UvA-DARE (Digital Academic Repository)

Parasitoids follow herbivorous insects to a novel host plant, generalist predators less so

Grosman, A.H.; Holtz, A.M.; Pallini, A.; Sabelis, M.W.; Janssen, A.

DOI

[10.1111/eea.12545](https://doi.org/10.1111/eea.12545)

Publication date

2017

Document Version

Final published version

Published in

Entomologia Experimentalis et Applicata

License

Article 25fa Dutch Copyright Act

[Link to publication](#)

Citation for published version (APA):

Grosman, A. H., Holtz, A. M., Pallini, A., Sabelis, M. W., & Janssen, A. (2017). Parasitoids follow herbivorous insects to a novel host plant, generalist predators less so. *Entomologia Experimentalis et Applicata*, 162(3), 261-271. <https://doi.org/10.1111/eea.12545>

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (<https://dare.uva.nl>)

Parasitoids follow herbivorous insects to a novel host plant, generalist predators less so

Amir H. Grosman^{1§}, Anderson Mathias Holtz^{2§§}, Angelo Pallini², Maurice W. Sabelis^{1#} & Arne Janssen^{1*}

¹Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands, and ²Department of Entomology, Federal University of Viçosa, Minas Gerais, Brazil

Accepted: 18 January 2017

Key words: host shift, enemy-free space, Lepidoptera, community structure, parasitism, tritrophic interactions, guava, eucalyptus, Myrtaceae, *Thyriniteina leucoceraea*, Geometridae

Abstract

The ‘enemy-free space’ hypothesis predicts that herbivorous insects can escape their natural enemies by switching to a novel host plant, with consequences for the evolution of host plant specialisation. However, if natural enemies follow herbivores to their novel host plants, enemy-free space may only be temporary. We tested this by studying the colonisation of the introduced tree *Eucalyptus grandis* (Hill) Maiden (Myrtaceae) by insects in Brazil, where various species of herbivores have added eucalyptus to their host plant range, which consists of native myrtaceous species such as guava. Some herbivores, for example, *Thyriniteina leucoceraea* Ringe (Lepidoptera: Geometridae), cause outbreaks in eucalyptus plantations but not on guava, possibly because eucalyptus offers enemy-free space. We sampled herbivores (mainly Lepidoptera species) and natural enemies on eucalyptus and guava and assessed parasitism of Lepidoptera larvae on both host plant species during ca. 2 years. Overall, predators were encountered more frequently on guava than on eucalyptus. In contrast, parasitoids were encountered equally and parasitism rates of Lepidoptera larvae were similar on both host plants. This indicates that herbivores may escape some enemies by moving to a novel host plant. However, this escape may be temporary and may vary with time. We argue that studying temporal and spatial patterns of enemy-free space and the response of natural enemies to host use changes of their herbivorous prey is essential for understanding the role of natural enemies in the evolution of host plant use by herbivorous arthropods.

Introduction

The effect of natural enemies on patterns of host plant use by herbivorous insects has received considerable attention in the past decades (Price et al., 1980; Bernays & Graham, 1988; Ehrlich & Murphy, 1988; Thompson, 1988; Berdegue et al., 1996; Singer & Stireman, 2005). According to the ‘enemy-free space’ (EFS) hypothesis, herbivorous

insects may escape from their natural enemies when switching to a novel plant species (Jeffries & Lawton, 1984; Berdegue et al., 1996). Whereas herbivorous arthropods may experience lower performance when colonising a novel host plant (Gould, 1979; Thompson, 1996; Grosman et al., 2015), the associated fitness reduction may be compensated by lower predation and parasitism on this plant, thereby facilitating its colonisation. Although this idea was much criticised initially (Ehrlich & Murphy, 1988; Rausher, 1988; Schultz, 1988; Thompson, 1988), the fact that herbivore vulnerability to natural enemies varies consistently between host plant species (Lill et al., 2002) and the notion that predation risk may play a role in host plant selection has been recognised since (Ohsaki & Sato, 1994; Stamp, 2001). Several studies indeed demonstrate that by shifting to a novel host plant, herbivores may escape from their enemies (Feder, 1995; Gratton & Welter, 1999; Ballabeni et al., 2001; Mulatu et al., 2004; Murphy, 2004; Diamond & Kingsolver, 2010; Meijer et al., 2016). It is less

*Correspondence: Arne Janssen, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands. E-mail: arne.janssen@uva.nl

#In memory of Maurice Sabelis, who sadly passed away before the final version of this ms was prepared.

§Present address: Bio-Bee Sde Eliyahu LTD, Bet Shean Valley 10810, Israel

§§Present address: Federal Institute of Espírito Santo, Campus Itapina, Espírito Santo, Brazil

well-studied, however, whether natural enemies can ‘catch up’ with the herbivores on the novel host plant after many generations. Gratton & Welter (1999) demonstrated that a leaf miner was attacked less by parasitoids on novel host plants than on native hosts, but this effect varied among years. Hartley et al. (2010) showed that novel host plants had a lower associated abundance of parasitoids, but not of predators. If natural enemies indeed follow herbivores to novel host plants, EFS is a temporary ‘window of opportunity’ (Gratton & Welter, 1999) and its effect on the evolution of host plant use may be constrained.

