

# Do invasive ants respond more strongly to carbohydrate availability than co-occurring non-invasive ants? A test along an active *Anoplolepis gracilipes* invasion front

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**Abstract** Invasions by non-native insects can have important ecological impacts, particularly on island ecosystems. However, the factors that promote the success of invaders relative to co-occurring non-invasive species remain unresolved. For invasive ants, access to carbohydrate resources via interactions with both extrafloral nectary-bearing plants and honeydew-excreting insects may accelerate the invasion process. A first step towards testing this hypothesis is to determine whether invasive ants respond to variation in the availability of carbohydrate resources, and whether this response differs from that of co-occurring, non-invasive ants. We investigated the effect of carbohydrate subsidies on the short-term foraging and hemipteran-tending behaviours of the invasive ant *Anoplolepis gracilipes* (Formicidae) and co-occurring ant species on an extrafloral nectary-bearing plant by experimentally manipulating carbohydrate levels and tracking ant recruitment. We conducted experiments in 2 years at two sites: one site was invaded by *A. gracilipes* prior to 2007 and the other became invaded during the course of our study, allowing pre- (2007) and post-invasion (2009) comparisons. Short-term increases in carbohydrate availability increased the density of *A. gracilipes* workers on plants by as much as 400% and reduced tending of honeydew-excreting insects by this species by up to 89%, with similar responses across years. In contrast, ants at the uninvaded site in 2007 showed a weak and non-significant forager recruitment response. Across all sites, *A. gracilipes* workers were the only ants that responded to carbohydrate manipulations in 2009. Furthermore, ant-carbohydrate dynamics at a site newly invaded by *A. gracilipes* quickly diverged from dynamics at uninvaded sites and converged on those of the site with an established invasion. These findings suggest that carbohydrate resources may be particularly important for *A. gracilipes* invasions, and underscore the importance of species interactions, particularly putative mutualisms, in facilitating exotic species invasions.

**Key words:** ant-plant, extrafloral nectar, honeydew, mutualism, species invasion.

## INTRODUCTION

Invasive species pose a major threat to global biodiversity and may significantly alter the composition of native communities as well as important ecosystem processes and services (Fritts & Rodda 1998; Pimentel *et al.* 2001; Holway *et al.* 2002; Abbott 2006; Dunham & Mikheyev 2010). Among insect groups, invasive ants have had especially strong, negative effects on native communities, altering community dynamics across multiple trophic levels and leading to the dissolution of important ecosystem processes (reviewed by Holway *et al.* 2002). Currently, nine of the ~150 ant species that have been introduced around the world are considered invasive (McGlynn 1999). Five of these species were included in the IUCN's list of the world's 100 worst invasive species: *Solenopsis invicta* (the red imported fire

ant), *Wasmania auropunctata* (the little fire ant), *Linepithema humile* (the Argentine ant), *Pheidole megacephala* (the big-headed ant) and *Anoplolepis gracilipes* (the yellow crazy ant) (Lowe *et al.* 2000). Although few ant species have become invasive, when they have, there have been widespread and strong negative impacts on native communities. For example, some ant invasions have been associated with reductions of >90% in native ant abundances (Porter & Savignano 1990; Hoffmann & Parr 2008, but see King & Tschinkel 2008). Invasive ants may also negatively affect other native invertebrates, birds and reptiles (Holway *et al.* 2002).

Identifying factors that promote ant invasions is of critical importance. At least two common traits are shared among invasive ant species: a wide diet breadth and the ability to form high-density supercolonies in which intraspecific competition is low (reviewed by Holway *et al.* 2002). A third factor that may promote the success of invasive ants is their ability to exploit carbohydrate resources (Lach 2003). Invasive ants

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Accepted for publication March 2010.

commonly exhibit tending behaviour towards extrafloral nectary (EFN)-bearing plants (Koptur 1979; Lach 2003; Ness & Bronstein 2004) and honeydew-excreting insects (Helms & Vinson 2002; Styrsky & Eubanks 2007). It has been hypothesized that these carbohydrate-rich resources promote ant invasions by providing high-energy fuel for greater activity, growth and the establishment of dominant supercolonies (Holway *et al.* 2002; Lach 2003). Furthermore, carbohydrates may drive the aggressive behaviours of invading ants. For example, laboratory colonies of the invasive Argentine ant (*L. humile*) became more aggressive and had higher exploration rates when provided with sucrose (Grover *et al.* 2007). The relative importance of diet breadth, supercolony formation and carbohydrate exploitation in ant invasions remains unclear; however, comparisons between the ecology of invasive ants and that of co-occurring, non-invasive ants may help to disentangle these factors.

Here we focus on the mechanism of carbohydrate exploitation and compare responses of invasive and non-invasive ants in an island ecosystem. Like many island groups, the Samoan archipelago (South Pacific Ocean) is dominated by non-native ants. Our recent survey of six islands and 35 sites across the archipelago revealed that the yellow crazy ant, *A. gracilipes*, was the most dominant non-native species (Savage *et al.* 2009). Furthermore, *A. gracilipes* abundance was positively correlated with natural levels of EFN availability and negatively associated with the species richness of native ants across sites. Neither of these associations were displayed by any other non-native ant species (Savage *et al.* 2009).

Here, we used manipulations of nectar to evaluate the hypothesis that invasive ants respond differently to plant-based carbohydrate resources than co-occurring, non-invasive ants. We conducted experiments along an active invasion front, yielding the ability to examine non-invasive ant behaviour both before and after the arrival of the invasive species. Specifically, we replicated nectar manipulations: (i) at a site with an established *A. gracilipes* population in 2007 and 2009; (ii) at a nearby site both before (2007) and after (2009) *A. gracilipes* had invaded; and (iii) at the same nearby site, but in an area outside of the invasion front that remained free of *A. gracilipes* in 2009. We conducted our experiments on *Morinda citrifolia*, an EFN-bearing shrub that reaches ecological dominance in the lava fields in Savaii where this study was conducted. Previous work demonstrated that both *A. gracilipes* and co-occurring ants commonly forage at the nectaries of *M. citrifolia* (Savage *et al.* 2009).

