

SHORT COMMUNICATION

Azteca instabilis ants and the defence of a coffee shade tree: an ant–plant association without mutual rewards in Chiapas, Mexico

David J. Gonthier¹, Gabriella L. Pardee and Stacy M. Philpott

Department of Environmental Sciences, University of Toledo, 2801 W Bancroft Rd Mail Stop 604, Toledo, OH 43606, USA
(Accepted 11 December 2009)

Key Words: Alticinae, beetles, Chrysomelidae, *Conostegia xalapensis*, tri-trophic interaction

Ants (Hymenoptera: Formicidae) are important predators of herbivorous insects on plants (Rosumek *et al.* 2009). Ant removal or absence may result in negative indirect effects on plants, as herbivore abundance and herbivory increase and plant growth and reproduction decline (Rosumek *et al.* 2009, Schmitz *et al.* 2000). Ant presence on plants often results from a mutualistic interaction. For example, strong highly coevolved ant–plant mutualisms are found on myrmecophytic plants that house ants in domatia (specialized nesting sites). Weaker mutualistic associations are found with myrmecophilic plants that only offer extra-floral nectaries (EFNs) or food bodies to attract ants, or on other plants hosting honeydew-producing hemipterans (indirect ant–plant interactions) that mediate ant abundance (Hölldobler & Wilson 1990). However, in most cases, plants and arboreal ants form more passive associations, where ants nest in the natural cavities of branches or bark, or construct carton nests on plant substrates (Hölldobler & Wilson 1990) and the only reward plants offer these ants is the use of their substrates. In these situations the indirect effect of ants on plants is merely by chance, a byproduct of ant presence (byproduct association).

Although these ‘byproduct’ ant–plant associations are common, few document the importance of ant absence or removal to plants. On the contrary, recent meta-analyses reveal the removal of ants from myrmecophytic plants resulted in greater herbivore damage than the removal of ants from more facultative associations (Chamberlain & Holland 2009, Rosumek *et al.* 2009). Although these findings are compelling, the literature

ignores the large class of interactions in which herbivore removal by ants is by chance. In fact, many of these interactions include aggressive numerically dominant ant species and if foraging patterns of ants disrupt herbivore feeding then this disruption may cascade down to reduce host plant damage regardless of how co-evolved the interaction between ant and plant may be. Here we ask if the aggressive arboreal ant, *Azteca instabilis* F. Smith (Formicidae: Dolichoderinae) can reduce the abundance of a herbivorous beetle and its damage to a coffee shade tree when only forming a ‘byproduct’ ant–plant association?

Investigation into a tri-trophic interaction between shade tree, herbivore and *A. instabilis* was conducted at Finca Irlanda (15°11′N, 92°20′W; 900 m asl; 4500 mm y⁻¹ rain), a shaded organic coffee farm in Chiapas, Mexico in May to July of 2009. *Conostegia xalapensis* (Bonpl.) D. Don ex DC. (Melastomataceae) is a neotropical tree distributed from southern Mexico to northern South America in natural and managed areas (Zuchowski 2007). *Conostegia xalapensis* has never been noted to contain EFNs or ant-housing domatia across a broad geographical range (F. Almeda pers. comm.) and makes up ~8% (670/8500) of shade trees at our study site. We commonly observed many small holes (shot-hole damage) penetrating partially through *C. xalapensis* leaves, characteristic of a herbivorous flea beetle, *Margaridisa* sp. (Coleoptera: Chrysomelidae: Alticinae). *Azteca instabilis* constructs carton nests in shade trees, including 2% (13/649) of *C. xalapensis* trees, but shows no preference across tree species; nests are distributed at random with respect to tree identity (Vandermeer *et al.* 2008) despite some trees offering EFN rewards.

To determine the relationship between *A. instabilis*, flea beetle abundance and shot-hole damage, we compared

¹ Corresponding author. Email: Gonthier.david@gmail.com, david.gonthier@Rockets.Utoledo.edu

C. xalapensis trees with naturally occurring nests of *A. instabilis* ($N = 6$) and nearby trees without *A. instabilis* ($N = 6$). On each tree, we counted the number of flea beetles on the underside of 20 base leaves (< 1 m above ground) and 100 canopy leaves (3–6 m). Trees were often on steep slopes allowing access to view the canopy and we only counted undersides of clearly visible leaves. The frequency of flea beetles relative to other small beetles (< 5 mm) on *C. xalapensis* trees was high (97% = 129/133 beetles sampled) in a few thoroughly sampled trees, suggesting they account for the majority of beetles on *C. xalapensis* (unpubl. data). Further, because flea beetles prefer leaf undersides we felt confident in visual and binocular surveying (Gonthier pers. obs.).