Natural enemies may benefit from tracking herbivores to a novel host plant. With the build-up of populations of herbivores on novel host plants, any predator that would start exploiting these populations would escape from competition with con- and heterospecifics, and would thus experience initial fitness gains. In fact, it would offer competitor-free space (Messing & Wang, 2009). However, there are several potential reasons for the absence or ineffectiveness of natural enemies on novel host plants. First, the localisation of prey on novel host plants may not be easy because foraging efficiency may be compromised: the blends of volatiles produced by novel host plants in response to attack by the herbivore may not be recognised as cues by the natural enemy complex. Yet, selection for attraction of predators by volatiles can be rapid (Margolies et al., 1997) and predators can learn to respond to volatiles of novel host plants (Papaj & Lewis, 1993; Turlings et al., 1993; Vet et al., 1995; Drukker et al., 2000; Takabayashi et al., 2006; Janssen et al., 2014). Second, natural enemies may have difficulties in consuming prey on a novel host plant because herbivores use toxins from their host plants for their own protection (Smiley et al., 1985; Denno et al., 1990; Rank, 1994; Nishida, 2002; Singer et al., 2004a,b; Zvereva & Rank, 2004). However, the sequestration of secondary plant compounds may require a complex scenario of adaptation of the herbivores (Nishida, 2002). Thus, natural enemies that follow the switch of their prey may often continue feeding on what is essentially the same diet. Third, the novel host plants may not provide the natural enemies with protection against adverse conditions or hyperpredators (Walter & O’Dowd, 1992; Matos et al., 2006; Ferreira et al., 2011), or alternative food such as pollen or nectar (Koptur, 1992). Such alternative food can sustain a population of natural enemies even in the absence of herbivorous prey, which will lead to higher predator/prey ratios and subsequent lower herbivore levels (Sabelis et al., 2005). Yet, if neighbouring plants do provide domatia and alternative food, the effect of their absence on a novel host plant may be mitigated. Thus, the hypothetical hurdles that natural enemies may face when switching to novel host plants, may in reality not be much of a hindrance.

In Brazilian eucalyptus plantations, a huge unplanned experiment is taking place, which is ideally suited to study the colonisation of novel host plants by herbivorous insects and their natural enemies. Some 10 species of *Eucalyptus* (Myrtaceae) were introduced from Australia into southern Brazil since 1905 (Reis & Reis, 1993) and eucalyptus forests now cover about 7 million ha (Instituto Brasileiro de Geografia e Estatística, 2014), with large monoculture stands resulting in reduced biodiversity and ecosystem services (Bragança et al., 1998b; Fagundes et al., 2011; Gerber, 2011). Eucalyptus foliage is renowned for its variety of plant secondary metabolites (Marsh et al., 2003; Burchfield et al., 2005; Wiggins et al., 2006) and it was therefore expected that eucalyptus would be well-protected from herbivores in Brazil. However, many insect herbivores, mainly moths (Lepidoptera) but also beetles (Coleoptera), leaf-cutter ants (Hymenoptera), and termites (Isoptera), readily colonised eucalyptus and have become serious pests of this wood crop in the past century (Pereira et al., 1994; Zanuncio et al., 1994; Bragança et al., 1998a,b; Ribeiro et al., 2005). Nowadays, *Thyrinitea leucoceraea* Ringe (Lepidoptera: Geometridae) is considered one of the major pests of eucalyptus and frequently shows outbreaks in eucalyptus plantations (Zanuncio et al., 1994). Outbreaks have not been reported on native host plants of this insect, such as guava, *Psidium guajava* L. (Myrtaceae). Holtz et al. (2003) suggested that this may be caused by higher levels of plant defences in guava than in eucalyptus. Indeed, they found a somewhat higher performance of *T. arnobia*, a close relative of *T. leucoceraea*, on eucalyptus than on guava, but the herbivore had been collected from eucalyptus and may therefore have lost its adaptation to guava (Grosman et al., 2015). Unfortunately, a comparison with a strain of *T. arnobia* from guava was missing, which might have shown that such a strain has higher performance on guava.

Lack of control of *T. leucoceraea* and other pest species by natural enemies in eucalyptus plantations suggests that herbivores acquire EFS on this novel host plant. We tested whether natural enemies have difficulties in following Lepidoptera to eucalyptus by comparing the densities of herbivores and natural enemies on guava and eucalyptus trees occurring in adjacent areas. We also compared the rates of parasitism of Lepidoptera larvae on both host plants.

Materials and methods

Field sites

We sampled insect communities and collected caterpillars in two areas with guava trees (ca. 2 550 and 6 100 m²) and two areas with eucalyptus, *Eucalyptus grandis* (Hill)

Maiden (ca. 4 150 and 9 100 m²). The areas were at a distance of 0.5–2 km from each other on the campus of the Federal University of Viçosa, Minas Gerais, Brazil (20°45'S, 42°51'W; Grosman et al., 2015). Eucalyptus is common in the study area and was introduced around 80 years ago (Prof. Acelino Couto Alfenas, pers. comm.). Neither of the areas had other tree species, and the main vegetation covering the soil consisted of grasses, but the areas were surrounded by more diverse native vegetation. One of the guava areas was managed organically whereas the other areas were not managed, except for the removal of a few trees from the eucalyptus areas. Sampling and collection started on January 2004 in one guava and one eucalyptus area and 3 months later in one additional area for each of the two plant species. Except for an interruption from October 2004 to February 2005, the field study continued until February 2006. In one eucalyptus area, however, sampling had to be stopped early, because all trees were logged in June 2005.

Densities of herbivores and natural enemies

To study the densities of Lepidoptera and their natural enemies on eucalyptus and guava trees, weekly observations were carried out in areas of each of the plant species. On each observation day, six randomly chosen trees were searched for insects for 20 min on average, excluding the time to process insects. The numbers of Lepidoptera and their natural enemies were scored for each of the trees. Observations alternated between host species, each area was usually visited once per 2 weeks. The height of the guava trees did not exceed 4 m, whereas the eucalyptus trees had a maximum height of ca. 15 m. Only the branches that were accessible from the ground were inspected. We searched for insects on the branches and leaves by observation. We used this method because it minimised the disturbance of insects and thus allowed us to observe insects that would fly off if we had sampled by shaking the branches and collected the falling insects. Moreover, it allowed for repeated and frequent sampling in the same area, which would not have been possible if we sampled by spraying insecticides to collect insects.

The predator species that were included in the study were those observed attacking Lepidoptera eggs, larvae, or pupae in the field sites. These included mantids (Mantidae), predatory wasps (Hymenoptera), predatory bugs (Heteroptera), lacewing larvae (Chrysopidae), ants (Formicidae), spiders (Araneae), and grasshoppers (Orthoptera). Of ants and spiders, we recorded the presence/absence data per tree. All species of parasitic wasps and Lepidoptera encountered were included in the study. Lepidoptera included *T. leucoceraea* and five other

geometrid species, three tortricid species (caterpillars that construct shelters by connecting one or several leaves with silken threads), *Eupseudosoma* spp. (Arctiidae), *Blera varana* Schaus (Notodontidae), and a minority of unidentified species. Other herbivores that were included were Thysanoptera and Hemiptera, of which presence/absence data were collected per tree.