Our experiments addressed the following specific questions: (1) Do invasive *A. gracilipes* workers differ from co-occurring non-invasive ants in their response to increasing carbohydrate levels? While we expected most ants to recruit to experimental manipulations of

carbohydrate resources, we predicted that *A. gracilipes* workers would display the strongest positive response. Next, in addition to nectar provided by *M. citrifolia*, the ants in this study had access to honeydew-excreting insects that used *M. citrifolia* as a host plant. Therefore, we also asked (2) Does increasing plant-based carbohydrate levels change the honeydew-excreting insect-tending behaviours of *A. gracilipes* and/or non-invasive ants? We expected that the proportion of ants tending honeydew-excreting insects would decline with increasing plant-based carbohydrates, irrespective of ant species identity, if our artificial treatments designed to mimic plant nectar were more attractive than honeydew-excreting insects rewards (as predicted by Becerra & Venable 1989). We expected that ant tending behaviours would remain unchanged if plant-based and honeydew-excreting insects-based resources were equivalently attractive to ants. Because our experiments were conducted both before and after a new *A. gracilipes* invasion as well as at a previously invaded site, our study also provided insight into how ant responses may vary during different stages of *A. gracilipes* invasions.

## METHODS

### Study organisms

A pan-tropical 'tramp ant', *A. gracilipes* has a broad diet breadth, can form supercolonies, and is considered invasive in many island groups, including Samoa, Hawaii, Christmas Island and Tokelau (Holway *et al.* 2002; Lester & Tavite 2004; Abbott 2006). Although its native range is thought to include Africa or Asia, this species' origin is currently undetermined (Wetterer 2005). In Samoa, *A. gracilipes* workers were first recorded in 1925 at very low abundances near ports and plantations (Emery 1925 cited in Wilson & Taylor 1967). However, recent studies have demonstrated that this species is currently widely distributed in Samoa, occasionally occurring at very high densities (Lester & Tavite 2004; Savage *et al.* 2009). For example, Lester and Tavite (2004) found that *A. gracilipes* reached abundances of 191–1060 per trap within 24 h.

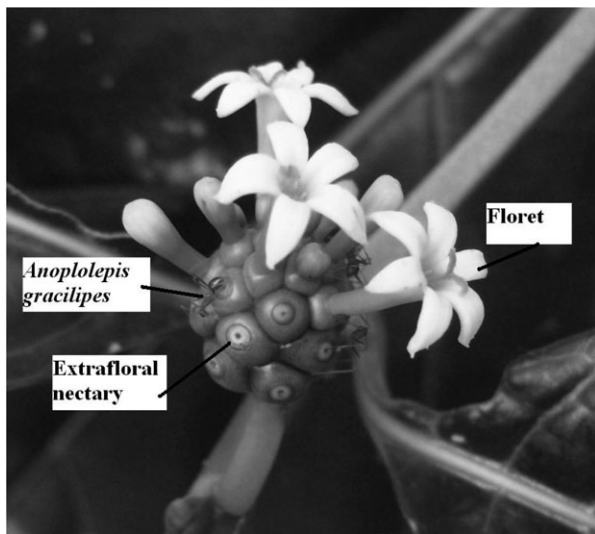
*Morinda citrifolia* (Nonu, Rubiaceae) is an abundant EFN-bearing plant that commonly co-occurs with *A. gracilipes* in Samoa (Savage *et al.* 2009). Native to Southeast Asia, *M. citrifolia* may have spread to many Pacific islands via historical migrations of indigenous peoples ~3000 years ago (Whistler 1993). This plant possesses annular disk nectaries clustered on an inflorescence (Figure 1, Waki *et al.* 2007). Some of these nectaries are surrounded by a small white floret, and are thus functionally floral. However, other nectaries begin secreting nectar soon after bud burst and never become surrounded by a floret, making them functionally extrafloral. Finally, floral nectaries continue to produce nectar after florets drop; these nectaries are therefore post-floral (A. Savage, pers. obs., 2007–2009). Here, we adopt the inclusive term 'nectary body' to refer to the pre-fruit struc-

ture that bears all three nectary types (Figure 1). At our sites in Samoa, nectary bodies contained 2 to >50 nectaries and reached a maximum size of 80 cm<sup>3</sup>. *Morinda citrifolia* plants produce nectary bodies year-round, the three nectary types are commonly active simultaneously, and we observed ants visiting all nectary types.

**Study sites**

Experiments were conducted on the island of Savaii, Samoa during July–August 2007 and September 2009. Study sites were located on lava flows dating from 1907 to 1911, and plant communities were dominated by *M. citrifolia*. In 2007, we studied one site invaded by *A. gracilipes* in the village of Saleaula (13°27'31"S, 172°19'19"W) and a similar reference site lacking *A. gracilipes* in the village of Mauga (13°29'11"S, 172°19'07"W), where the dominant ant species was *P. megacephala* (Table 1). The two sites were separated by ~5 km and had similarly low levels of anthropogenic disturbance, almost entirely limited to the collection of *M. citrifolia* fruit by local people. We returned to both sites monthly from July 2007 to June 2008. In November 2007, we first recorded the arrival of *A. gracilipes* in the southern portion of the previously uninvaded Mauga site. By August 2009, *A. gracilipes* workers were abundant and dominant over ~1.5 km<sup>2</sup> in Mauga ('Mauga South'). However, at the northernmost end of the site ('Mauga North'), there were no *A. gracilipes* workers and *P. megacephala* remained the dominant ant species (Table 1), corresponding to the front of the *A. gracilipes* invasion.

Despite the co-occurrence of other non-native ants at our sites, we only classified *A. gracilipes* as highly invasive because, when present, *A. gracilipes* reached very high abundances and comprised >98% of all ground foragers (see Table 1). Across both invaded and uninvaded sites, no other ant species approached these high abundances (Table 1).



**Fig. 1.** *Morinda citrifolia* plant showing florets and extrafloral nectaries, and *Anoplolepis gracilipes* workers. We refer to the entire structure (inflorescence and nectaries) as a 'nectary body' (see text for more details).

**Table 1.** Densities of *Anoplolepis gracilipes* and other co-occurring ants at study sites in both 2007 and 2009

Site	2007				2009			
	Density of <i>A. gracilipes</i>		Density of other ants		Density of <i>A. gracilipes</i>		Density of other ants	
	Ground	<i>Morinda citrifolia</i>	Ground	<i>M. citrifolia</i>	Ground	<i>M. citrifolia</i>	Ground	<i>M. citrifolia</i>
Saleaula	15.38 ± 3.99	29.44 ± 4.37	0.22 ± 0.22	2.33 ± 1.0	30.5 ± 6.43	53.6 ± 7.69	12.4 ± 2.49	3.67 ± 2.01
Mauga	0	0	0	6.40 ± 0.80	–	–	–	–
Mauga North	–	–	–	–	0	0	9.4 ± 1.60	11.32 ± 0.98
Mauga South	–	–	–	–	38.75 ± 1.32	53.21 ± 4.65	0	0

During the 2007 experiment, Saleaula and Mauga were invaded and uninvaded by *A. gracilipes*, respectively. The southern region of Mauga became invaded by 2009 (see text for more information). Densities (mean ± SE) on the ground are reported per 10 cm<sup>2</sup> and per sub-sampled plant for *M. citrifolia* plants (see text for more detail).

