh those of genotypes, and our results suggest that such effects are stronger on specialist than generalist herbivores.

Evidence has mounted for the effects of intra- (reviewed by Bailey et al. 2009) and inter-specific (Siemann 1998, Haddad et al. 2009) plant diversity on higher trophic levels. Numerous studies have found effects of plant diversity on arthropod species richness and abundance (Koricheva et al. 2000, Crutsinger et al. 2006, Haddad et al. 2009) and on consumptive interactions at higher trophic levels (Moreira and Mooney 2013, Abdala-Roberts and Mooney 2014), with the basis of such effects being variation in ecologically important traits among plant species or genotypes within species (Hare 2002, Mooney and Singer 2012). Within this context, a widely documented pattern is that greater plant diversity frequently leads to reductions in herbivory (reviewed by Andow 1991, Barbosa et al. 2009). Two hypotheses have been offered to explain this phenomenon and invoke the influence of resource heterogeneity on consumers. According to the ‘enemies hypothesis’ (hereafter EH; Root 1973), greater habitat complexity at high plant diversity favors increased predator recruitment (e.g. because of greater availability of

shelters or prey) resulting in stronger top-down suppression of herbivore populations and thus lower herbivory (reviewed by Russell 1989). Alternatively, the ‘resource concentration hypothesis’ (RCH) (Root 1973) holds that herbivore foraging is density-dependent and increasing plant species diversity at a constant plant density reduces the probability of finding a preferred host plant, which lowers herbivore recruitment and damage on individual plants. While both hypotheses have received considerable attention, evidence from natural systems has generated mixed support (Bommarco and Banks 2003, Underwood et al. 2014).

The inconsistent support for the EH and RCH could be due to unaccounted variation in herbivores traits (Vehviläinen et al. 2007, Plath et al. 2012). For example, the dynamics predicted by the EH should be more likely to occur for generalist herbivores because they are more susceptible to natural enemies as they frequently lack defense mechanisms found in specialists (e.g. crypsis, sequestration of plant toxic compounds; Mooney et al. 2012, Singer et al.

2014). Similarly, the dynamics predicted by the RCH should also depend on herbivore dietary specialization (Root 1973); in this case, however, specialist herbivores should be negatively influenced by plant diversity whereas generalist herbivores should exhibit weak (or variable) responses since they are not limited to feeding on a specific host plant (Jactel and Brockerhoff 2007, Castagneyrol et al. 2013, Salazar et al. 2013). In addition, other traits such as mobility and feeding mode are also thought to be important predictors of plant diversity effects on herbivores (Koricheva et al. 2000, Bommarco and Banks 2003).

Plant diversity effects on herbivores may also vary depending on the magnitude of underlying plant trait variation. We expect that because trait variation is typically greater among species than among genotypes within a species (Albert et al. 2010), plant species diversity should lead to stronger effects due to greater resource heterogeneity influencing predator (as predicted by the EH) or herbivore (as predicted by the RCH) foraging behaviors. However, only two studies have tested this prediction and found that genotypic diversity effects were equally (Cook-Patton et al. 2011) or more (Crawford and Rudgers 2013) important than species diversity in structuring arthropod communities. Further research comparing various sources of plant diversity is necessary in order to assess the relative strength and combined action of plant intra- and interspecific diversity effects on consumers.

We report on the results of a large-scale (7.2-ha, 4780 plants) tree diversity experiment testing for the effects of big-leaf mahogany *Swietenia macrophylla* genotypic diversity and tree species diversity on two groups of insect herbivores: immobile, mandibulate specialists that feed exclusively on mahogany, *Hypsipyla grandella* stem borers and *Phyllocnistis meliacella* leaf miners (hereafter 'specialists'), and mobile, phloem-feeding generalists (leafhoppers, Cicadellidae; hereafter 'generalists'). In addition, the specialists are concealed or internal feeders whereas the generalists are external feeders. Therefore, effects of plant diversity on each group may reflect differences not only in diet breadth but also in other traits such as mobility and feeding mode. A side from herbivores and to evaluate the EH, we also tested for diversity effects on spiders, a major predator group in the system which could mediate diversity effects on herbivores. Sampling was conducted on tree saplings at an early time point in the experiment, prior to direct interactions among widely-spaced plants. Thus, any effect of diversity on consumers would be mostly due to plant-based habitat heterogeneity influencing consumer recruitment, rather than effects of increased plant biomass as a result of resource partitioning or facilitative interactions among plants. First, we predicted that the strength of diversity effects corresponds to the magnitude of plant trait variation underlying each source of diversity, i.e. species diversity exerts a stronger effect on herbivores and predators than genotypic diversity (predicted by both the EH and RCH). Second, we predicted that genotypic diversity effects on these consumer groups are weaker at high species diversity because the effects of genotype variation are overridden by increased variation at the tree species level. Third, our predictions for the response of the two herbivore groups differ between the EH and the RCH. Following the RCH, we predicted that specialist herbivores are more strongly (negatively) influ-

enced by tree species diversity than generalists, as the former are more sensitive to changes in density of a particular host (i.e. mahogany) across levels of species diversity. Similarly, the effects of mahogany genotypic diversity (albeit weaker overall) are expected to be stronger on specialists because of reductions in the density of a preferred mahogany genotype at high genotypic diversity, whereas generalists are less influenced by genotype variation (and thus diversity) within any one tree species. Alternatively, following the EH, generalist herbivores are expected to be more strongly affected by diversity than specialists because the former are more susceptible to enhanced effects by predators at high diversity. Overall, this study builds towards a predictive understanding of plant diversity–herbivory relationships and more broadly of consumer responses to resource heterogeneity.