In total, there were 511 observations of guava trees and 434 of eucalyptus trees, which do not correspond to the number of individual trees involved, as trees were inspected more than once. However, we avoided inspecting the same trees successively. To calculate the densities of herbivores, predators, and parasitoids, we divided the numbers observed (or presence/absence) by the time spent searching the trees. We subsequently averaged these densities per observation date and per area (i.e., the average of six trees per observation date). These average densities were used for statistical analysis.

Lepidoptera parasitism

We estimated the rates of parasitism of lepidoptera caterpillars on guava and eucalyptus by collecting them during the field observations (see previous section) and rearing the collected caterpillars in the laboratory. Caterpillars were reared individually in cups (500 ml) kept at ambient temperature and natural light conditions (25 ± 5 °C, L13:D11 photoperiod). The cups contained small twigs of the host plant as food, which were inserted into moist vermiculite on the bottom of the cup to maintain leaf turgor (Grosman et al., 2015). New twigs were added twice per week until the caterpillars pupated or died owing to parasitism or other causes. Pupae of Lepidoptera and parasitoids were incubated in similar cups and the emergence of adult moths and parasitoids was scored. It was unknown whether caterpillars and pupae that died in the laboratory were parasitised, as they were not dissected after death. These caterpillars were therefore omitted from the analysis. Caterpillar death occurred with comparable frequencies among caterpillars from the two host plants (guava, 29.5%; eucalyptus, 34.5%). Caterpillars were collected approximately once every 15 days from each of the areas described above.

The majority of the 1 282 caterpillars collected concerned species that occurred on both host plants: *T. leucoceraea* (60% of collected caterpillars), Tortricidae (18%), *B. varana* (2%), and *Eupseudosoma* spp. (1%). Of the remaining unidentified collected species (19%), several species, among which a few geometrid species, occurred only on one of the two host plant species. Tortricidae were almost never parasitised (four of the 247 caterpillars collected) and were therefore omitted from the analysis. The remaining Lepidoptera were divided in '*T. leucoceraea*'

and ‘other species’ as not enough specimens of the latter species were collected to justify a species-wise analysis.

Statistical analysis

Because none of the commonly used error structures resulted in a good error distribution, data (densities and rates of parasitism) were $\sqrt{(y+0.1)}$, $\sqrt{(y+0.01)}$, or $\sqrt{(y+0.001)}$ transformed before further analysis. The densities and rates of parasitism varied irregularly with time, and we therefore first analysed the data with a generalised additive model (GAM of the *mgcv* package in R Wood, 2004, R version 3.1.0) with time as smoothed predictor variable, host plant species as fixed factor, and a Gaussian error distribution. The error distribution and model fit was checked with the *gam.check* function of the same package, and the dimension of the smooth term was adjusted when needed. We tested for significant effects of host species through the *summary* function of GAM, subsequently removed host species from the model, and compared this reduced model with the full model with Akaike’s Information Criterion (AIC) and an analysis of deviance (*anova* function of R). The effect of time was highly significant in all analyses ($P < 0.0001$), and is not reported separately for each analysis below. We analysed the data of those groups and species that were encountered frequently.

We also used linear-mixed effects models (LME of the *nlme* package in R; Pinheiro et al., 2014) with time, host species, and their interaction as factors, and area as random factor, and compared their AICs with those of the GAM models. We assessed the significance of the effect of the interaction of time and host species by comparing models with and without this interaction with a likelihood ratio test (*anova* function of R; Crawley, 2013). We used general linear hypothesis testing to compare the two host plant species (function *glht* of the package *lsmeans*; Lenth, 2016). Below, we present the results of both analyses. Parasitism data of Lepidoptera species other than *T. leucoceraea* were too haphazard to analyse as above; they were therefore analysed with a simple generalised linear model (GLM) with host plant species as factor, hence, omitting temporal variation.

Results

Densities of herbivores and natural enemies

Most herbivores occurred on both host plant species (Figure 1). All Lepidoptera together occurred in similar densities on both host plant species with somewhat higher densities on eucalyptus during the first 200 days (Figure 1A), causing a significant effect of host plant with the GAM analysis (Table 1). *Thyrintina*

Table 1 Effect of plant species and time on densities of the most abundant groups of herbivores and natural enemies on guava and eucalyptus trees

Group or species	GAM ¹	LME ²	
	Host	Host × time	Host
Herbivores			
Lepidoptera	3.32 (1.05)*	1.72 ns	1.21 ns
<i>Thyrinteina leucoceraea</i>	2.11 (0.99) ns	2.88 ns	0.20 ns
Other Geometridae	10.9 (1.99)***	21.5***	5.71 *
Tortricidae	0.04 (0.96) ns	17.5***	0.68 ns
Thysanoptera	18.1 (1.03)***	0.43 ns	5.73*
Homoptera	2.71 (1.02)**	1.24 ns	5.73*
Natural enemies			
Parasitoids	2.07 (1.17)*	0.93 ns	1.42 ns
All predators	1.03 (1.14)**	5.07*	2.49 ns
Ants	0.69 (1.91)***	19.1***	1.15 ns
Spiders	0.15 (1.07)**	7.83**	0.47 ns
Heteroptera	0.55 (1.01) ns	0.26 ns	0.53 ns
Predatory wasps	0.06 (0.97) ns	4.76*	0.01 ns

¹For the effect of host plant analysed with generalised additive models (GAM), the deviance (estimated d.f. in parentheses) and significance are given based on a comparison of models with and without host plant species (Host), models were also compared with Akaike’s Information Criterion (AIC).

²For linear-mixed effects models (LME), the significance of the interaction between host plant species and time was assessed by comparing models with and without this interaction. The *glht* function of the *lsmeans* package was used to assess significant differences between host plants. Given are the t-values and their significance. Per group or species, the model with the best fit (based on AIC) is given in bold.