Additionally, in a recent survey of 35 sites spanning six islands of the Samoan archipelago, we documented a total of 11 non-native ant species. However, *A. gracilipes* was the only non-native ant species significantly associated with lower native ant species richness (Savage *et al.* 2009).

### Experimental design: 2007

At each site, we established five 4 × 4 m plots. Plots were located a minimum of 8 m apart (range 8–11 m) to reduce the probability that ants in different plots would belong to the same colony. Within each plot, five *M. citrifolia* plants of similar size were selected and randomly assigned to one of five carbohydrate availability treatments (see next section). We also measured total branch length and evaluated differences in the abundances of ant-tended hemipterans between sites. For each plant, we haphazardly selected five leaves, two stems and two nectary bodies and counted the number of aphids (Aphididae), scale insects (Coccididae) and mealybugs (Pseudococcidae). Finally, ant assemblages were assessed outside treatment plots (<0.5 m from edge of plots) using 10 × 10 cm cards placed on the lava surface and observed for 30 s, following methods in Abbott (2005). We used this approach because the lava was impenetrable; thus, it was not possible to set out pitfall traps. Ant assemblage assessments were conducted on 12 October 2007 at Mauga and at 15 October 2007 at Saleaula (Table 1).

To control variation in ambient nectar availability, we bagged all nectary bodies in each plot using dark poplin fabric secured at the base with a plastic cable tie. Bags were ~1.5–2× bigger than nectary bodies to minimize contact between nectaries and bagging material. These bags excluded ants from floral, extrafloral and post-floral nectaries.

To inform the design of our carbohydrate treatments, we assessed natural nectar production and concentration for *M. citrifolia*. We excluded insect visitors from nectary bodies for 24 h and collected nectar in microcapillary tubes. Average nectar production per plant per day was 2249 µL ± 642 SE (range = 645–5226, *n* = 6 plants). The concentration of *M. citrifolia* nectar using a field refractometer (EZ-Red B1, EZ Red Co., Deposit, NY) was 28.06% ± 1.04 % SE (*n* = 8 plants).

We manipulated carbohydrate availability by adding artificial nectary tubes that were either empty or contained 2000 µL of a 30% sucrose solution. In a previous study, Freeman *et al.* (1991) demonstrated that *M. citrifolia* nectar is dominated by sucrose, with sucrose contributing an average of 72.6–88.9% to total nectar carbohydrates. Therefore, we considered the 30% sucrose solution in our artificial nectaries to be a reasonable approximation of naturally occurring *M. citrifolia* nectar. Treatments manipulated the number of filled artificial nectar tubes with five levels: 0, 5, 10, 15 or 20 filled tubes. To control for the presence of the nectary tube structures, all plants received 20 artificial nectary tubes (2000 µL, MCT-200-C Microtubes, Axygen Scientific, Union City, CA) with a small hole in the lid, through which a cotton wick was threaded and allowed to reach the bottom of the tube; wicks were trimmed to ~3 mm. The 20 tubes were strung on a strip of poplin fabric (~2 cm wide) in randomized order, then wrapped around the main stem and branches of *M. citrifolia* to mimic the distribution of nectary

bodies. To maximize the potential to detect ant responses, our nectar treatments substantially increased nectar availability, for example, the five tubes treatment produced 450% higher nectar levels (10 000 µL/day) than would occur naturally on an average plant.

Artificial nectaries were established between 1530 and 1700 (five replicates) on 26 July 2007 at the Saleaula, and 1500–1700 on 8 August 2007 (two replicates) and 1700–1830 on 9 August 2007 (three replicates) at Mauga. During this time period, there was no measurable rainfall and average daily temperatures at Avao weather station ranged from 28°C to 30°C. At both sites, artificial nectaries were refreshed once after the first census at 16 h, because of depletion of nectar in the tubes.

Counts of ant visitors to plants were obtained at three times: 16 h, 24 h and 40 h after the initial establishment of the nectar treatment. All counts occurred during daylight hours because of cultural restrictions. While this sampling scheme provided a good estimate of the relative foraging rates for diurnal and crepuscular foragers, it did not account for the nocturnal activities of local ant assemblages. During counts, each individual ant was classified by behaviour (tending hemipterans *vs.* any other behaviour), by species, and by location (at artificial nectaries, nectary bodies, stems and leaves, or hemipteran aggregations). Counts took ~5 min per plant. We collected specimens of each ant species from nearby non-treatment plants and identified them using Wilson and Taylor (1967) and Shattuck (1999).

### Experimental design: 2009

We returned to the same villages during 16–17 September 2009 (Saleaula) and 24–25 September 2009 (Mauga) to conduct similar experiments. We selected three sites for these trials: (i) Saleaula; (ii) Mauga South (inside *A. gracilipes* invasion front); and (iii) Mauga North (outside *A. gracilipes* invasion front). The sites selected at Mauga were separated by ~150 m. Average daily temperatures during these dates ranged between 26°C and 29°C, and there was no measurable precipitation at the Avao weather station. As in 2007, treatments were applied at random to individual plants chosen haphazardly from among plants of similar size bearing at least two nectary bodies. Treatment plants were again located in blocks that were separated from each other by ~10 m (*n* = 5 blocks for Saleaula and Mauga South and *n* = 3 blocks for Mauga North because of the local extent of the new invasion). We measured plant height, counted the total number of nectary bodies and counted the number of honeydew-excreting insects per plant. Prior to nectar manipulations, we also assessed ant assemblages using card counts (as described for 2007).

The experimental design for 2009 was the same as for 2007, with the following exceptions. The carbohydrate manipulations were modified to more closely mimic natural variation in *M. citrifolia* extrafloral nectar availability at the per plant scale. Thus, the levels were 0%, 50%, 100%, 150% and 200% of ambient levels per plant. To reduce nectar, we again secured bags to the base of nectary bodies (as described above), but bags were constructed from lightweight organza instead of poplin. We bagged all nectary bodies, regardless of treatment assignment to control for any

effects of the bags. However, we cut holes in control bags to allow ants to access actual *M. citrifolia* nectar. Thus, for plants in the 50% treatment, we cut holes in half of the bags, and all bags had holes in the 100%, 150% and 200% treatments.