Methods

Study system

Big-leaf mahogany and its herbivores

Big-leaf mahogany *Swietenia macrophylla* (Meliaceae), the target tree species and the component of genotype variation evaluated in this study, is a self-compatible, long-lived perennial tree distributed from southern México to Bolivia (Pennington and Sarukhán 2005). Adult tree density of mahogany is low in tropical forests, but seedlings occur at higher densities and can be found in mono-specific patches in forest gaps (Grogan et al. 2003). The main herbivores of this species in tropical forests are insect leaf chewers (Norghauer et al. 2010) and small mammals (Grogan et al. 2003), whereas in managed systems the most common herbivores are *Hypsipyla grandella* (Lepidoptera: Pyralidae) stem-boring caterpillars, *Phyllocnistis meliacella* (Lepidoptera: Gracillariidae) leaf-mining caterpillars and phloem-feeding leafhoppers (Cicadellidae). These two specialist caterpillars as well as the generalist phloem-feeders were the most abundant groups of herbivores in our system, and we thus chose to focus on them in this study.

Hypsipyla grandella is a specialist herbivore that only feeds on a handful of species of Meliaceae (Newton et al. 1993). Larvae carve tunnels through the terminal shoots of saplings and juvenile plants, resulting in deformation of the main stem and reduced growth, and a single caterpillar can produce multiple damage sites per plant (Mo et al. 1997). Likewise, *P. meliacella* is a caterpillar that also specializes on a few species of Meliaceae (Becker 1976, Arguedas 2007). Leafminer larvae produce characteristic serpentine galleries throughout the leaf surface (Becker 1976). Finally, leafhoppers (Hemiptera: Cicadellidae) are frequently found feeding on mahogany in disturbed sites (Sánchez-Soto et al. 2009). Most of the leafhopper species found in our study system are dietary generalists (Maes and Godoy 1993), and at the study site the most common species belong to the genera *Homalodisca*, *Oncometopia* and *Pseudophera*. Abundance and damage by these generalist leafhoppers and by specialist caterpillars is greatest during the rainy season, which spans from June to October.

In tropical forests of the Yucatan Peninsula, big-leaf mahogany co-occurs with five other tree species (CICY

2010) that were used to manipulate tree species diversity in this experiment, namely: *Tabebuia rosea* (Bignoniaceae), *Ceiba pentandra* (Malvaceae), *Enterolobium cyclocarpum* (Fabaceae), *Piscidia piscipula* (Fabaceae) and *Cordia dodecandra* (Boraginaceae). These species are long-lived, deciduous, and adult trees generally reach from 20 m (*P. piscipula*) to 40 m (*C. pentandra*) in maximum height, depending on the species (Pennington and Sarukhán 2005), and are distributed from central México to Central and South America (Pennington and Sarukhán 2005).

Experimental design

Across 7.2 ha we planted 74 plots of 21 × 21 m each at a density of 64 plants per plot, 3 m spacing among trees, for a total of 4780 plants. Aisles between plots were 6-m wide. The experiment was established on a recently cleared site where vegetation was composed mostly of grasses and shrubs, and is currently surrounded by a matrix of secondary tropical forest. For this study, we restricted our sampling to the 59 plots where mahogany was planted (ignoring 15 plots without mahogany). These plots were classified into four types, depending on the diversity treatment combination: 1) mahogany monocultures of a single genotype (12 plots, two replicate plots/genotype), 2) mahogany monocultures of four genotypes (20 plots), 3) polycultures of four species within which all mahogany saplings planted were of one genotype (12 plots, two plots/genotype), and 4) polycultures of four species within which mahogany plants were represented by four genotypes (15 plots) (Supplementary material Appendix 5 Fig. A1). Treatments of both species and genotypic diversity included equal numbers of individuals of four species and four mahogany genotypes drawn randomly from pools of six species and six genotypes, respectively. All non-mahogany species were equally represented across polycultures (each species present in six polyculture plots). Likewise, mahogany genotypes were represented in a similar number of mahogany monocultures of four genotypes (8–9 plots per genotype), and also in a similar number of species polycultures where mahogany plants were of four genotypes (9–10 plots per genotype). Plots of each treatment combination were randomly interspersed throughout the experimental landscape.

Seed sources and collection

From January 2011 to March 2011, we collected seeds of all species from adult plants located in southern Quintana Roo (México), and germinated at the INIFAP (Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias) campus in Mocochoá, Yucatán (México) (21°6'40"N, 89°26'35"W). For all species, we collected seed from six mother trees (distance among trees ranged from 0.5 to 50 km, depending on the species; 3 to 50 km for mahogany). In the case of mahogany, these seed source distances fall within the range used by previous studies to define distinct populations of this species (Gillies et al. 1999, Loveless and Gullison 2003). Accordingly, we found significant variation among mahogany maternal families (mixture of full- and half-sibs; hereafter referred to broadly as 'genotypes') in growth-related traits (e.g. canopy size: 2.5-fold), herbivore resistance (e.g.

stem borer attack: 3.8-fold) (Supplementary material Appendix 1 Table A1), and chemical defenses (polyphenolics: five-fold variation 10.44 mg g⁻¹ to 50.39 mg g⁻¹; F_{5,50} = 6.30, p < 0.0001; data from Moreira et al. 2014). As such, these genotype identity effects represent the basis of expected genotypic diversity effects on herbivores. In December 2011, we established the diversity experiment by planting four month-old seedlings (40–60 cm in height) at a site owned by INIFAP near Muna, Yucatán (100 km southwest of Mocochoá; 20°24'44"N, 89°45'13"W). Plants were fertilized once in January 2012 with N, P, and K (20:30:10), and irrigated with 2 l of water three times per week from January 2012 until June 2012. Seedlings were sampled from June 2012 until November 2012.

Measurements of plant growth and consumer abundance

We measured the size of mahogany plants at the start (June 2012; all mahogany plants n = 2480) and end (October 2012: randomly chosen subset n = 944) of the rainy season. Plant size was estimated by measuring canopy height, width, and length, and calculating the product of these three measures which represented a proxy of canopy volume (m³). Given the short time since the establishment of the plantation (six months), we found no effects of mahogany genotypic diversity or tree species diversity on mahogany canopy volume at the end of the growing season, suggesting weak effects of diversity on plant biomass via plant–plant interactions (analyses based upon plot-level-means; see results in Supplementary material Appendix 2 Table A2). Likewise, analyses showed no initial differences in mahogany canopy volume between diversity treatments (Supplementary material Appendix 2 Table A2).