Significance: *0.005 < P < 0.05, **0.0001 < P < 0.005, ***P < 0.0001; ns, not significant (P > 0.05).

leucoceraea, the most abundant lepidopteran species, occurred in similar densities on both host plants (Table 1, Figure 1B). Likewise, Tortricidae occurred overall in similar densities on both host plants, but more on guava than on eucalyptus during the first 200 days, causing a significant effect of the interaction between host plant species and time (Table 1, Figure 1C). The other Geometridae (i.e., not *T. leucoceraea*) occurred in higher densities on eucalyptus, but not so much towards the end of the study (Table 1, Figure 1D). Thysanoptera occurred more frequently on the native host guava than on the introduced host throughout the sampling period (Table 1, Figure 1E). Hemiptera tended to occur in higher densities on eucalyptus than on guava (Table 1, Figure 1F). The densities of all herbivores varied significantly through time (Table 1, Figure 1). In conclusion, the most abundant herbivores

Figure 1 Densities (numbers found per min searching a tree) of herbivores on guava (closed circles, drawn line) and eucalyptus trees (open circles, broken line) as a function of time. Each point is the average density on six trees. (A) All Lepidoptera, (B) *Thyriniteina leucoceraea*, (C) Tortricidae, (D) other Geometridae than *T. leucoceraea*, (E) Thysanoptera, and (F) Hemiptera. Curves were fit for each host plant species separately with a generalised additive model with time as smoothed predictor. See Table 1 for significance of differences in densities.

from guava also occur on the novel host plant eucalyptus.

The densities of parasitoids varied significantly through time, but did not differ significantly between host plants (Table 1, Figure 2A). The analysis with general additive models suggests that parasitoids occurred somewhat more on eucalyptus than on guava (Table 1, Figure 2A). In contrast, the density of predators was significantly lower on eucalyptus than on guava (Table 1, Figure 2B). The interaction between time and host plant species was also

significant (Table 1) because predator densities were equally low on both host plants during the second part of the study period (Figure 2B). The difference in predator occurrence on eucalyptus and guava was mainly caused by the presence or absence of ants (excluding leaf-cutter ants) and spiders, which were more abundant on guava than on eucalyptus (Table 1, Figure 2C and D), although not consistently through time. Densities of Heteroptera and predatory wasps did not differ significantly between the two host plants (Table 1, Figure 2E and F). The densities

Figure 2 Densities (numbers found per min searching a tree) of predators and parasitoids on guava (closed circles, drawn line) and eucalyptus trees (open circles, broken line) as a function of time. Each point is the average density on six trees. (A) Parasitoids, (B) all predators, (C) ants, (D) spiders, (E) Heteroptera, and (F) predatory wasps. Curves were fit for each host plant species separately with a generalised additive model with time as smoothed predictor. See Table 1 for significance of differences in densities.

of these last four groups of predators varied significantly through time, often asynchronously on the two host plant species (Table 1, Figure 2C–F).

Lepidoptera parasitism

The rates of parasitism of Lepidoptera did not differ between the two host plant species, but varied significantly throughout the sampling period (Table 2, Figure 3A). A similar pattern was found for *T. leucoceraea* (Table 2, Figure 3B). A gregarious endoparasitoid from the genus *Glyptapanteles* (Hymenoptera: Braconidae) attacked *T. leucoceraea* on both host plant species (Grosman et al.,

2008; Janssen et al., 2010). The remaining parasitoid species attacking *T. leucoceraea* are not yet identified, but judged by their general morphological appearance there were two species, both found on the two host plants. One of the species is a gregarious ectoparasitoid, normally killing the third host instar. The other is a solitary endoparasitoid species, killing the fourth or fifth host instar.

The low numbers of caterpillars of other Lepidoptera species collected did not allow for a more in-depth analysis of parasitism through time, but on average, the incidence of parasitism in this group was (mean $\pm$ SE =) 0.12 ± 0.0037 on guava and 0.2 ± 0.0058 on eucalyptus;

Table 2 Effect of plant species and time on rates of parasitism of all Lepidoptera and the most abundant species *Thyriniteina leucoceraea* on guava and eucalyptus trees

Group or species	GAM ¹ Host	LME ²	
		Host × time	Host
Lepidoptera	0.18 (1.00) ns	1.37 ns	0.00 ns
<i>T. leucoceraea</i>	0.69 (0.97) ns	0.94 ns	0.00 ns

¹For the effect of host plant analysed with generalised additive models (GAM), the deviance (estimated d.f. in parentheses) and significance are given based on a comparison of models with and without host plant species (Host), models were also compared with Akaike's Information Criterion (AIC).

²For linear-mixed effects models (LME), the significance of the interaction between host plant species and time was assessed by comparing models with and without this interaction. The glht function of the lsmeans package was used to assess significant differences between host plants. Given is the t-value and its significance. Per group or species, the model with the best fit (based on AIC) is given in bold. ns, not significant ($P > 0.05$).

this difference was not significant (GLM with binomial error distribution: deviance = 0.66, d.f. = 1, $P = 0.42$).

Discussion

We investigated the occurrence of EFS for herbivores on the novel host tree eucalyptus compared with the native host tree guava. In general, most herbivores occurring on guava also occurred on eucalyptus, of which Hemiptera and Geometridae (except *T. leucoceraea*) occurred in higher densities on eucalyptus than on guava. Thysanoptera were the only taxon that occurred more frequently

on the native host plant. All the natural enemy species attacking Lepidoptera on guava have colonised eucalyptus, but not all of them followed the herbivores to the same extent. In periods with high densities of predators, densities were lower on the novel host plant than on the native host. When predator densities were low, the densities on both host plants differed less, resulting in a significant effect of the interaction between host plant species and time. In contrast, densities of parasitoids did not differ significantly between host plants, and there was a trend towards higher densities on the novel host plant. Of the most abundant predators, ants and spiders were encountered more often on guava than on eucalyptus, whereas densities of predatory wasps and Heteroptera did not differ significantly between the two host plants. Parasitism of Lepidoptera larvae was similar on the novel and the native host plants, which is in agreement with the similar densities of parasitoids encountered on both host plants. Thus, parasitoids followed the moth species to eucalyptus, but the density of most predatory insects was higher on guava. However, higher predator densities do not necessarily result in higher predation risk in the short term. There is a clear need for an estimate of predation risks of the various life stages of the herbivores on the novel and native plants – this will be the subject of a future publication.