As in 2007, we used artificial nectarials to supplement nectar levels (50 µL per tube – Seal-Rite microcentrifuge tubes, USA Scientific 1605-0000, Ocala, FL USA, filled with a 30% sucrose solution). We inserted a 5 µL microcapillary tube into the centre of each microcentrifuge tube instead of using string in order to reduce evaporation of the sucrose solution. Tubes were affixed to trees using twist ties, all plants received 10 tubes, and as in 2007, control tubes were empty. Thus, the 150% treatment had five empty tubes and five filled tubes for a total supplement of 2500 µL over the course of the experiment (48 h). Tubes were not replenished at any time during the experiment. As a result, some of the tubes were empty by the end of the experiment. However, this was rare, as microcapillary tubes reduced evaporation rates. Air bubbles occasionally formed inside the microcapillary tubes. Therefore, all microcapillary tubes were cleared (removed and then re-inserted) ~15 min before each census.

We conducted six censuses for each plant: morning (~600–800), mid-afternoon (~1200–1400) and evening (~1600–1800) over two consecutive days. Response variables were the same as in 2007.

## Data analyses

Because we used different methods in 2007 and 2009, we analysed responses for each year separately. To test (1) Do invasive *A. gracilipes* workers differ from co-occurring non-invasive ants in their response to increasing carbohydrate levels?, we examined ant density (number of workers per plant) for each of two ant dominance status categories: the most abundant ant species per site (dominant) or all other co-occurring ants (subordinate). Using dominance status allowed us to compare the responses of both dominant and non-dominant ants between both invaded (*A. gracilipes*-dominated) and reference (*P. megacephala*-dominated) sites. Data were analysed by site and year using repeated measures general linear models following recommendations in von Ende (2001). Models included the following independent variables: time elapsed since the addition of nectar tubes (16, 24, 40 h for 2007 or 12, 18, 24, 32, 36, 48 h for 2009), the random effect of block, the fixed effects of ant dominance status and the carbohydrate treatment (0, 5, 10, 15, 20 filled tubes for 2007 or 0%, 50%, 100%, 150%, 200% ambient levels for 2009), and all interactions with time (SAS Institute 2003, version 9.1, Cary, NC). Ant dominance status and block were treated as categorical factors, and the carbohydrate treatment was treated as a continuous factor. Plant size (total branch length for 2007 or height for 2009), the number of nectary bodies per plant and the abundance of honeydew-excreting insects per plant (2009 only) were tested as possible covariates, but none had significant effects on ant abundances, perhaps because plants were of similar sizes. Thus, we did not include these covariates in the final analyses. When the factor of elapsed time had no significant influence on ant responses to our treatments, we averaged across time to simplify data presentation. Statistical analyses met assumptions of multivariate normality of residuals and homogeneity of variances following square-root transformation of ant density.

doi:10.1111/j.1442-9993.2010.02152.x

Using dominance status as a factor in the previous analyses allowed us to compare the within-site differences of *A. gracilipes* and co-occurring non-invasive ants. However, we needed a separate test to compare *A. gracilipes* with other non-invasive ants across sites with different invasion statuses. Using the analysis described above, we first confirmed that dominant and subordinate ants at sites where *A. gracilipes* were absent did not differ in their responses to our carbohydrate manipulations (see below). We then pooled their responses and compared them with *A. gracilipes* responses at invaded sites, again using repeated measures analysis of covariance. Subordinate ants at *A. gracilipes*-invaded sites were excluded from these cross-site comparisons. Site was treated as a categorical factor, our carbohydrate treatments were again treated as continuous, and block was incorporated in the model as a nested factor within site. When ant responses to carbohydrate manipulations were significantly different between sites, as indicated by a statistically significant site by carbohydrate treatment interaction, then for each site we conducted regressions of ant density per plant as a function of carbohydrate treatment.

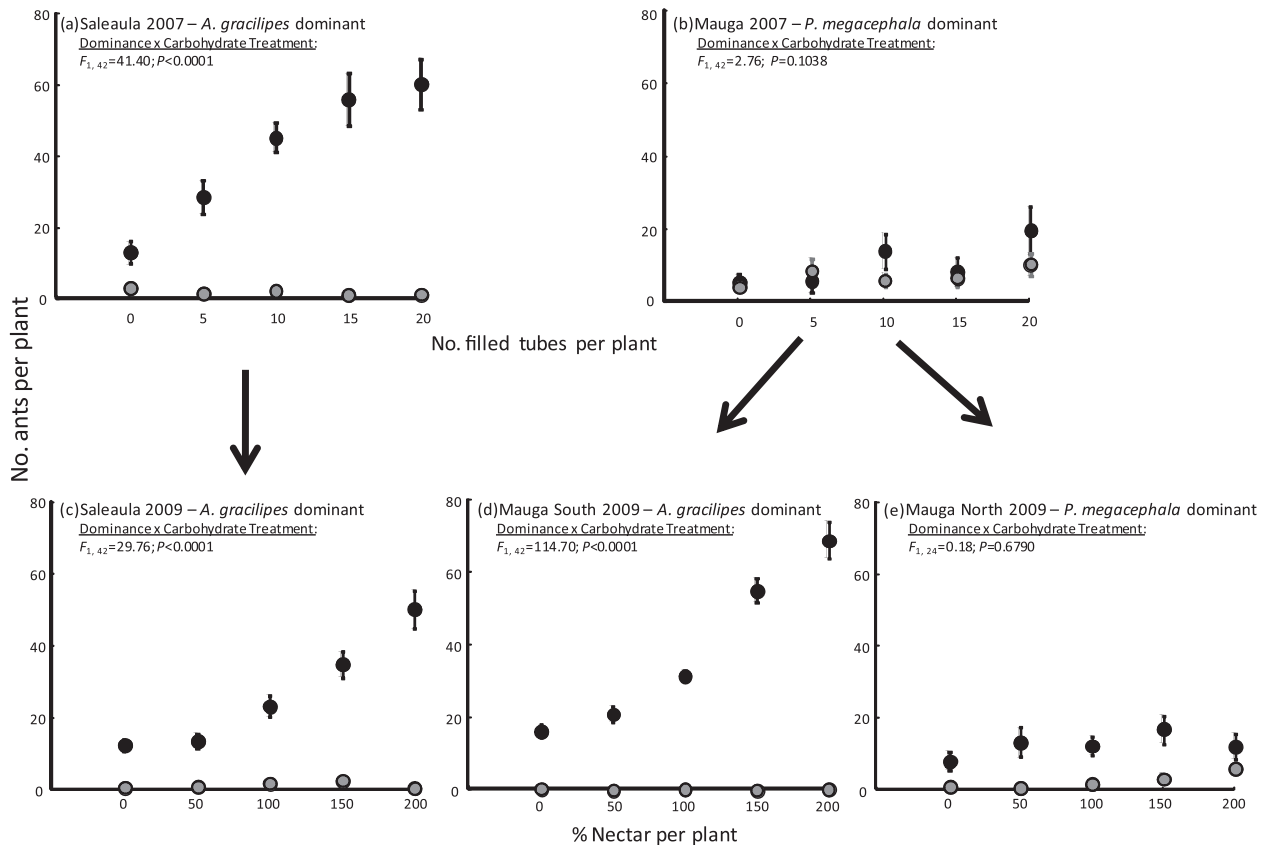
To test (2) Does increasing plant-based carbohydrate levels change the honeydew-excreting insects-tending behaviours of *A. gracilipes* and/or non-invasive ants?, we examined the proportion of workers tending honeydew-excreting insects using the same repeated measures analyses as described for question (1). Proportion data were arcsine square-root transformed to meet assumptions of normality and heterogeneity.