We conducted three surveys of *H. grandella* stem borer attack throughout the rainy season of 2012, approximately every 45 days, in late July (early season), mid-September (mid-season), and late October (late season). During each survey, we inspected the entire canopy of each mahogany sapling (n = 2480 plants, 59 plots) and recorded the number of new attack sites (following Taveras et al. 2004). At the start of the sampling season, mahogany height and canopy volume were 76.04 ± 1.21 cm and 0.17 ± 0.01 m³, respectively (mean ± SE). For *P. meliacella* leaf miners, we randomly selected 16 mahogany plants from each plot (n = 944 plants, 59 plots), examined the entire canopy of each plant, and counted the number of leaves with mines (in > 90% of cases we observed one mine per leaf). We conducted this survey only once at the end of the rainy season of 2012 (mid-October). Because mahogany leaves are long-lived, both old and new mines were present on a given plant at the time of sampling, and our survey thus represented an estimate of cumulative leaf miner abundance throughout the rainy season. Adults of this herbivore were identified following Becker (1976).

We used the same plants surveyed for leaf miner abundance to record leafhopper (Cicadellidae) and spider abundance by examining the entire canopy of each mahogany plant three times throughout the rainy season of 2012 (late July, late September, and late October). Across tree species, sap feeders represented 42.9% of all recorded

specimens of generalist herbivores (Campos-Navarrete unpubl.), and they were the most abundant guild of generalist herbivores found on mahogany during the sampling season (representing 88.2% of all generalist herbivore specimens recorded for this tree species). *Oncometopia* sp., *Homalodisca hambletoni* and *Pseudophera atra* accounted for 45.3% of all generalist sap-feeding specimens and were observed feeding on all tree species (Supplementary material Appendix 3 Table A3; Campos-Navarrete unpubl.). These three generalist herbivores were the subject of this study and exhibited up to 15-fold variation in abundance among tree species thus emphasizing the importance of tree species identity effects (Campos-Navarrete unpubl.). The genus and species of these leafhoppers were identified following Young (1968). On the other hand, spiders were the most abundant predator group on mahogany (87% of predatory arthropods surveyed, excluding ants; Abdala-Roberts unpubl.). Although ants were more abundant than spiders, some ant species tended leafhoppers for their honeydew and thus their role as predators or mutualists is difficult to assess (Abdala-Roberts unpubl.). Based on this, we chose to focus only on spiders to test if natural enemies mediate plant diversity effects on herbivores. Spiders were frequently observed feeding on leafhoppers, as well as moths and butterflies (Abdala-Roberts unpubl.), the latter suggesting that they likely fed upon adults of the studied specialist stem borer and leaf miner. It is important to note that by sampling leafhoppers and spiders only on mahogany (rather than sampling all tree species), our test of species diversity effects on these generalist consumers addresses the influence of tree species neighborhood on one of the component species in the system rather than the overall effect of species diversity on these consumers (i.e. across all tree species). In addition, while this study focuses on diversity effects on herbivore and predator abundance we acknowledge that measuring effects on herbivory (while different in nature and depending on the questions being asked) is desirable to obtain a more complete understanding of diversity effects on plant–herbivore interactions.

Data analyses

Specialist herbivores

We used a repeated measures general linear model with the MIXED procedure in SAS ver. 9.2 using plot as subject, to test for the effects of mahogany genotypic diversity (fixed, two levels), tree species diversity (fixed, two levels), survey (fixed, three levels), all two-way interactions, and the three-way interaction on the proportion of plants with new *H. grandella* attack sites per plot during each survey. We used a repeated measures analysis (as opposed to cumulative or mean values across surveys) because we observed a significant change through time in the effects of diversity on stem borer attack. Although a parallel repeated-measures model using the plot-level mean number of attacks per plant, per survey yielded qualitatively similar results (not shown), the frequency of attack sites does not provide a reliable estimate of stem borer abundance as a single *Hypsipyla* larva can produce multiple damage sites on a plant. Therefore, we only report results for the proportion of attacked plants which is in keeping with our goal of measuring effects on herbivore abundance. If we found a significant interaction between

either source of diversity and survey date, we performed component models testing for diversity effects separately within each survey date. In the case of *P. meliacella* leaf miners, we performed a general linear model with PROC GLM in SAS to test for effects of genotypic diversity, species diversity, and their interaction on the plot-level mean number of leaves with mines (i.e. average of all sampled mahogany plants within each plot).

Generalist herbivores

We performed a general linear model with PROC GLM in SAS using the same model previously described for leaf miner abundance. We summed leafhopper counts across species and surveys for each plant and used the mean value across plants per plot as response variable. We did not use a repeated measures model because preliminary analyses showed no temporal variation in the strength or direction of diversity effects on leafhopper abundance (i.e. non-significant survey date \times species diversity and survey date \times genotypic diversity interactions; $F_{2,112} = 0.14$, $p = 0.86$ and $F_{2,112} = 0.89$, $p = 0.41$, respectively). Analyses performed separately for each of the three leafhopper species yielded qualitatively identical results relative to a model based upon pooled data across species (Supplementary material Appendix 4 Table A4).

Spiders

We performed a general linear model with PROC GLM in SAS using the same model previously described for leaf miners and leafhoppers. We summed spider counts across surveys for each plant and used the mean value across plants per plot as response variable. As for leafhoppers, we did not find temporal variation in the strength or direction of diversity effects on spider abundance (i.e. non-significant survey date \times species diversity and survey date \times genotypic diversity interactions; $F_{2,111} = 0.16$, $p = 0.85$ and $F_{2,111} = 1.13$, $p = 0.32$, respectively).

