ORIGINAL PAPER

Plant-based food resources, trophic interactions among alien species, and the abundance of an invasive ant

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Abstract   Recent research on invasive ants suggests that their success may be facilitated by increased resources at introduced locations stemming from the emergence of novel trophic interactions with abundant honeydew-producing Hemiptera. Moreover, those Hemiptera may themselves often be introduced or invasive. To test the importance of mutualisms for invasive species, we conducted a study in the southeastern United States of factors hypothesized to affect the abundance of an invasive ant native to South America, *Solenopsis invicta*. The study was conducted within grazing pastures, where *S. invicta* can be extremely abundant while also exhibiting substantial variability in abundance. A path analysis showed that the abundance of *S. invicta* was strongly and positively affected by the abundance of an invasive honeydew-producing mealybug native to Asia, *Antonina graminis*, and by the mealybugs' host grasses because of their strong positive effect on mealybug abundance. Abundance of the mealybug was primarily attributable to an invasive host grass native to Africa, *Cynodon dactylon*. The abundance of *S. invicta* was also positively affected by the abundance of other arthropods that they are likely to consume, and those arthropods were positively affected by the abundance of both the *A. graminis* host grasses and other plants. Thus the study shows that the distribution and abundance of different plant species could have important effects on the abundance of *S. invicta* through their effect on the ants' food resources. The results are also consistent with the hypothesis that the emergence of novel trophic interactions among invasive species can promote the abundance of invasive ants.

Keywords   *Antonina graminis* · Ants ·
Hemiptera · Honeydew · Invasional meltdown ·
Solenopsis invicta

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Introduction

Populations of invasive species are often much larger at introduced locations than within their native range. Comparisons of their native and introduced ranges suggest that increased abundance at introduced locations may result from a relaxed competitive environment, or a reduction or absence of natural

enemies, or an increase in suitable habitat or other resources (Elton 1958; Vitousek et al. 1996; Mack et al. 2000; Sakai et al. 2001; Shea and Chesson 2002; Torchin et al. 2003). One or more of these factors could play a role in the success of invasive ants. Evidence suggests that the natural enemies of invasive ants can be rare or absent in the introduced range (Porter et al. 1997; Cremer et al. 2008), and invasive ants can also be well adapted to disturbed habitats that occur frequently at introduced locations (King and Tschinkel 2006, 2008). Moreover, behavioral characteristics such as multiple queen colonies (polygyny) that reproduce by budding can limit intraspecific competition and facilitate large populations (Macom and Porter 1996; Tsutsui et al. 2000; Abbott 2005; Ross et al. 1996; Cremer et al. 2008; Vogel et al. 2009). A number of factors, some general and applicable to other invasive organisms and some specific to social insects can promote their success, and multiple factors could work in concert.

A relatively new insight into the success of invasive ants is that they may encounter increased resource abundance in the introduced range from forming novel associations with honeydew-producing Hemiptera whose populations can also be very large (Helms and Vinson 2002; Holway et al. 2002; O'Dowd et al. 2003; Kaplan and Eubanks 2005; Abbott and Green 2007; Tillberg et al. 2007; Paris and Espadaler 2009). The Hemiptera with which invasive ants associate are sometimes introduced or invasive themselves, and when this is the case, both parties could lack substantial natural enemies from their native ranges (Simberloff and Von Holle 1999; Helms and Vinson 2002 (and references therein); O'Dowd et al. 2003). Large populations of honeydew-producing Hemiptera can provide an abundant source of carbohydrate-rich honeydew for invasive ants, while the ants may in turn provide services to the Hemiptera promoting their mutual abundance (Helms and Vinson 2003, 2008; Abbott and Green 2007). Honeydew allows for increased colony growth over that possible from consuming only arthropod tissues (Porter 1989; Helms and Vinson 2008), and they may fuel increased worker activity, perhaps allowing colonies to more quickly find and monopolize other food sources (Davidson 1997; Grover et al. 2007).

One of the most ecologically and economically important invasive ants is the red imported fire ant, *Solenopsis invicta* Buren, introduced into the southeastern United States from South America sometime in the 1930s (ISSG 2006). It is now also established in the southwestern United States, as well as parts of Asia, Australia, and the Caribbean Islands (ISSG 2006). Comparative study of *S. invicta* within its native and introduced ranges suggests that an absence of their natural enemies may be an important factor promoting its success at introduced locations (Porter et al. 1997; Williams et al. 2003; Hashimoto and Valles 2008). A preference for disturbed habitats, common in the introduced range, may also be important (Tschinkel 1987a, b; King and Tschinkel 2008). One disturbed habitat where *S. invicta* is particularly abundant in this region is grazing pastures (e.g. Tschinkel 2006). Their success in this environment may be facilitated by its openness that results in abundant solar radiation to warm the elevated mound structures within which immature *S. invicta* are reared (Vogt et al. 2008), and perhaps by a reduction in native species with which it would compete (King and Tschinkel 2006). Moreover, grazing pastures can harbor abundant populations of other introduced species occurring at high densities that can serve directly or indirectly as food resources for *S. invicta* (Hu and Frank 1996; Helms and Vinson 2008). Such species can include honeydew-producing Hemiptera, and honeydew of an invasive mealybug, *Antonina graminis*, has been estimated to supply from 16% to 48% of the nutritional energy utilized by *S. invicta* colonies in the southeastern United States (Chada and Wood 1960; Helms and Vinson 2002). Moreover, an experiment showed that *A. graminis* can serve as an important intermediary in the transfer of carbohydrates to *S. invicta* from grasses, and that *A. graminis* honeydew substantially increases colony growth over that possible from the consumption of only arthropod prey (Helms and Vinson 2008).