## RESULTS

Assessments of ground and plant foragers outside of treatment blocks confirmed that the relative abundances of *A. gracilipes* and co-occurring non-invasive ants were similar both on *M. citrifolia* plants and on the ground, although there were more ants observed on plants than on the ground (Table 1). These data confirmed that the patterns below are likely indicative of site-level ant assemblage characteristics. However, we focused on the plant-foraging ant community in order to present our results conservatively.

### (1) Do invasive *A. gracilipes* workers differ from co-occurring non-invasive ants in their response to increasing carbohydrate levels?

Experimental increases in carbohydrate availability strongly increased the short-term densities of *A. gracilipes* on plants but had no significant effects on other ant species. In 2007, *A. gracilipes* recruited to increasing carbohydrate availability at Saleaula, with an increase in worker density per plant of ~400% for the 20 tubes treatment relative to zero tubes. However, few workers of other ant species were observed on treatment plants and their numbers did not respond to carbohydrate availability (Table S1a, Fig. 2a). Mean-



**Fig. 2.** Ant density as a function of the carbohydrate treatment. Black circles (●) represent the abundance of the dominant species in a site per plant and grey circles (●) represent the combined abundances of all subordinate ant species per plant. Error bars represent  $\pm 1$  SE of the mean.

while, at Mauga, the reference site, there was no significant difference between the response of the dominant species (*P. megacephala*) and co-occurring, subordinate ant species. Although both dominant and subordinate ants were somewhat more abundant on plants with high carbohydrate levels at Mauga, the carbohydrate treatment effect was not statistically significant for either (Table S1a, Fig. 2b).

In 2009, *A. gracilipes* workers again responded strongly to our carbohydrate manipulations, while workers of co-occurring ant species were infrequent visitors to *M. citrifolia* plants and non-responsive to carbohydrate treatments. This effect was consistent at both the site with the established *A. gracilipes* invasion (Saleaula) and in the newly invaded region of Mauga South (Table S1a, Fig. 2c,d). However, outside of the *A. gracilipes* invasion front in Mauga (Mauga North), neither the dominant or subordinate ants significantly recruited to increasing carbohydrates, although both dominant *P. megacephala* and subordinate ants were observed on *M. citrifolia* plants (Table S1a, Fig. 2e). Over the course of the experiments in both 2007 and 2009, there were some cases in which the magnitude of the carbohydrate treatment effect strengthened through time. This resulted in significant 2- and 3-way

interactions with time, but did not change the relative rankings of treatments during any given census (Table S1a).

Across-site differences in the response of *A. gracilipes* versus non-invasive ants to carbohydrate treatments were significant in both 2007 and 2009 (Table S1b). Across both years and sites, *A. gracilipes* displayed a strong, positive foraging recruitment response to increasing levels of carbohydrates (Saleaula 2007:  $r = 0.74$ ,  $P < 0.0001$ ; Saleaula 2009:  $r = 0.80$ ,  $P < 0.0001$ ; Mauga South 2009:  $r = 0.93$ ,  $P < 0.0001$ ). However, there was only a slight, positive response of ant assemblages at the site that lacked *A. gracilipes* in 2007 (Mauga 2007:  $r = 0.45$ ,  $P = 0.0232$ ), and no foraging response of non-*A. gracilipes* workers in 2009 (Mauga North 2009:  $r = 0.37$ ,  $P = 0.1800$ ).

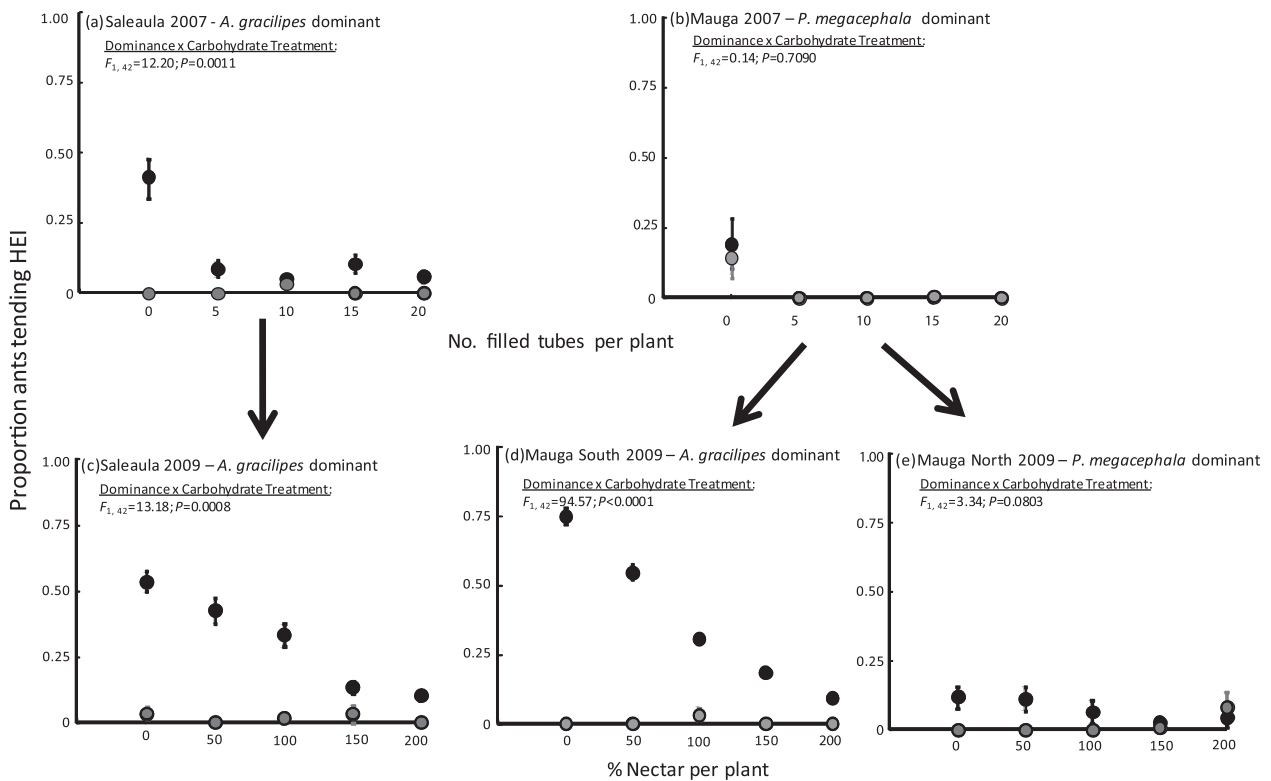
## (2) Does increasing plant-based carbohydrate levels change the honeydew-excreting insects-tending behaviours of *A. gracilipes* and/or non-invasive ants?