Testing for non-additive effects of diversity

Whenever a genotypic or species diversity effect was significant in the above models, we determined if such effect was additive, where diversity effects were due to sampling effects, or non-additive, where diversity results in higher or lower values relative to monoculture (Houston 1997, Tilman et al. 2002). Following Johnson et al. (2006), we calculated mahogany genotype means for each consumer group at low diversity (i.e. expected values), and compared these values to the mean of each genotype at high diversity (i.e. observed values). For mahogany genotypic diversity, we compared the mean of each genotype across mahogany monocultures with one genotype and species polycultures with one mahogany genotype (expected values) to observed values of the corresponding genotype in mahogany monocultures of four genotypes and species polycultures with four genotypes (observed values). For species diversity, we compared the mean of each genotype across mahogany monocultures of one genotype and mahogany monocultures of four genotypes (expected values) to observed values of the corresponding genotype in species polycultures with either one or four mahogany genotypes (observed values). We performed genotypic and species diversity tests separately by comparing observed and expected values with one-way general linear models using

the MIXED procedure in SAS. These models also included the effects of plot and genotype nested within plot which made 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). For stem borer attack, we performed these tests separately for each survey whenever a diversity effect was significant on a given date.

General considerations

For all models we used plot-level data and included plot-level mean canopy volume (mean across plants per plot) as a covariate to account for residual variation in mahogany size influencing herbivore and spider abundance. If the genotypic diversity \times species diversity interaction was significant for the herbivore abundance or non-additivity models, we performed component models testing for an effect of each source of diversity separately within each level of the other source of diversity. For all models, we used a normal distribution with the identity as link function. The assumption of normality was met in all cases following verification of residuals and Kolmogorov–Smirnov tests. In addition, although we found evidence of heteroscedasticity for the models testing for non-additivity of diversity effects, non-parametric Wilcoxon tests showed qualitatively similar results (not shown). Thus, we report results for parametric models only. We present least-square means and standard errors as descriptive statistics and report results for type III sums of squares.

Results

Effects of plant diversity on specialist herbivores

The mean percent of mahogany plants with new attack sites by *Hypsipula grandella* stem borers per plot was $18.5 \pm 2.0\%$ (mean and SE across surveys). Although there was no overall effect of tree species diversity (mahogany monocultures versus species polycultures) on the proportion of plants with new attacks per plot (Table 1, Fig. 1A), we found significant species diversity \times survey interaction (Table 1), indicating

a temporal shift in the effect of tree species diversity (Fig. 1B). Specifically, we found that during the second survey date species polycultures exhibited a 55% lower proportion of attacked plants relative to monocultures ($F_{1,53} = 5.35$, $p = 0.02$) (Fig. 1B), while for the third survey this pattern reversed as polycultures exhibited a 24% higher proportion of attacked plants relative to monocultures ($F_{1,53} = 4.20$, $p = 0.04$) (Fig. 1B). The negative effect of species diversity on stem borer attack during the second survey date was non-additive (observed versus expected: $F_{1,67} = 10.64$, $p = 0.002$), whereas for the third survey the effect was additive ($F_{1,65} = 2.66$, $p = 0.11$). There was no overall effect of genotypic diversity (genotype monocultures vs. plots with four genotypes) on stem borer attack as well as no evidence of a genotypic diversity \times species diversity interaction (Table 1, Fig. 1A).

There was a significant effect of species diversity on the plot-level mean number of *Phyllocnistis meliacella* leaf mines per plant (Table 1), with species polycultures showing a 48% lower mean value relative to mahogany monocultures (Fig. 2A). Such reduction in leaf miner abundance for species polycultures was greater than expected based upon leaf miner abundance in mahogany monocultures ($F_{1,66} = 7.51$, $p = 0.008$), indicating non-additive effects of species diversity on this specialist herbivore. In contrast, there was no effect of mahogany genotypic diversity (Fig. 2A). Likewise, we found no evidence of a genotypic diversity \times species diversity interaction on leaf miner abundance (Table 1, Fig. 2A).

Effects of plant diversity on generalist herbivores

Oncometopia sp. was by far the most abundant of the three focal leafhopper species feeding on mahogany (1470 individuals, 82.5% of specimens recorded), followed by *H. hambletoni* (177 individuals, 9.2%), and *P. atra* (161 specimens, 8.3%). Based upon pooled abundances across species and contrary to the results for the two specialist caterpillars, there was no evidence of tree species diversity on the abundance of generalist leafhoppers (Table 1, Fig. 2B). Likewise, there was no effect of genotypic diversity, as well as no evidence of a genotypic diversity \times species diversity interaction on leafhopper abundance (Table 1, Fig. 2B). As mentioned previously, models conducted separately for each leafhopper species yielded equivalent results (Supplementary material Appendix 4 Table A4).

Table 1. Effects of tree species diversity (SD) and big-leaf mahogany genotypic diversity (GD) on attack by specialists (S) stem-boring caterpillar and leaf-mining caterpillars, generalist (G) sap-feeding herbivores (leafhoppers), and spiders. Shown are F- (with numerator and denominator degrees of freedom) and p-values (in parenthesis). Significant ($p < 0.05$) results are in bold. Plant size = canopy volume (measured in m^3).

Predictor	Response variable			
	Stem borers (S)	Leaf miners (S)	Leafhoppers (G)	Spiders
SD	$F_{1,53} = 0.89$ (0.35)	$F_{1,54} = 8.26$ (0.006)	$F_{1,54} = 0.17$ (0.69)	$F_{1,54} = 0.73$ (0.39)
GD	$F_{1,53} = 0.22$ (0.63)	$F_{1,54} = 1.13$ (0.29)	$F_{1,54} = 0.01$ (0.99)	$F_{1,54} = 1.89$ (0.17)
SD \times GD	$F_{1,53} = 0.15$ (0.69)	$F_{1,54} = 0.006$ (0.99)	$F_{1,54} = 2.58$ (0.11)	$F_{1,54} = 0.45$ (0.50)
Time	$F_{2,105} = 64.61$ (< 0.0001)	–	–	–
Time \times SD	$F_{2,105} = 6.08$ (0.003)	–	–	–
Time \times GD	$F_{2,105} = 0.22$ (0.80)	–	–	–
Time \times SD \times GD	$F_{2,105} = 1.42$ (0.24)	–	–	–
Plant size	$F_{1,53} = 9.18$ (0.003)	$F_{1,54} = 22.17$ (< 0.0001)	$F_{1,54} = 1.67$ (0.20)	$F_{1,54} = 7.84$ (0.007)