Eucalyptus is thus not completely free of enemies of the herbivores, and should therefore be considered as 'enemy-scarce space' (sensu Takabayashi et al., 2006) relative to the native host plant. Our findings are in contrast with another study, which found that an introduced tree species harboured fewer herbivore species, fewer parasitoids, and no difference in predator abundance compared with the native trees (Hartley et al., 2010). The novel trees in this

Figure 3 Average rates of parasitism of caterpillars of (A) all Lepidoptera and (B) *Thyriniteina leucoceraea* through time. Caterpillars were collected from guava trees (closed circles) or eucalyptus trees (open circles) in the field and reared to adulthood or until parasitoids emerged. Points are average parasitism rates per collection date. Curves were fit for each host plant species separately with a generalised additive model with time as smoothed predictor. See Table 2 for significance of differences in parasitism rates.

latter system were introduced into the USA more than 100 years ago (Hartley et al., 2010), so the contrasting outcome cannot be attributed to differences in the time-scale of adaptation between the two systems. Nevertheless, both novel hosts have been present for a considerable period, so initial communities of herbivores and natural enemies on novel host trees may have differed more from those on native host trees than the current communities.

Field observations and results from an earlier study (Grosman et al., 2005) provide some possible explanations for the lower densities of predators on eucalyptus. *Podisus nigrispinus* (Dallas) is an omnivorous predatory bug that was observed on guava and eucalyptus in the current study. In choice experiments, foraging predators that were collected from eucalyptus areas were more attracted to guava than to eucalyptus plants, independent of the presence of prey on the plants (Grosman et al., 2005). Hence, when both host plants are present in the environment, these predators tend to move to guava rather than eucalyptus, which will result in higher predator densities on this native host plant. One possible reason for this host plant preference is that herbivores that feed on eucalyptus are an inferior diet to predators, which is the subject of a further study. Another possibility is that guava trees may offer more alternative food and shelter to the natural enemies. Both guava and eucalyptus flowers produce nectar and pollen (Nicolson, 1994; Gressler et al., 2006), which may serve as alternative food for predators. However, whereas eucalyptus fruits are woody and inedible (Hodgson, 1976), guava produces edible fruits that attract large numbers of predatory wasps and bugs that feed on the fruits (AH Grosman, pers. obs.). Leaves of both host plants can be used as a water source by omnivorous predators, and the quality of the two host plants may differ in this respect. Hence, natural enemies possibly found more alternative resources on guava than on eucalyptus, and this is expected to result in higher densities of natural enemies, thus higher predation risk for the herbivores (Holt, 1977; van Rijn et al., 2002). In our study sites, the diversity of plants covering the soil was higher in guava areas than in eucalyptus areas, which may further increase the abundance of alternative food sources in guava areas.

Guava trees also provide shelter to predators. Ants were the most frequently observed and certainly most abundant predators on guava. They were present on most guava trees, where they often nested in cavities in the trunk, patrolled the branches and leaves, and attacked eggs, larvae, and pupae of *T. leucoceraea* and other insects (AH Grosman, pers. obs.). No ants or other natural enemies were observed using cavities in eucalyptus trunks as a shelter (AH Grosman, pers. obs.). Spiders, the second most common predator group on guava, frequently used guava

leaves as shelter. Especially leaves that suffered mechanical damage by insect feeding are curved and structurally complex, making them suitable as shelter for spiders and other insects. In contrast, eucalyptus leaves are flat and smooth and were seldom used as shelter by spiders (AH Grosman, pers. obs.).

Unlike most predators, parasitoids successfully followed Lepidoptera to eucalyptus. In the case of *T. leucoceraea*, at least one of the three parasitoid species, *Glyptapanteles* spec., is the same on guava and eucalyptus. This parasitoid manipulates the behaviour of its host (Grosman et al., 2008; Janssen et al., 2010), but the behavioural changes are similar on both host plants and only occur after the parasitoid larvae have egressed from the caterpillar (Grosman et al., 2008). Hence, it is unlikely that these changes affected the relative differences in parasitism between eucalyptus and guava, as measured in this study.

It is unclear why parasitoids apparently colonised eucalyptus quicker than predators. Possibly, specialist parasitoids, which depend on a specific host for their reproduction, are under stronger selection to follow the host to a novel host plant than are generalist natural enemies. Indeed, there are several examples of parasitoids that followed their host to a new host plant. For instance, the apple maggot fly, *Rhagoletis pomonella* Walsh, formed a host race by adapting to a novel host plant within 150 generations (Feder & Filchak, 1999), and the specialist parasitoid *Diachasma alloeum* Muesebeck (Braconidae) has followed it to the novel host plant, indicating similar host-plant-specific adaptation and host-related genetic variation (Forbes et al., 2009). Hartley et al. (2010), however, show the opposite: predator abundance and species richness was equally high on one novel tree species than on three native species, but parasitoid abundance and species richness was lower on the novel tree than on two out of three native trees. Thus, it seems that the pattern found here and elsewhere (Forbes et al., 2009) is not general.

One might expect that lower densities of predators on eucalyptus would result in higher densities of Lepidoptera as a consequence of lower predation. However, this pattern varied among Lepidoptera groups. Whereas the overall densities of Lepidoptera and those of *T. leucoceraea* did not differ between the two host plants, the densities of other Geometridae were higher on eucalyptus and densities of Tortricidae were higher on guava. These differences may reflect different levels of adaptation of these herbivores to eucalyptus, independent of the natural enemies, or different degrees of interaction with natural enemies. A previous study with the same strains of *T. leucoceraea* showed that the herbivore's performance is lower on eucalyptus (Grosman et al., 2015). Hence, lower predation on eucalyptus may be offset by lower herbivore performance.