Carbohydrate manipulations altered ant behaviours towards honeydew-excreting insects. At Saleaula in

2007, the percentage of *A. gracilipes* workers that tended honeydew-excreting insects decreased from 42% ( $\pm 7\%$ ) in the zero filled tubes treatment to 9% ( $\pm 3\%$ ) in the treatment with five filled tubes (450% ambient levels) and remained below 10% for all other carbohydrate levels (Fig. 3a). Only one non-*A. gracilipes* worker (*Monomorium pharoensis*) was observed tending honeydew-excreting insects during the entire course of the experiment, resulting in a significant ant dominance status-carbohydrate treatment interaction (Table S2, Fig. 3a). Ants at the reference site displayed similar patterns, with the only discernable honeydew-excreting insects tending occurring at the zero tubes treatment by the species *P. megacephala* and *Paratrechina longicornis*. However, both dominant *P. megacephala* workers and workers from co-occurring ant species responded similarly by abandoning tending when carbohydrates increased (Table S2, Fig. 3b). *Anoplolepis gracilipes* workers did not respond significantly differently to carbohydrate treatments in terms of honeydew-excreting insects-tending behaviours than ants at the reference (uninvaded) site in 2007 (Table S2).

Carbohydrate treatments for the 2009 trials were closer to ambient levels (0–200%). However, even with these more subtle carbohydrate subsidies, there was a significant linear decrease in honeydew-

excreting insects tending as nectar levels increased at both Saleaula (~81% reduction) and within the *A. gracilipes* invasion front at Mauga South (~89% reduction; Table S2, Fig. 3c,d). Outside of the invasion front (Mauga North), there was no effect of the carbohydrate treatment on honeydew-excreting insects-tending rates, which were generally low (Table S2, Fig. 3e). Outside of the invasion front, more *P. megacephala* workers were observed tending honeydew-excreting insects than any other ant species, there was no consistent effect of our carbohydrate treatment through time, and this high variability resulted in a significant interaction between time and the carbohydrate treatment (Table S2). In contrast to the 2007 trials, there was a significant difference between the honeydew-excreting insects-tending responses of *A. gracilipes* and ants at the uninvaded site. Across both Saleaula and Mauga South, *A. gracilipes* workers displayed a strong, negative response to carbohydrate treatments (Saleaula 2009:  $R^2 = 0.6117$ ,  $P < 0.0001$ ; Mauga South 2009:  $R^2 = 0.9230$ ,  $P < 0.0001$ ). Conversely, ants outside of the *A. gracilipes* invasion front in Mauga did not change their honeydew-excreting insects-tending behaviours as carbohydrate availability increased (Mauga North 2009:  $R^2 = 0.0742$ ,  $P = 0.3259$ ).



**Fig. 3.** Proportion of ants tending honeydew-excreting insects (HEI) as a function of the carbohydrate treatment. The proportion of the dominant species at each site is denoted with black circles (●) and the proportion of all subordinate ants that were observed tending honeydew-excreting insects are represented by grey circles (●). Error bars represent  $\pm 1$  SE of the mean.

## DISCUSSION

Many invasive ants readily form facultative associations with carbohydrate-excreting plants and insects. These putatively mutualistic interactions may accelerate the progression of non-native ant invasions, particularly if invasive ant species respond more strongly to carbohydrate resources than co-occurring non-invasive ant species (Holway *et al.* 2002). As an initial test of the predictions of this nectar subsidy hypothesis, our experiments created variability in plant-based carbohydrate resources and demonstrated that short-term increases in carbohydrate availability can increase the density of foraging ants on plants. Importantly, the effects of carbohydrate manipulations were significantly stronger for invasive *A. gracilipes* ants than for non-invasive ants, a pattern that was consistent across and within five site-year combinations along an active *A. gracilipes* invasion front.

### Importance of carbohydrate resources to invasive ants

For all cases in which *A. gracilipes* dominated the local ant assemblage, this species responded strongly to experimentally increased carbohydrate levels while non-invasive ants that co-occurred at the same or at nearby sites did not respond strongly. Several mechanisms could underlie this difference in response between the invader species and other ant species. First, a strong *A. gracilipes* response could be a simple consequence of dominance, as dominant ant species can exclude subordinate ants from resources (Andersen 1992). However, when *A. gracilipes* was absent from the site, there was no significant influence of carbohydrate manipulations on the total density of other ant species observed on *M. citrifolia* plants, including the dominant species, *P. megacephala*. Recently, Lach (2005) evaluated the relative foraging behaviours of co-occurring *A. gracilipes*, *P. megacephala* and *L. humile* (Argentine ant, also considered a dominant invasive in many regions) at floral nectaries across multiple sites in Hawaii. Consistent with our results, *A. gracilipes* visited flowers at the highest densities and were the least likely of the three species to abandon nectaries. Moreover, *P. megacephala* visited flowers at the lowest densities and were the most likely to abandon nectaries of the three species Lach compared. Together, these results suggest that nectar may be particularly important for invasive *A. gracilipes* workers, and particularly unimportant for *P. megacephala* workers. Additional studies that manipulate carbohydrate availability for ant species that vary in their degree of invasiveness will help to elucidate the mechanism(s) of these patterns.

The importance of carbohydrate resources to ants has been hypothesized to vary during the temporal progression of ant invasions. Specifically, some research has suggested that increased carbohydrate availability may allow exotic ant invasions to progress past benign lag phases (Holway *et al.* 2002; O'Dowd *et al.* 2003). For example, the diets of invasive *L. humile* workers shifted towards plant-based resources and honeydew-producing hemipterans only after invading populations became established (Tillberg *et al.* 2007). However, we rarely know exactly when a species is introduced into a new region or the rate of progression for any particular invasion, making it difficult to evaluate the validity of this hypothesis. In our study, the approximate date of arrival of *A. gracilipes* to Mauga was known. In less than 2 years, *A. gracilipes*-dominated ant assemblages at this site displayed a response to carbohydrate manipulations that closely resembled the response of ants at a site with an older, established *A. gracilipes* invasion (Saleaula). The responses of ants in *A. gracilipes*-dominated regions differed markedly from those in areas that were not dominated by *A. gracilipes*. This finding suggests that strong recruitment to carbohydrates may be a characteristic of invasive *A. gracilipes* generally, rather than specific to a particular stage of its invasion. However, replication of the age of the invasion would be needed to directly address this question.