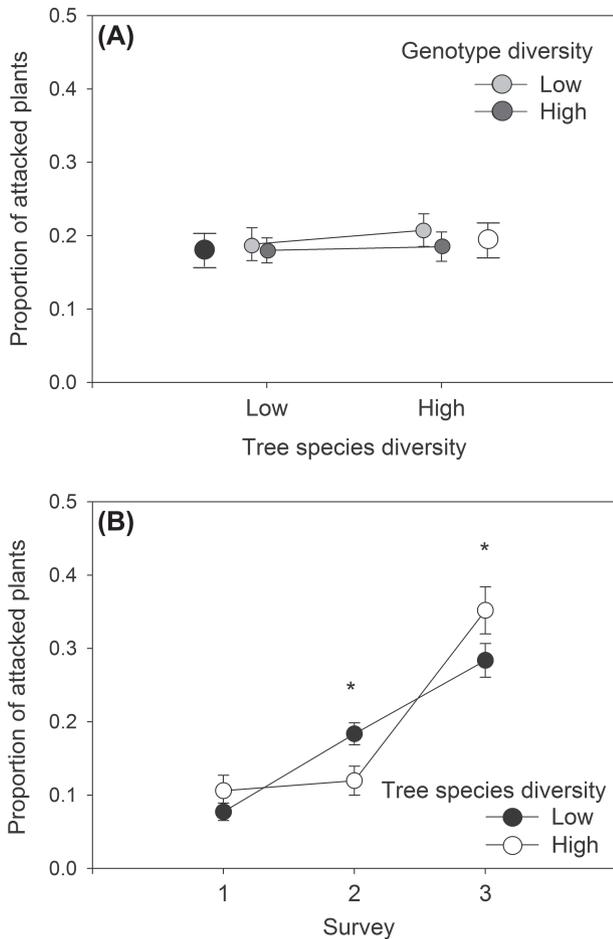


Figure 1. (A) Effects of big-leaf mahogany *Swietenia macrophylla* genotypic diversity and tree species diversity on the proportion of mahogany sapling attacked by the specialist stem-boring caterpillar *Hypsipyla grandella*. Grand least-square means \pm SE for each level of species diversity (i.e. mahogany monocultures and species polycultures) are shown on each side using a different color code to differentiate from levels of genotypic diversity (black = low species diversity; white = high species diversity; light grey = low genotypic diversity; dark grey = high genotypic diversity). Symbols are offset for purposes of visual clarity. (B) Effects of tree species diversity on the proportion of plants attacked by *H. grandella* shown separately for each of three surveys conducted in July, September, and October of 2012. For both panels, circles represent plot-level least-square means \pm SE from a repeated measures analysis accounting for plant size. For panel B, the asterisk indicates significant differences ($p < 0.05$) between levels of species diversity (mahogany monocultures and species polycultures) during a particular survey date.

Effects of plant diversity on predators

There was no evidence of either tree species diversity (monocultures = 0.70 ± 0.05 spiders; polycultures = 0.76 ± 0.06) or mahogany genotypic diversity (plots with one genotype = 0.78 ± 0.06 spiders; four genotypes = 0.68 ± 0.05) on spider abundance (Table 1). Likewise, the genotypic diversity \times species diversity interaction on spider abundance was not significant (Table 1).

Discussion

Our study provides insight into how herbivore species vary in their response to different sources of plant diversity (i.e.

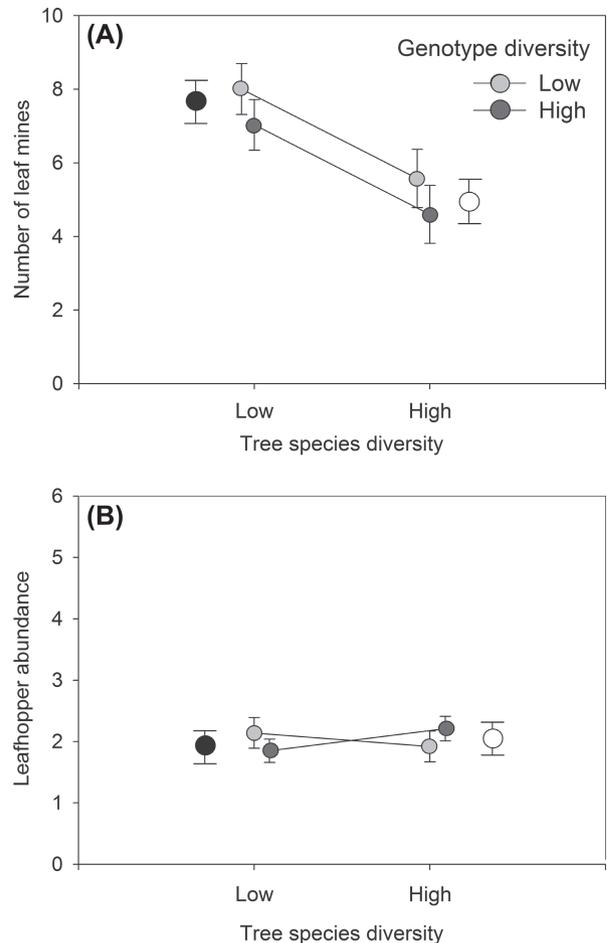


Figure 2. (A) Effects of tree species diversity and big-leaf mahogany (*Swietenia macrophylla*) genotypic diversity on the abundance of the specialist leaf-mining caterpillar *Phyllocnistis meliacella* attacking mahogany. (B) Effects of tree species diversity and big-leaf mahogany (*Swietenia macrophylla*) genotypic diversity on the abundance of generalist leafhoppers (Cicadellidae) feeding on mahogany (pooled data across three leafhopper species). Circles are plot-level least-square means \pm SE from a general linear model accounting for plant size. Symbols are offset for purposes of visual clarity. Grand least-square means \pm SE for each level of species diversity (mahogany monocultures and species polycultures) are shown on each side using a different color code to differentiate from levels of genotypic diversity (black = low species diversity; white = high species diversity; light grey = low genotypic diversity; dark grey = high genotypic diversity).

intra- and interspecific). Because of presumably weak plant-plant interactions at the time of insect monitoring, diversity effects on the specialist herbivores likely occurred not through increased mahogany growth via plant resource partitioning or facilitation, but rather through the effects of greater habitat heterogeneity. We found that specialist herbivores (i.e. immobile stem borers and leaf miners) were responsive to tree species but not to mahogany genotypic diversity, whereas generalist herbivores (i.e. mobile leafhoppers) were not influenced by either form of diversity. Because diversity effects were observed for specialists only, they likely operated directly on herbivore foraging behaviors (as predicted by the RCH) rather than indirectly through suppression by predators (as predicted by the EH). The lack of diversity effects on spider abundance further supports this interpretation.