Next, to examine leaf damage on trees with and without *A. instabilis*, we cut two branches from the base and three branches from the canopy of each tree using a pole cutter. For each branch, we measured the first five leaves (starting from the meristem) > 4 cm wide on basal and > 3 cm wide on canopy branches, by counting the number of shot-holes on each leaf. We also estimated total leaf area with the equation for an ellipse (area = length/2 \times width/2 $\times \pi$) and the number of shot-holes per unit area. If it appeared there were more than 500 shot holes per given leaf, we estimated the amount of damage per leaf by averaging shot-hole damage within two randomly placed 2-cm² grids. To determine whether there was any relationship between honeydew-producing insects and the effects of *A. instabilis* on leaf damage, we counted the number of honeydew-producing insects per clipped branch and found the mean for canopy and base branches of each tree.

To compare data across sampling positions on trees we adjusted flea beetle counts to number per 40 leaves. We determined the relationship between flea beetle abundance and shot-hole damage for both the base and canopy levels with linear regressions (SPSS 16.0). To determine the relationship between *A. instabilis* presence and flea beetle abundance, we compared mean abundance with a two-factor analysis of variance with position and *A. instabilis* as factors. To determine the relationship between *A. instabilis* presence and herbivory, we compared mean shot-hole damage with a two-factor ANOVA with position and *A. instabilis* as factors. To determine if honeydew-producing hemipterans mediated *A. instabilis* effect we correlated the mean number of hemipterans with the mean shot-hole damage at base and canopy positions using Spearman's correlation coefficient (r_s) (SPSS 16.0). We also compared the mean shot-hole damage on trees housing *A. instabilis* with hemipterans ($N = 3$) and without hemipterans ($N = 3$) with one-factor ANOVA. Finally, to compare the effect of *A. instabilis* to other observational ant–plant studies, we calculated the size of the effect of *A. instabilis* presence on herbivore abundance and shot-hole damage for canopy

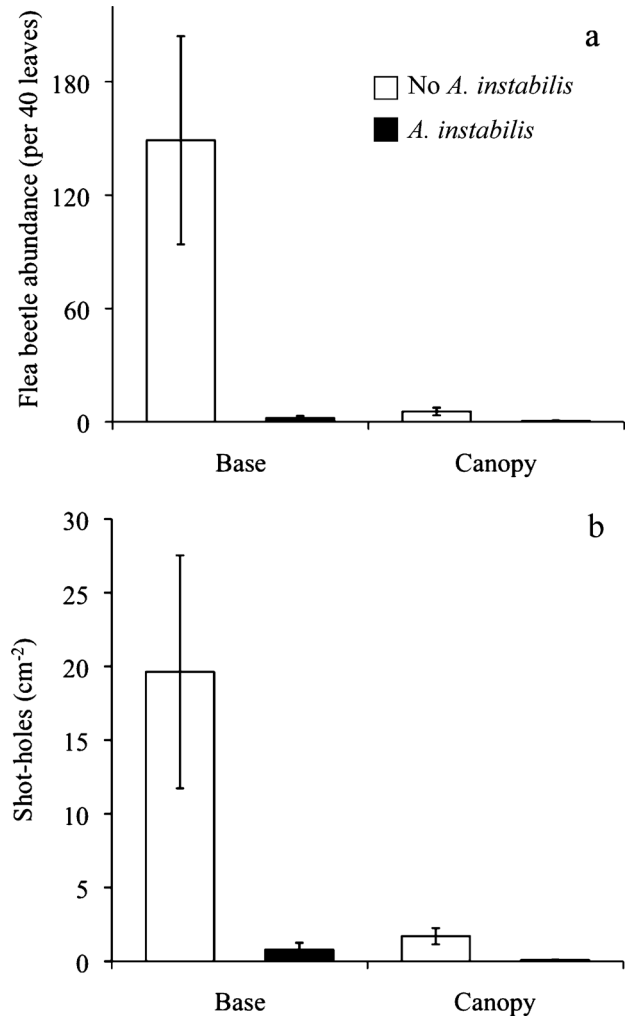


Figure 1. Difference in flea beetle abundance and shot-hole damage across *Conostegia xalapensis* trees with and without *Azteca instabilis* ants at base and canopy positions. Flea beetle abundance on base and canopy leaves of *C. xalapensis* with and without *A. instabilis* nests (a). Shot-hole damage on base and canopy leaves of *C. xalapensis* with and without *A. instabilis* nests (b).

and base leaves using the equation: $\ln(\text{mean without } A. \text{ instabilis} / \text{mean with } A. \text{ instabilis})$ as in Rosumek *et al.* (2009). To meet assumptions of normality for all tests (except hemipteran correlations) we log transformed ($\log[\text{variable} + 1]$) flea beetle abundance and shot-hole damage.