### Extrafloral nectar availability and Hemiptera tending by ants

During the 2007 trials, all ants, regardless of site, reduced honeydew-excreting insects-tending behaviours when they were provided with additional carbohydrates. For these trials, carbohydrate supplements were, at minimum, 450% of ambient *M. citrifolia* nectar levels and the only treatment level in which an appreciable proportion of ants tended honeydew-excreting insects was zero. This abandonment of tending was displayed by ants at both the invaded and the reference (uninvaded) site. Interestingly, the zero filled tubes treatment represented a carbohydrate level that was lower than ambient levels. This suggests that the attractiveness of honeydew-excreting insects to local ant assemblages increases as other sources of carbohydrates on *M. citrifolia* plants decline. Because artificial nectaries closely mimicked the composition of naturally occurring *M. citrifolia* nectar (Freeman *et al.* 1991), but lacked the additional amino acids that are more commonly found in hemipteran honeydew (e.g. Blüthgen *et al.* 2004), we interpret this result as a preference for plant-based carbohydrate resources over those derived from honeydew-excreting insects.



Because changes in ant behaviour occurred at both the *A. gracilipes*-dominated and *P. megacephala*-dominated sites, *A. gracilipes* and other ant species appeared to respond similarly to the choice between plant nectar and hemipterans on *M. citrifolia*. As with overall forager recruitment to *M. citrifolia* plants, *A. gracilipes* workers displayed a stronger response to carbohydrate manipulations in terms of reduced honeydew-excreting insects tending than did co-occurring non-invasive ants within the same site. However, the amount of artificial nectar that we provided ants in the 2007 trials was higher than ambient levels produced by *M. citrifolia* plants, making it difficult to extrapolate to ant behaviours under more natural conditions.

In response to more realistic nectar manipulations, *A. gracilipes* workers again displayed a strong negative honeydew-excreting insects-tending response, while ants outside of the *A. gracilipes* invasion front in 2009 no longer changed their honeydew-excreting insects tending as carbohydrate resources increased. These 2009 trials not only included more realistic levels of artificial nectar, but also allowed ants to access actual *M. citrifolia* nectar. Tending of honeydew-excreting insects at both *A. gracilipes*-dominated sites decreased linearly as carbohydrate availability increased. At sites that were not dominated by *A. gracilipes*, this trend was less clear, but a weak trend for reduced honeydew-excreting insects tending with increased carbohydrate availability remained. These results suggest that an increase in the plant's nectar resources can distract ants from the honeydew resources of hemipterans and redirect foraging efforts to the potentially more abundant or higher-quality plant-derived nectar, as proposed by Becerra and Venable (1989). Furthermore, the fact that honeydew-excreting insects were abandoned at high levels of carbohydrate availability suggests that there may be a limit to the amount of carbohydrate resources that *A. gracilipes* and co-occurring non-invasive ants will collect. Perhaps at high levels of carbohydrate availability, workers become more protein-starved (e.g. Ness *et al.* 2009) or costs associated with collecting carbohydrates (in terms of time or effort expended) outweigh the benefits provided by collecting both honeydew and nectar. Alternatively, these patterns may be due to the ease of obtaining artificial nectar from tubes, relative to honeydew and EFN. However, the linear decline in tending behaviours during the 2009 trials makes this unlikely because tending behaviours were reduced when ants were allowed access to different levels of natural plant nectar. More generally, if decreased tending of honeydew-excreting insects leads to declines in the abundance of hemipterans, this change in ant behaviour could cascade to the plant as well as to other community members (Styrsky & Eubanks 2007).

## CONCLUSIONS

Our experiments showed that increases in carbohydrate resources can produce strong, short-term changes in the relative foraging densities and honeydew-excreting insects-tending behaviours of ants on plants. Furthermore, invasive *A. gracilipes* workers were more responsive to carbohydrate subsidies than were other ant species at our sites. These findings provide a first step towards confirming that interactions with carbohydrate-excreting species promote ant invasions. If further studies spanning more sites and different invasion histories confirm the strong patterns reported here, then ant-plant protective mutualisms may have community-wide consequences by promoting *A. gracilipes* invasions. Previous studies have found impacts of this species to cascade to canopy tree survival and seedling establishment (O'Dowd *et al.* 2003), and to affect the abundance, behaviours and reproduction of native birds (Davis *et al.* 2008) and crabs (O'Dowd *et al.* 2003; McNatty *et al.* 2009).

Caution should be exercised in the interpretation of these results for two reasons. We tested the responses of *A. gracilipes* workers and workers from co-occurring non-invasive ant species to carbohydrate subsidies along an invasion history gradient, spanning two different years. However, within each year, each invasion category was only represented by one (or two) replicate. Future studies with greater replication are therefore needed to determine the generality of our findings. Additionally, we focused on plant-foraging ants, not on population-level dynamics (which would have included ground foragers and nests).

Nonetheless, our results demonstrate that ant species can differ markedly in their responses to carbohydrate-rich resources. Evidence for stronger responses of invasive *A. gracilipes* compared with other ant species marks a first step towards understanding the importance of carbohydrate resources in fuelling this and other ant invasions.

## ACKNOWLEDGEMENTS

We thank Levao Fa'a Malo and Paogo Malo in the villages of Saleaula and Mauga for graciously allowing us to use their land for our experiments. Thanks also to Meki Tauai, Maiava Veni, Pati Liu, Afele Failagi and Susau Siolo in the Samoan Ministry of Natural Resources and Environment for logistical and field assistance. We also thank Phil Lester for thoughtful comments on previous versions of this manuscript. This work was supported by the National Geographic Society grant 8237-07, by the Godwin Assistant Professorship to JAR, and by a Rice University Wray-Todd Fellowship to AMS.