Effects of tree species diversity on herbivores

Had the effects of diversity operated via natural enemies (according to the EH), we would expect an increase in predation or parasitism at high diversity which would have in turn led to stronger reductions in the abundance of generalist herbivores (relative to specialists) because these are generally more susceptible to natural enemies (Bernays 1998, Mooney et al. 2012, Singer et al. 2014). However, we found the opposite pattern, as only the specialists were influenced by tree species diversity. In addition, tree species diversity did not influence spider abundance suggesting that diversity effects on the specialist herbivores were not mediated by this predator functional group. Recent work conducted at the study plantation has similarly shown no effect of tree species diversity on parasitism associated with *P. meliacella* (mahogany monocultures: $49.1 \pm 11.2\%$; species polycultures: $42.9 \pm 9.2\%$; Abdala-Roberts unpubl.), and previous studies conducted in mahogany and Mexican cedar *Cedrela odorata* plantations located near our study site (Chan-Basto 2006) have found low parasitism rates for *H. grandella* (2 to 6%). Although these findings suggest that predators and parasitoids probably did not mediate the effect of tree species diversity on the specialist herbivores, we cannot entirely rule out their influence. Further work is necessary to assess whether natural enemies become a predominant force controlling herbivore populations in subsequent successional stages as observed in previous forest studies (Jactel and Brockerhoff 2007).

That species diversity affected specialist but not generalist herbivores is in keeping with the RCH, which predicts that specialists are more strongly influenced by plant diversity. For the two specialist herbivores, we found species diversity reduced *P. meliacella* leaf miner abundance (48%) and *H. grandella* stem borer abundance for the middle survey (55%). With respect to stem borers, we speculate that attack occurred in accordance to the mechanisms predicted by the RCH at the middle of the sampling season, with greater attack occurring at low diversity because of easier host location under higher densities of mahogany. This effect was non-additive supporting the idea that such diversity effect was mediated by emergent patch-level properties (i.e. resource heterogeneity). The subsequent reversal of this pattern may have been due to reduced host availability in the low-diversity plots (which exhibited greater attack rates earlier).

At the same time, our finding of generalist leafhoppers being insensitive to species diversity runs counter to results from several previous studies showing positive effects of plant species diversity on generalist herbivores (Loranger et al. 2014). These effects have been attributed to diet mixing (Singer et al. 2004, Unsicker et al. 2008), increased encounter rates with preferred hosts (Salazar et al. 2013), or greater plant biomass (Crutsinger et al. 2006, Cook-Patton et al. 2011). Our results are unresponsive of this last mechanism in particular because there were no effects of diversity on tree sapling growth. Accordingly, if plant diversity effects on generalist herbivores typically operate via increased plant biomass, this might explain or failure to detect such effects for leafhoppers.

The studied herbivore groups varied not only in diet breadth, but also in their degree of mobility; the specialist

caterpillars have relatively immobile larval stages whereas the generalist leafhoppers have highly mobile nymphs (in addition to adults). Dispersal ability can influence herbivore responses to plant diversity, with mobile herbivores responding more than sedentary herbivores because they can more readily disperse and choose among plant patches (Bommarco and Banks 2003). While we cannot separate the effects of diet breadth and mobility, had the latter predominated then this would have presumably led to a stronger effect of tree species diversity on (mobile) generalist leafhoppers which runs counter to our findings. This suggests that diet breadth (and not dispersal ability) was responsible for the reduction in damage by stem borers and leaf miners at high diversity. However, a robust evaluation of the linkage between plant diversity and herbivore diet breadth requires testing for effects on generalist and specialist species within the same taxon, as well as controlling for other factors such as feeding guild or feeding mode (Ali and Agrawal 2012, Plath et al. 2012).

Effects of plant genotypic diversity on herbivores

Despite using a mahogany parental tree sampling scheme that provided equal or greater variation than that found at the population level, and despite observing detectable variation among mahogany genotypes in growth and chemical defenses (Moreira et al. 2014; data from this study), we found no evidence of mahogany genotypic diversity effects on herbivores. This finding runs counter to previous work showing strong effects of plant genotypic diversity on arthropods (Crutsinger et al. 2006, Parker et al. 2010, McArt and Thaler 2013). Such discordant results are perhaps not surprising given that we did not find significant variation among mahogany genotypes for leafhopper or leaf miner abundance (only for stem borers). In addition, we speculate that genotypic diversity effects on consumers were weak because of the spatial scale of our experiment (Bommarco and Banks 2003) and how scale relates to the magnitude of plant trait variation. In particular, the magnitude of plant trait variation needed to manifest a diversity effect must increase with increasing spatial scale (relative to herbivore movement). Because heterogeneity in forest systems occurs at a greater spatial scale relative to past experiments with herbaceous species, our design may straddle this threshold such that the effects of tree species diversity were strong enough to matter at the studied scale whereas genotypic diversity effects were not.