The lower densities of predators on eucalyptus may offer a partial EFS and this may have played a role in the process of adding this plant species to the host plant range of Lepidoptera in Brazil. Enemy-scarce space may also explain the emergence of pest outbreaks in eucalyptus rather than guava plantations. In the absence of alternative food on eucalyptus, natural enemies have no means of surviving on this plant prior to its colonisation by herbivores. Indeed, numbers of natural enemies were found to decrease in eucalyptus plantations with increasing distance to the surrounding native vegetation (Bragança et al., 1998a,b). Hence, the first herbivores that colonised eucalyptus after its introduction to Brazil have most likely experienced a full escape from their enemies. However, within a century, all natural enemy groups that were observed attacking Lepidoptera on guava have colonised eucalyptus, with some groups already reaching higher or equal densities on this novel host plant. If natural enemies continue colonising eucalyptus and improve their foraging efficiency through adaptation, it is likely that the EFS on eucalyptus will eventually disappear, as this vacant niche will be 'filled' with natural enemies. The question then is, why outbreaks of herbivores still occur in eucalyptus plantations. We suggest that this is a matter of scale and limited biodiversity in large plantations, resulting in limited alternative resources for the natural enemies (Bragança et al., 1998a,b). The eucalyptus plantations studied here are small and more diverse vegetation was nearby, so natural enemies may have been relatively common compared with large-scale monocultures of eucalyptus (Bragança et al., 1998a,b). Hence, EFS may continue to exist in such large plantations.

There is a tendency in the literature to treat EFS as a static pattern. However, this study shows that some natural enemies can follow herbivores to a novel host plant, and possibly make EFS a transient ecological effect. In fact, our study – and most other studies on EFS – presents a snapshot of the ecological interactions occurring on novel and native hosts. It is unclear whether eucalyptus initially offered true EFS to the herbivores, or whether natural enemies (especially parasitoids) immediately followed the herbivores to the new host. However, our data were sampled over a sufficiently long period to accurately reflect the current situation, which is that parasitism rates are comparable on the two host plants, whereas predator densities are lower on the novel host. It is difficult to envisage a scenario where predator densities would have been similar on both host plants upon introduction of the new host whereas these densities are now lower. A more plausible scenario is that predator densities have lagged behind consistently, perhaps even more in the past than at present.

We also found a significant effect of the interaction between plant species and time of the year for densities of several predator species, indicating that EFS on eucalyptus may be a dynamic rather than a static pattern. Heard et al. (2006) also found no evidence for consistent EFS patterns, rather, parasitoids attacks showed strong temporal and spatial variation. It is therefore important to study the occurrence of EFS or enemy-scarce space on larger temporal and spatial scales as was done here (Heard et al., 2006). Whereas research on EFS has focused on herbivore mortality on different host plants, understanding the mechanisms causing this different mortality is essential in unravelling the spatial and temporal dynamics of EFS and its potential to shape the evolution of host plant range in herbivorous insects. A better understanding of the mechanisms that shape EFS patterns requires insight in the ability of predators and parasitoids to adapt or learn to cope with host use changes of herbivores, which still receives little attention in research on EFS.

Acknowledgements

Amir Grosman was supported by The Netherlands Organisation for Scientific Research (NWO-WOTRO grant nr. W 84-520). Amir Grosman and Arne Janssen thank the Federal University of Viçosa for providing facilities. Arne Janssen was supported by FAPEMIG (CBB-30003/09). Angelo Pallini was supported by CNPq and FAPEMIG. Eduardo G. Cordeiro, Felipe Colares, and Claudinei Lima Oliveira are thanked for their assistance in collection and rearing of insects. Steph Menken, Martijn Egas, Astrid Groot, Paul Harvey, Jan Wolf, and members of the lab of Jay Rosenheim are thanked for discussions and suggestions. Special thanks to Seu Geraldo, for allowing us to observe and collect insects from his guava area for this study.

References

- Ballabeni P, Włodarczyk M & Rahier M (2001) Does enemy-free space for eggs contribute to a leaf beetle's oviposition preference for a nutritionally inferior host plant? *Functional Ecology* 15: 318–324.
- Berdegue M, Trumble JT, Hare JD & Redak RA (1996) Is it enemy-free space? The evidence for terrestrial insects and freshwater arthropods. *Ecological Entomology* 21: 203–217.
- Bernays E & Graham M (1988) On the evolution of host specificity in phytophagous arthropods. *Ecology* 69: 886–892.
- Bragança M, De Souza O & Zanuncio JC (1998a) Environmental heterogeneity as a strategy for pest management in *Eucalyptus* plantations. *Forest Ecology and Management* 102: 9–12.
- Bragança MAL, Zanuncio JC, Picanco M & Laranjeiro AJ (1998b) Effects of environmental heterogeneity on Lepidoptera