Flea beetle abundance explained 66.7% and 91.3% of the variability in shot-hole damage on *C. xalapensis* canopy ($r^2 = 0.697$, $P = 0.001$) and base leaves ($r^2 = 0.913$, $P = 0.001$), respectively. *Azteca instabilis* presence reduced flea beetle abundance ~ 75 fold on base leaves and ~ 12 fold on the canopy leaves (Figure 1a, $F_{1,11} = 53.2$, $P < 0.001$). There were also more flea beetles on base leaves than on canopy leaves ($F_{1,19} = 26.5$, $P < 0.001$). There was an *A. instabilis* \times position interaction

($F_{1,19} = 13.8$, $P = 0.002$) with the effect of *A. instabilis* presence on flea beetles stronger on the base leaves. Additionally, *A. instabilis* presence reduced shot-hole damage ~ 25 fold on base leaves and ~ 22 fold on canopy leaves (Figure 1b, $F_{1,11} = 49.1$, $P < 0.001$). There was also more damage on base leaves relative to canopy leaves ($F_{1,19} = 27.4$, $P < 0.001$) and an *A. instabilis* \times position interaction ($F_{1,19} = 11.1$, $P = 0.004$) with the effect of *A. instabilis* on damage stronger on base leaves. Four species of honeydew-producing hemipterans were observed on *C. xalapensis* branches: *Coccus viridis*, white flies (Aleyrodidae) and two other species of scale insect (Coccidae), but all occurred in low densities (0–23 per branch) and on only three of six trees with *A. instabilis* and none without *A. instabilis*. There was no relationship between the number of hemipterans and shot-hole damage at base ($r_s = -0.548$, $P = 0.127$) and canopy ($r_s = -0.524$, $P = 0.08$) positions. Additionally, there was no difference between shot-hole damage on *A. instabilis* trees with and without hemipterans ($F_{1,5} = 0.75$, $P = 0.435$). The size of the effect of *A. instabilis* on beetle abundance on base leaves was 4.31 and on canopy leaves 2.47. The size of the effect of *A. instabilis* on shot-hole damage for base leaves was 3.21 and for canopy leaves 3.09.

The observed reduction in flea beetle abundance and shot-hole damage on *C. xalapensis* trees with *A. instabilis* suggests *A. instabilis* negatively affects beetles and has an indirect positive effect on *C. xalapensis*. The indirect effect of *A. instabilis* did not appear to be mediated by honeydew-producing insects because there was no correlation between their abundance and shot-hole damage; however, our sample size was small. Further, *C. xalapensis* does not bear food bodies, EFNs, or domatia, suggesting the indirect effect of *A. instabilis* on *C. xalapensis* is a byproduct of the mere presence of *A. instabilis*.

The size of the effect of *A. instabilis* is comparable to many strong ant–plant mutualisms reported in observational studies (observational studies should not be compared with experiments because observation produces a higher magnitude of effect; Chamberlain & Holland 2009). The size of the effect of *A. instabilis* on flea beetle abundance was stronger than the sizes of the effect for indirect ant–plant associations reported in observational studies in a meta-analysis by Schmitz *et al.* (2000), but was weaker than the one myrmecophytic ant–plant study reported (Letourneau *et al.* 1993). The size of the effect of *A. instabilis* on shot-hole damage was stronger than the mean size of the effect for ants on herbivory in observational studies investigating myrmecophytic and myrmecophilic ant–plant associations reported in Chamberlain & Holland (2009), suggesting the size of the effect of *A. instabilis* is on par with some of the strongest ant–plant mutualisms.

There are several reasons we believe that *A. instabilis* has such strong indirect effects on *C. xalapensis*. First,

A. instabilis is a very aggressive arboreal-nesting ant species, and therefore has a large effect on the vegetation immediately near nests. Second, *A. instabilis* is efficient at removing small (~ 2 – 3 mm) rather than large (~ 5 – 8 mm) prey items (Philpott *et al.* 2008), suggesting that this flea beetle (< 3 mm) would be easily removed. Additionally, flea beetles are amazing jumpers and at the slightest disturbance will leap and re-colonize a new location, indicating *A. instabilis* may limit flea beetles mainly through displacement rather than predation. Finally, the damage caused by the flea beetle is easily identifiable and quantifiable allowing for sensitive measurement.

Beetle abundance on canopy leaves was lower than on base leaves. It is possible our visual sampling method underestimated beetle abundance on the canopy. However, because damage and beetle abundance were highly correlated, this is unlikely. It is more likely that our flea beetles, like many other flea beetles, feed on the roots of host plants as larvae (White 1983), and therefore adults congregate on leaves closest the ground for oviposition.