Mahogany genotypic diversity effects may have also been weak because our study was conducted prior to genotypic diversity affecting producer biomass via plant-plant interactions (Crutsinger et al. 2006, Cook-Patton et al. 2011). This could be a particularly important factor in early successional forests dominated by widely-spaced tree saplings (Schuldt et al. 2011), where the effects of resource partitioning of plant biomass and of habitat heterogeneity on herbivores and predators take longer to emerge relative to systems dominated by herbaceous plants (Siemann 1998, Haddad et al. 2009). At the same time, however, herbivore effects during initial plant developmental stages can strongly influence subsequent plant growth and survival, and examinations such as ours of plant-herbivore interactions early in the establishment of forest systems are important from both a basic and an applied (forestry) perspective.

Concluding remarks

The effects of plant diversity in this study, particularly in the case of specialist herbivores, were likely caused by effects of habitat (plant-based) heterogeneity and not due to either positive effects of diversity on plant biomass or stronger top-down effects of predators. Within this context, our findings indicate that herbivore traits are important predictors of the effects of plant diversity on herbivore foraging behavior. Accordingly, we speculate that the effects of plant diversity on higher trophic levels may vary among systems depending on the degree of dietary specialization and mobility within key life stages of the dominant herbivores in each community. In addition, whereas plant species diversity was expected to have stronger effects on herbivores relative to plant genotypic diversity, comparisons of these sources of diversity might depend upon the spatial scale of analysis. Overall, this study shows that resource heterogeneity effects on consumer behaviors represent a prevalent mechanism of plant diversity effects on herbivores, and that our ability to predict plant diversity–herbivory relationships depends on linkages between the magnitude of plant trait variation and herbivore traits.

Acknowledgements – The authors would like to thank N. Salinas, R. Mena, V. Solís, P. Medina, A. Durán, V. Hernández and D. Marrufo for field assistance. To J. Berny-Mier y Teran for the initial motivation to develop this experiment, and to R. Centeno and R. Rivera for advice on seed germination methods and for providing accommodations for seed germination and seedling establishment. X. Moreira provided valuable comments during manuscript preparation. J. Quinto and E. Reyes kindly helped with the identification of leaf miner and leafhopper specimens, respectively. This research was supported by a CONACyT grant awarded to VPT (128856). INIFAP provided logistic accommodations and infrastructure for the establishment of the experiment, subsequent sampling and maintenance. LAR was funded by a GAANN fellowship through the Univ. of California-Irvine and a UCMEXUS-CONACyT fellowship. KAM was supported by NSF DEB 1120794, and KAM and VPT were supported by a UCMEXUS collaborative grant (UCM-55592).

References

- Abdala-Roberts, L. and Mooney, K. A. 2014. Ecological and evolutionary consequences of plant genotype diversity in a tri-trophic system. – *Ecology* 95: 2879–2893.
- Albert, H. C. et al. 2010. A multi-trait approach reveals the structure and the relative importance of intra- vs interspecific variability in plant traits. – *Funct. Ecol.* 24: 1192–1201.
- Ali, J. G. and Agrawal, A. A. 2012. Specialist versus generalist insect herbivores and plant defense. – *Trends Plant Sci.* 17: 293–302.
- Andow, D. A. 1991. Vegetational diversity and arthropod population response. – *Annu. Rev. Entomol.* 36: 561–586.
- Arguedas, M. 2007. Plagas y enfermedades forestales en Costa Rica. – *Kurú Revista Forestal* 4.
- Bailey, J. et al. 2009. From genes to ecosystems: a synthesis of the effects of plant genetic factors across levels of organization. – *Phil. Trans. R. Soc.* 364: 1607–1616.
- Barbosa, P. et al. 2009. Associational resistance and associational susceptibility: having right or wrong neighbors. – *Annu. Rev. Ecol. Evol. Syst.* 40: 1–20.
- Becker, O. B. 1976. Microlepidópteros asociados con *Carapa*, *Cedrela* y *Swietenia* en Costa Rica. – In: Whitmore, L. (ed.), *Studies on the shootborer *Hypsipyla grandella* (Zeller) Lep. Pyralidae*. Volume II. CATIE, Costa Rica, pp. 75–101.
- Bernays, E. A. 1998. Evolution of feeding behavior in insect herbivores: success seen as different ways to eat without being eaten. – *Bioscience* 48: 35–44.
- Bommarco, R. and Banks, J. E. 2003. Scale as modifier in vegetation diversity experiments: effects on herbivores and predators. – *Oikos* 102: 440–448.
- Castagneyrol, B. et al. 2013. Plant apparency, and overlooked driver of associational resistance to insect herbivory. – *J. Ecol.* 101: 418–429.
- CICY (Centro de Investigación Científica de Yucatán) 2010. Flora de la Península de Yucatán. – Catálogo de Flora, Yucatán, México. <www.cicy.mx/sitios/flora%20digital/index.php>, accessed 8 April 2014.
- Chan-Basto, F. 2006. Parasitismo de *Hypsipyla grandella* Zeller (Lepidoptera: Pyralidae), Barrenador de las Meliáceas en Plantaciones de Cedro (*Cedrela odorata* L.) en Yucatán. – Masters thesis, Univ. Autónoma de Yucatán, México.
- Cook-Patton, S. C. et al. 2011. A direct comparison of the consequences of plant genotypic and species diversity on communities and ecosystem function. – *Ecology* 92: 915–923.
- Crawford, K. M. and Rudgers, J. A. 2013. Genetic diversity within a dominant plant outweighs plant species diversity in structuring an arthropod community. – *Ecology* 94: 1025–1035.
- Crutsinger, G. M. et al. 2006. Plant genotypic diversity predicts community structure and governs an ecosystem process. – *Science* 313: 966–968.
- Gillies, A. C. M. et al. 1999. Genetic diversity in Mesoamerican populations of mahogany (*Swietenia macrophylla*), assessed using RAPDs. – *Heredity* 83: 722–732.
- Grogan, J. et al. 2003. Regeneration of big-leaf mahogany in closed and logged forests of southeastern Pará, Brazil. – In: Lugo, A. E. et al. (eds), *Big-leaf mahogany. Genetics, ecology and management*. Springer, pp. 193–208.
- Haddad, N. et al. 2009. Plant species loss decreases arthropod diversity and shifts trophic structure. – *Ecol. Lett.* 12: 1029–1039.
- Hare, J. D. 2002. Plant genetic variation in tritrophic interactions. Multitrophic level interactions. – In: Tscharrntke, T. and Hawkins, B. A. (eds), *Multitrophic level interactions*. Cambridge Univ. Press, pp. 8–43.
- Houston, M. A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. – *Oecologia* 110: 449–460.
- Jactel, H. and Brockerhoff, E. G. 2007. Tree diversity reduces herbivory by forest insects. – *Ecol. Lett.* 10: 835–848.
- Johnson, M. T. J. et al. 2006. Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. – *Ecol. Lett.* 9: 23–34.
- Koricheva, J. et al. 2000. Numerical responses of different trophic groups of invertebrates to manipulations of plant diversity in grasslands. – *Oecologia* 125: 271–282.
- Loranger, H. et al. 2014. Invertebrate herbivory increases along an experimental gradient of grassland plant diversity. – *Oecologia* 174: 183–193.
- Loveless, M. D. and Gullison, R. E. 2003. Genetic variation in natural mahogany populations in Bolivia. – In: Lugo, A. E. et al. (eds), *Big-leaf mahogany. Genetics, ecology and management*. Springer, pp. 9–28.
- Maes, P. J. and Godoy, C. 1993. Catálogo de los Cicadellidae (Homoptera) de Nicaragua. – *Rev. Nicarag. Entomol.* 24: 5–34.
- McArt, S. H. and Thaler, J. S. 2013. Plant genotypic diversity reduces the rate of consumer resource utilization. – *Proc. R. Soc. B* 280: 20130639.
- Mo, J. et al. 1997. Within-tree distribution of attack by *Hypsipyla robusta* Moore (Lepidoptera: Pyralidae) in Australian red cedar