- and Hymenoptera populations in *Eucalyptus* plantations in Brazil. *Forest Ecology and Management* 103: 287–292.
- Burchfield E, Agar NS & Hume ID (2005) Effect of terpenes and tannins on some physiological and biochemical parameters in two species of phalangerid possums (Marsupialia: Phalangeridae). *Australian Journal of Zoology* 53: 395–402.
- Crawley MJ (2013) *The R Book*. John Wiley & Sons, Chichester, UK.
- Denno RF, Larsson S & Olmstead KL (1990) Role of enemy-free space and plant quality in host-plant selection by willow beetles. *Ecology* 71: 124–137.
- Diamond SE & Kingsolver JG (2010) Fitness consequences of host plant choice: a field experiment. *Oikos* 119: 542–550.
- Drukker B, Bruin J & Sabelis MW (2000) Anthocorid predators learn to associate herbivore-induced plant volatiles with presence or absence of prey. *Physiological Entomology* 25: 260–265.
- Ehrlich PR & Murphy DD (1988) Plant chemistry and host range in insect herbivores. *Ecology* 69: 908–909.
- Fagundes C, Di Mare R, Wink C & Manfio D (2011) Diversity of the families of Coleoptera captured with pitfall traps in five different environments in Santa Maria, RS, Brazil. *Brazilian Journal of Biology* 71: 381–390.
- Feder JL (1995) The effects of parasitoids on sympatric host races of *Rhagoletis pomonella* (Diptera: Tephritidae). *Ecology* 76: 801–813.
- Feder JL & Filchak KE (1999) It's about time: the evidence for host plant-mediated selection in the apple maggot fly, *Rhagoletis pomonella*, and its implications for fitness trade-offs in phytophagous insects. *Entomologia Experimentalis et Applicata* 91: 211–225.
- Ferreira JAM, Cunha DFS, Pallini A, Sabelis MW & Janssen A (2011) Leaf domatia reduce intraguild predation among predatory mites. *Ecological Entomology* 36: 435–441.
- Forbes AA, Powell TH, Stelinski LL, Smith JJ & Feder JL (2009) Sequential sympatric speciation across trophic levels. *Science* 323: 776–779.
- Gerber J-F (2011) Conflicts over industrial tree plantations in the South: who, how and why? *Global Environmental Change* 21: 165–176.
- Gould F (1979) Rapid host range evolution in a population of the phytophagous mite *Tetranychus urticae*. *Evolution* 33: 791–802.
- Gratton C & Welter SC (1999) Does “enemy-free space” exist? Experimental host shifts of an herbivorous fly. *Ecology* 80: 773–785.
- Gressler E, Pizo MA & Morellato LPC (2006) Polinização e dispersão de sementes em Myrtaceae do Brasil. *Revista Brasileira de Botânica* 29: 509–530.
- Grosman AH, van Breemen M, Holtz A, Pallini A, Rugama AM et al. (2005) Searching behaviour of an omnivorous predator for novel and native host plants of its herbivores: a study on arthropod colonization of eucalyptus in Brazil. *Entomologia Experimentalis et Applicata* 116: 135–142.
- Grosman AH, Janssen A, de Brito EF, Cordeiro EG, Colares F et al. (2008) Parasitoid increases survival of its pupae by inducing hosts to fight predators. *PLoS ONE* 3: e2276.
- Grosman AH, Molina-Rugama AJ, Mendes-Dias R, Sabelis MW, Menken SBJ et al. (2015) No adaptation of a herbivore to a novel host but loss of adaptation to its native host. *Scientific Reports* 5: 16211.
- Hartley MK, Rogers WE & Siemann E (2010) Comparisons of arthropod assemblages on an invasive and native trees: abundance, diversity and damage. *Arthropod-Plant Interactions* 4: 237–245.
- Heard SB, Stireman JO, Nason JD, Cox GH, Kolacz CR & Brown JM (2006) On the elusiveness of enemy-free space: spatial, temporal, and host-plant-related variation in parasitoid attack rates on three gallmakers of goldenrods. *Oecologia* 150: 421–434.
- Hodgson LM (1976) Some aspects of flowering and reproductive behaviour in *Eucalyptus grandis* (Hill) Maiden at JDM Keet Forest Research Station (formerly Zomerkomst Forest Research Station). 1. Flowering, controlled pollination methods, pollination and receptivity. *South African Forestry Journal* 97: 18–28.
- Holt RD (1977) Predation, apparent competition, and structure of prey communities. *Theoretical Population Biology* 12: 197–229.
- Holtz AM, de Oliveira HG, Pallini A, Venzon M, Zanuncio JC et al. (2003) Desempenho de *Thyrineina arnobia* Stoll (Lepidoptera: Geometridae) em eucalipto e goiaba: o hospedeiro nativo não é um bom hospedeiro? *Neotropical Entomology* 32: 427–431.
- Instituto Brasileiro de Geografia e Estatística (2014) Banco de Dados Agregados. Available at: <http://www.sidra.ibge.gov.br/bda/tabela/listabl.asp?c=5930&z=p&o=30> (accessed 24 April 2016).
- Janssen A, Grosman AH, Cordeiro EG, de Brito EF, Fonseca JO et al. (2010) Context-dependent fitness effects of behavioral manipulation by a parasitoid. *Behavioral Ecology* 21: 33–36.
- Janssen A, Fonseca JO, Colares F, Silva L, Pedrosa ARP et al. (2014) Time scales of associating food and odor by predator communities in the field. *Behavioral Ecology* 25: 1123–1130.
- Jeffries MJ & Lawton JH (1984) Enemy free space and the structure of ecological communities. *Biological Journal of the Linnean Society* 23: 269–286.
- Koptur S (1992) Plants with extrafloral nectaries and ants in Everglades habitats. *Florida Entomologist* 75: 38–50.
- Lenth R (2016) Lsmeans: least-squares means. *Journal of Statistical Software* 69: 1–33.
- Lill JT, Marquis RJ & Ricklefs RE (2002) Host plants influence parasitism of forest caterpillars. *Nature* 417: 170–173.
- Margolies DC, Sabelis MW & Boyer JE (1997) Response of a phytoseiid predator to herbivore-induced plant volatiles: selection on attraction and effect on prey exploitation. *Journal of Insect Behavior* 10: 695–709.
- Marsh KJ, Foley WJ, Cowling A & Wallis IR (2003) Differential susceptibility to *Eucalyptus* secondary compounds explains feeding by the common ringtail (*Pseudocheirus peregrinus*) and