## REFERENCES

- Abbott K. L. (2005) Supercolonies of the invasive ant, *Anoplolepis gracilipes*, on an oceanic island: forager activity patterns, activity and biomass. *Insectes Soc.* **52**, 266–73.
- Abbott K. L. (2006) Spatial dynamics of supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean. *Divers. Distrib.* **12**, 101–10.
- Andersen A. N. (1992) Momentary diversity by dominant species in exceptionally rich ant communities of the Australian seasonal tropics. *Am. Nat.* **140**, 401–20.
- Becerra J. X. I. & Venable D. L. (1989) Extrafloral nectaries—a defense against ant-Homoptera mutualisms? *Oikos* **55**, 276–80.
- Blüthgen N., Gottsberger G. & Fielder K. (2004) Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian Rainforest. *Austral Ecol.* **29**, 418–29.
- Davis N. E., O'Dowd D. J., Green P. T. & MacNally R. (2008) Effects of an alien ant invasion on abundance, behavior, and reproductive success of endemic island birds. *Conservation Biology* **22**, 1165–76.
- Dunham A. E. & Mikheyev A. S. (2010) Influence of an invasive ant on grazing and detrital communities and nutrient fluxes in a tropical forest. *Divers. Distrib.* **16**, 33–42.
- Freeman C. E., Worthington R. D. & Jackson M. S. (1991) Floral nectar sugar compositions of some south and south-east Asian species. *Biotropica* **23**, 568–74.
- Fritts T. H. & Rodda G. H. (1998) The role of introduced species in the degradation of island ecosystems: a case history of Guam. *Annu. Rev. Ecol. Syst.* **29**, 113–40.
- Grover C. D., Kay A. D., Monson J. A., Marsh T. C. & Holway D. A. (2007) Linking nutrition and behavioural dominance: carbohydrate scarcity limits aggression and activity in Argentine ants. *Proc. R. Soc. B* **274**, 2951–7.
- Helms K. R. & Vinson S. B. (2002) Widespread association of the invasive ant *Solenopsis invicta* with an invasive mealybug. *Ecology* **83**, 2425–38.
- Hoffmann B. D. & Parr C. L. (2008) An invasion revisited: the African big-headed ant (*Pheidole megacephala*) in northern Australia. *Biol. Invasions* **10**, 1171–81.
- Holway D. A., Lach L., Suarez A. V., Tsutsui N. D. & Case T. J. (2002) The causes and consequences of ant invasions. *Annu. Rev. Ecol. Syst.* **33**, 181–233.
- King J. R. & Tschinkel W. R. (2008) Experimental evidence that human impacts drive fire ant invasions and ecological change. *Proc. Natl. Acad. Sci. USA* **51**, 20339–43.
- Koptur S. (1979) Facultative association between weedy vetches bearing extrafloral nectaries and weedy ants in California. *Am. J. Bot.* **66**, 1016–20.
- Lach L. (2003) Invasive ants: unwanted partners in ant-plant interactions? *Ann. Mo. Bot. Gard.* **90**, 91–108.
- Lach L. (2005) Interference and exploitation competition of three nectar-thieving invasive ant species. *Insectes Soc.* **52**, 257–62.
- Lester P. J. & Tavite A. (2004) Long-legged ants, *Anoplolepis gracilipes* (Hymenoptera: Formicidae), have invaded Tokelau, changing composition and dynamics of ant and invertebrate communities. *Pac. Sci.* **58**, 391–401.
- Lowe S., Browne M. & Boudjelas S. (2000) 100 of the world's worst invasive alien species. *Aliens* **12**, 1–12.
- McGlynn T. P. (1999) The worldwide transfer of ants: geographical distribution and ecological invasions. *J. Biogeogr.* **26**, 535–48.
- McNatty A., Abbott K. L. & Lester P. J. (2009) Invasive ants compete with and modify the trophic ecology of hermit crabs on tropical islands. *Oecologia* **160**, 187–94.
- Ness J. H. & Bronstein J. L. (2004) The effects of invasive ants on prospective ant mutualists. *Biol. Invasions* **6**, 445–61.
- Ness J. H., Morris W. F. & Bronstein J. L. (2009) For ant-protected plants, the best defense is a hungry offense. *Ecology* **90**, 2823–31.
- O'Dowd D. J., Green P. T. & Lake P. S. (2003) Invasional 'meltdown' on an oceanic island. *Ecol. Lett.* **6**, 812–17.
- Pimentel D., McNair S., Janecka J. *et al.* (2001) Economic and environmental threats of alien plant, animal, and microbe invasions. *Agric. Ecosyst. Environ.* **84**, 1–20.
- Porter S. D. & Savignano D. A. (1990) Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. *Ecology* **71**, 2095–106.
- SAS Institute (2003) *SAS Version 9.1.3*. SAS Institute, Cary.
- Savage A. M., Rudgers J. A. & Whitney K. D. (2009) Elevated dominance of extrafloral nectary-bearing plants is associated with increased abundances of an invasive ant and reduced native ant richness. *Diversity Distrib.* **15**, 751–61.
- Shattuck S. O. (1999) *Australian Ants: Their Biology and Identification*. Monographs on Invertebrate Taxonomy, Vol. 3. CSIRO Publishing, Collingswood.
- Styrsky J. D. & Eubanks M. D. (2007) Ecological consequences of interactions between ants and honeydew-producing insects. *Oecologia* **149**, 465–73.
- Tillberg C. V., Holway D. A., LeBrun E. G. & Suarez A. V. (2007) Trophic ecology of invasive Argentine ants in their native and introduced ranges. *Proc. Natl. Acad. Sci. USA* **104**, 20856–61.
- von Ende C. N. (2001) Repeated measures analysis: growth and other time dependent measures. In: *Design and Analysis of Ecological Experiments* (eds S. M. Scheiner & J. Gurevitch) pp. 134–57. Oxford University Press, New York.
- Waki J., Olkpu T. & Komolong M. K. (2007) A descriptor list for morphological characterization of Noni (*Morinda citrifolia* L.). *S. Pac. J. Nat. Sci.* **10**, 61–6.
- Wetterer J. K. (2005) Worldwide distribution and potential spread of the long-legged ant, *Anoplolepis gracilipes* (Hymenoptera: Formicidae). *Sociobiology* **45**, 77–96.
- Whistler W. A. (1993) *Flowers of the Pacific Island Seashore: A Guide to the Littoral Plants of Hawaii, Tahiti, Samoa, Tonga, Cook Islands, Fiji and Micronesia*. University of Hawaii Press, Honolulu.
- Wilson E. O. & Taylor R. W. (1967) The ants of Polynesia (Hymenoptera: Formicidae). *Pac. Insects Monogr.* **14**, 1–10.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Results from (a) within-site and (b) between-site repeated measures analyses of covariance (ANCOVAR) for ant density as a function of (a) ant status and carbohydrate treatment and (b) site and carbohydrate treatment. Data were square-root transformed prior to analyses.

**Table S2.** Results from within-site repeated measures analyses of covariance (ANCOVAR) for ant density as a function of ant status and carbohydrate treatment. Data were arc-sin square-root transformed prior to analyses.