- (*Toona australis* (F. Muell) Harmes). – For. Ecol. Manage. 96: 147–154.
- Mooney, K. A. and Singer, M. S. 2012. Plant effects on herbivore–enemy interactions in natural systems. – In: Ohgushi, T. et al. (eds), Trait-mediated indirect interactions: ecological and evolutionary perspectives. Cambridge Univ. Press, pp. 107–130.
- Mooney, K. A. et al. 2012. The tri-trophic interactions hypothesis: interactive effects of host plant quality, diet breadth and natural enemies on herbivores. – PLoS ONE 7: e34403.
- Moreira, X. and Mooney, K. A. 2013. Influence of plant genetic diversity on interactions between higher trophic levels. – Biol. Lett. 9: 20130133.
- Moreira, X. et al. 2014. Positive effects of plant genotypic and species diversity on anti-herbivore defences in a tropical tree species. – PLoS ONE 9: e105438.
- Newton, A. C. et al. 1993. The mahogany shoot borer: prospects of control. – For. Ecol. Manage. 57: 301–328.
- Norghauer, J. M. et al. 2010. Long-distance dispersal helps germinating mahogany seedlings escape defoliation by a specialist caterpillar. – Oecologia 162: 405–412.
- Parker, J. D. et al. 2010. Herbivory enhances positive effects of plant genotypic diversity. – Ecol. Lett. 13: 553–563.
- Pennington, T. and Sarukhán, J. 2005. Árboles Tropicales de México. Tercera edición. – Fondo de Cultura Económica. México.
- Plath, M. et al. 2012. Associational resistance and associational susceptibility: specialist herbivores show contrasting responses to tree stand diversification. – Oecologia 169: 477–487.
- Root, R. B. 1973. Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). – Ecol. Monogr. 43: 95–124.
- Russell, E. P. 1989. Enemies hypothesis: a review of the effect of vegetational diversity on predatory insects and parasitoids. – Environ. Entomol. 18: 590–599.
- Salazar, D. et al. 2013. Directed seed dispersal of *Piper* by *Carollia perspicillata* and its effect on understory plant diversity and folivory. – Ecology 94: 244–253.
- Sánchez-Soto, S. et al. 2009. Efecto de la sombra en plantas de caoba sobre la incidencia de *Hypsipyla grandella* Zeller y otros insectos, en Tabasco, México. – Univ. Ciencia 3: 225–232.
- Schuldt, A. et al. 2011. Predator diversity and abundance provide little support for the enemies hypothesis in forests of high tree diversity. – PLoS ONE 6: e22905.
- Siemann, E. 1998. Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. – Ecology 79: 2057–2070.
- Singer, M. S. et al. 2004. Roles of food quality and enemy-free space in host use by a generalist insect herbivore. – Ecology 85: 2747–2753.
- Singer, M. S. et al. 2014. Herbivore diet breadth mediates the cascading effects of carnivores in food webs. – Proc. Natl Acad. Sci. USA 111: 9521–9526.
- Taveras, R. et al. 2004. Population trends and damage patterns of *Hypsipyla grandella* (Lepidoptera: Pyralidae) in a mahogany stand, in Turrialba, Costa Rica. – Agric. For. Entomol. 6: 89–98.
- Tilman, D. et al. 2002. Plant diversity and composition: effects on productivity and nutrient dynamics of experimental grasslands. – In: Loreau, M. et al. (eds), Biodiversity and ecosystem functioning: synthesis and perspectives. Oxford Univ. Press, pp. 21–35.
- Underwood, N. et al. 2014. A conceptual framework for associational effects: when do neighbors matter and how would we know? – Q. Rev. Biol. 89: 1–19.
- Unsicker, S. B. et al. 2008. Complementarity effects through dietary mixing enhance the performance of a generalist insect herbivore. – Oecologia 156: 313–324.
- Vehviläinen, H. et al. 2007. Tree species diversity influences herbivore abundance and damage: meta-analysis of long-term forest experiments. – Oecologia 152: 287–298.
- Young, D. A. 1968. Taxonomic study of the Cicadellinae (Homoptera: Cicadellidae). Part 1 Proconiini. US Natl Mus. Bull. 261: 1–287. Smithsonian Inst., Washington D. C.

Supplementary material (available online as Appendix oik.02033 at <www.oikosjournal.org/readers/appendix>). Appendix 1–5.