- common brushtail possum (*Thichosurus vulpecula*). *Journal of Comparative Physiology B* 173: 69–78.
- Matos CHC, Pallini A, Chaves FF, Schroeder JH & Janssen A (2006) Do domatia mediate mutualistic interactions between coffee plants and predatory mites? *Entomologia Experimentalis et Applicata* 118: 185–192.
- Meijer K, Schilthuizen M, Beukeboom L & Smit C (2016) A review and meta-analysis of the enemy release hypothesis in plant–herbivorous insect systems. *PeerJ* 4: e2778.
- Messing RH & Wang X-G (2009) Competitor-free space mediates non-target impact of an introduced biological control agent. *Ecological Entomology* 34: 107–113.
- Mulatu B, Applebaum SW & Coll M (2004) A recently acquired host plant provides an oligophagous insect herbivore with enemy-free space. *Oikos* 107: 231–238.
- Murphy SM (2004) Enemy-free space maintains swallowtail butterfly host shift. *Proceedings of the National Academy of Sciences of the USA* 101: 18048–18052.
- Nicolson SW (1994) Pollen feeding in the eucalypt nectar fly, *Drosophila flavohirta*. *Physiological Entomology* 19: 58–60.
- Nishida R (2002) Sequestration of defensive substances from plants by Lepidoptera. *Annual Review of Entomology* 47: 57–92.
- Ohsaki N & Sato Y (1994) Food plant choice of *Pieris* butterflies as a trade-off between parasitoid avoidance and quality of plants. *Ecology* 75: 59–68.
- Papaj DR & Lewis AC (1993) *Insect Learning: Ecology and Evolutionary Perspectives*. Chapman & Hall, New York, NY, USA.
- Pereira JMM, Zanuncio JC, Schroeder JH & Gasperazzo WL (1994) Índices faunísticos e flutuação populacional de lepidópteros daninhos aos eucalyptos da região de Montes Claros, MG. *Anais Sociedade Entomológica Brasil* 23: 327–334.
- Pinheiro J, Bates D, DebRoy S & Sarkar D & R Core Team (2014) nlme: Linear and Nonlinear Mixed Effects Models. Available at: <http://CRAN.R-project.org/package=nlme> (accessed 19 December 2016).
- Price PW, Bouton CE, Gross P, McPherson BA, Thompson JN & Weis AE (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics* 11: 41–65.
- Rank NE (1994) Host-plant effects on larval survival of a salicin-using leaf beetle *Chrysomela aeneicollis* Schaeffer (Coleoptera, Chrysomelidae). *Oecologia* 97: 342–353.
- Rausher MD (1988) Is coevolution dead? *Ecology* 69: 898–901.
- Reis M & Reis GG (1993) A Contribuição de Pesquisa Florestal Para a Redução de Impactos Ambientais do Reflorestamento. *Anais do I Simpósio Brasileiro de Pesquisa Florestal*, Belo Horizonte, Minas Gerais, Brasil.
- Ribeiro GT, Mendonça MD, de Mesquita JB, Zanuncio JC & Carvalho GS (2005) Spittlebug *Cephus siccifolius* damaging eucalypt plants in the state of Bahia, Brazil. *Pesquisa Agropecuária Brasileira* 40: 723–726.
- van Rijn PCJ, van Houten YM & Sabelis MW (2002) How plants benefit from providing food to predators even when it is also edible to herbivores. *Ecology* 83: 2664–2679.
- Sabelis MW, van Rijn PCJ & Janssen A (2005) Fitness consequences of food-for-protection strategies in plants. *Plant-Provided Food for Carnivorous Insects: A Protective Mutualism and Its Applications* (ed. by FL Wäckers, PCJ van Rijn & J Bruin), pp. 109–134. Cambridge University Press, Cambridge, UK.
- Schultz JC (1988) Many factors influence the evolution of herbivore diets, but plant chemistry is central. *Ecology* 69: 896–897.
- Singer MS & Stireman JO (2005) The tri-trophic niche concept and adaptive radiation of phytophagous insects. *Ecology Letters* 8: 1247–1255.
- Singer MS, Carrière Y, Theuring C & Hartmann T (2004a) Disentangling food quality from resistance against parasitoids: diet choice by a generalist caterpillar. *American Naturalist* 164: 423–429.
- Singer MS, Rodrigues D, Stireman JO & Carrière Y (2004b) Roles of food quality and enemy-free space in host use by a generalist insect herbivore. *Ecology* 85: 2747–2753.
- Smiley JT, Horn JM & Rank NE (1985) Ecological effects of salicin at 3 trophic levels – new problems from old adaptations. *Science* 229: 649–651.
- Stamp N (2001) Enemy-free space via host plant chemistry and dispersion: assessing the influence of tri-trophic interactions. *Oecologia* 128: 153–163.
- Takabayashi J, Sabelis MW, Janssen A, Shiojiri K & van Wijk M (2006) Can plants betray the presence of multiple herbivore species to predators and parasitoids? The role of learning in phytochemical information networks. *Ecological Research* 21: 3–8.
- Thompson JN (1988) Coevolution and alternative hypotheses on insect/plant interactions. *Ecology* 69: 893–895.
- Thompson JN (1996) Trade-offs in larval performance on normal and novel hosts. *Entomologia Experimentalis et Applicata* 80: 133–139.
- Turlings TCJ, Wäckers FL, Vet LEM, Lewis WJ & Tumlinson JH (1993) Learning of host-finding cues by hymenopterous parasitoids. *Insect Learning: Ecology and Evolutionary Perspectives* (ed. by DR Papaj & AC Lewis), pp. 51–78. Chapman & Hall, New York, NY, USA.
- Vet LEM, Lewis WJ & Cardé RT (1995) Parasitoid foraging and learning. *Chemical Ecology of Insects 2* (ed. by RT Cardé & WJ Bell), pp. 65–101. Chapman & Hall, New York, NY, USA.
- Walter DE & O'Dowd DJ (1992) Leaves with domatia have more mites. *Ecology* 73: 1514–1518.
- Wiggins NL, Marsh KJ, Wallis IR, Foley WJ & McArthur C (2006) Sideroxylonal in *Eucalyptus* foliage influences foraging behaviour of an arboreal folivore. *Oecologia* 147: 272–279.
- Wood SN (2004) Stable and efficient multiple smoothing parameter estimation for generalized additive models. *Journal of the American Statistical Association* 99: 673–686.
- Zanuncio JC, do Nascimento EC, Carcia JF & Zanuncio TV (1994) Major lepidopterous defoliators of eucalypt in south-east Brazil. *Forest Ecology and Management* 65: 53–63.
- Zvereva EL & Rank NE (2004) Fly parasitoid *Megaselia opacicornis* uses defensive secretions of the leaf beetle *Chrysomela lapponica* to locate its host. *Oecologia* 140: 516–522.