Azteca instabilis forms different ant–plant associations depending on host tree species. For example, when *A. instabilis* colonizes *Inga* spp. it will visit EFNs forming a myrmecophilic association (Philpott *et al.* 2004). On coffee bushes it forms an indirect ant–plant mutualism with scale insects (Perfecto & Vandermeer 2006), and on *C. xalapensis*, *A. instabilis* forms a ‘byproduct’ association. Plants that offer EFN or indirect rewards to *A. instabilis* would be predicted to gain greater protection from *A. instabilis* than trees offering little or no reward. Despite this notion, *A. instabilis* does not have preference for one nest tree over another (Vandermeer *et al.* 2008) and here we suggest *A. instabilis* indeed still provides strong defences despite receiving little reward on trees such as *C. xalapensis*. However, fitness costs and benefits for hosting *A. instabilis* are unknown for all tree species. It is also unknown how important shot-hole damage is to *C. xalapensis* fitness, therefore further investigation is needed before full conclusions can be made.

There are few examples similar to the *A. instabilis*–*C. xalapensis* system in the literature. Most notable is *Oecophylla smaragdina*, which constructs nests by weaving leaves of host plants together, and provides defence against herbivores of mangroves and agricultural tree crops (Offenberg *et al.* 2004). Yet arboreal nesting ants are common, for example within our study site there are over 60 species within the coffee agro-ecosystem that do not nest in true domatia, many of which are observed to be predacious. Further work documenting the importance of other ant species to host plants is pending.

In conclusion, we suggest *A. instabilis* reduces flea beetle abundance and damage to *C. xalapensis* host trees, even though they only form ‘byproduct’ associations with *C. xalapensis* trees. Yet the size of the effect of *A. instabilis* was on par with some of the strongest effects reported in

the literature, suggesting in some cases factors other than plant rewards may be important to the size of the effect of ants on plants.

ACKNOWLEDGEMENTS

Thanks to the Peters family for allowing this research to be conducted at the Finca Irlanda coffee plantation. David Furth kindly identified Alticinae beetles. Gabriel Dominguez, D. Jackson, A. MacDonald and K. Ennis aided in finding *Azteca* nests. Financial support came from Grant in aid of research – Sigma Xi, Society of Integrative and Comparative Biology, the University of Toledo Study Abroad Travel Grants, USR & CAP, NSF DBI-0829252 URM, and NSF grant DEB-0349388 to I. Perfecto and J. Vandermeer.

LITERATURE CITED

- CHAMBERLAIN, S. A. & HOLLAND, J. N. 2009. Quantitative synthesis of context dependency in ant-plant protection mutualisms. *Ecology* 90:2384–2392.
- HÖLDOBLER, B. & WILSON, E. O. 1990. *The Ants*. Belknap Press and Harvard University Press, Cambridge. 732 pp.
- LETOURNEAU, D. K., ARIAS, F. & JEBB, M. 1993. Coping with enemy-filled space: herbivores on *Endospermum* in Papua New Guinea. *Biotropica* 25:95–99.
- OFFENBERG, J., HAVANON, S., AKSORNKOAE, S., MACINTOSH, D. J. & NIELSEN, M. G. 2004. Observations on the ecology of weaver ants (*Oecophylla smaragdina* Fabricius) in a Thai mangrove ecosystem and their effect on herbivory of *Rhizophora mucronata* Lam. *Biotropica* 36:344–351.
- PERFECTO, I. & VANDERMEER, J. 2006. The effect of an ant-hemipteran mutualism on the coffee berry borer (*Hypothenemus hampei*) in southern Mexico. *Agriculture Ecosystems & Environment* 117:218–221.
- PHILPOTT, S. M., GREENBERG, R., BICHER, P. & PERFECTO, I. 2004. Impacts of major predators on tropical agroforest arthropods: comparisons within and across taxa. *Oecologia* 140:140–149.
- PHILPOTT, S. M., PERFECTO, I. & VANDERMEER, J. 2008. Behavioral diversity of predatory arboreal ants in coffee agroecosystems. *Environmental Entomology* 37:181–191.
- ROSUMEK, F. B., SILVEIRA, F. A. O., NEVES, F. D. S., BARBOSA, N. P. D. U., DINIZ, L., OKI, Y., PEZZINI, F., FERNANDES, G. W. & CORNELISSEN, T. 2009. Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia* 160:537–549.
- SCHMITZ, O. J., HAMBÄCK, P. A. & BECKERMAN, A. P. 2000. Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. *American Naturalist* 155:141–153.
- VANDERMEER, J., PERFECTO, I. & PHILPOTT, S. M. 2008. Clusters of ant colonies and robust criticality in a tropical agroecosystem. *Nature* 451:457–460.
- WHITE, R. E. 1983. *A field guide to the beetles of North America*. Houghton Mifflin, Boston. 368 pp.
- ZUCHOWSKI, W. 2007. *Tropical plants of Costa Rica: a guide to native and exotic flora*. Zone Tropical, Ithaca. 529 pp.