That *S. invicta* is associated with honeydew producing Hemiptera, and especially *A. graminis*, across the southeastern United States (Helms and Vinson 2002), coupled with an experiment showing that *A. graminis* honeydew can greatly increase *S. invicta* colony growth and population size (Helms and Vinson 2008), serve as a foundation for the hypothesis that *A. graminis* and other honeydew-producing Hemiptera promote large populations of *S. invicta*. Moreover, because these Hemiptera utilize a limited number of available plant species as hosts (e.g. Helms and Vinson 2000, 2002), it follows that

their host plants could be indirectly important in promoting increases in population size in *S. invicta*. This hypothesis might be tested with a field experiment that removes Hemiptera and/or their host plants to determine if it decreases *S. invicta* populations. This is difficult, however, because of the largely subterranean locations of *A. graminis* as well as many other Hemiptera (Chada and Wood 1960; Helms and Vinson 2002). Their removal would necessitate extensive disturbance of soils and plants with unwanted side effects on *S. invicta* and their non-honeydew food resources. Chemical control of Hemiptera is another option, but also problematic because insecticides could have a direct negative impact on *S. invicta*, while also having a negative affect on the non-hemipteran arthropods that *S. invicta* consumes. Removal of Hemiptera host plants presents a similar problem because of extensive disturbance and because those plants can serve as food for herbivorous insects other than Hemiptera which *S. invicta* consume. The best currently available test for the importance of honeydew-producing Hemiptera and their host plants to *S. invicta* in the field is to determine if their population sizes are significantly correlated. No current study has tested whether this is the case.

Grazing pastures are an excellent system to test for association between the population size of *S. invicta* and honeydew-producing Hemiptera and their host plants. Within these pastures, the density of *S. invicta* can vary greatly between locations, with areas of high and low abundance occurring in close proximity. Moreover, the distribution and abundance of Hemiptera and their host plants can also vary spatially. We exploit these naturally occurring differences to test for association between the abundance of *S. invicta*, honeydew-producing Hemiptera, their host plants, and a number of other potentially important factors. To do so, we employ path analysis, a powerful technique for testing hypotheses of cause and effect relationships among variables with both direct and indirect effects (Li 1975; Shipley 2002).

Methods

In order to control for differences associated with region or habitat in determining the abundance of *S. invicta*, our study focused on a single habitat in south-central Texas. We hypothesized that differences in the abundance of *S. invicta* in our study would be determined by a number of different factors, including the frequency of single (monogyne) versus multiple queen (polygyne) colonies and the availability of food resources (Fig. 1). The source of food resources considered are honeydew produced by Hemiptera (carbohydrates) and arthropods that *S. invicta* may prey upon or scavenge (proteins; Fig. 1). Hemiptera abundance would be affected by the abundance of their host plants, while the
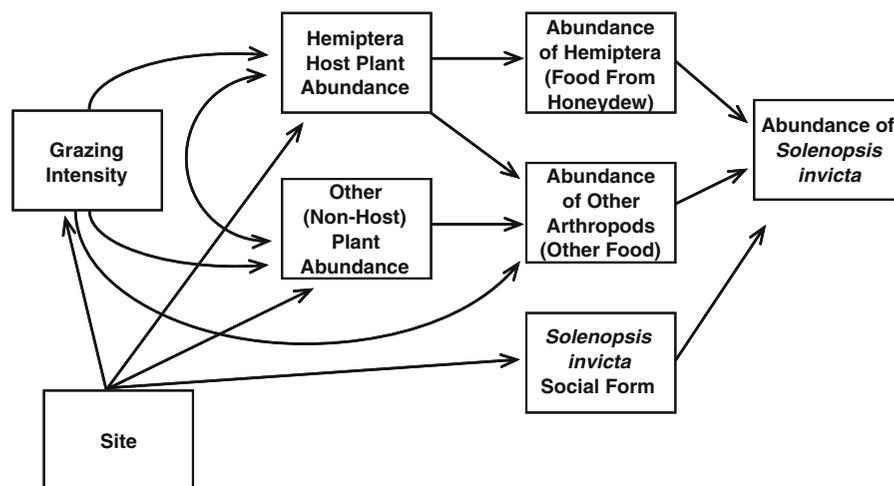

Fig. 1 Path diagram of factors hypothesized a priori and tested as important in affecting the abundance of *Solenopsis invicta* in grazing pastures. Note that the relationship between grazing intensity and the abundance of other arthropods is hypothesized because of the potential importance of arthropods associated with dung (Hu and Frank 1996)

abundance of prey and scavenge would be affected by the abundance of all plants, via their consumption by arthropod herbivores as well as the subsequent consumption of those herbivores by predator arthropods that *S. invicta* may also consume (Fig. 1). We also hypothesized that the productivity of plants would be affected by grazing intensity, and that grazing intensity would also affect the abundance of dung-inhabiting arthropods that *S. invicta* might consume (Hu and Frank 1996; Fig. 1). Differences in the abundance of host and non-host plants, as well as grazing intensity, and *S. invicta* social form, could also be affected by intrinsic differences among sites within which study plots occurred (Fig. 1).

Data were collected in September 2006, from 50 to 100 m^2 (10 × 10 m) plots located at five open grazing pasture sites (10 plots per site) in Brazos County, Texas. The dominant vegetation at all sites was grasses, often intermixed with low shrubby and weedy plants, primarily *Helenium amarum*, *Croton* sp., and *Solanum* sp. Mean distance between sites was 14.6 km (median = 12.4). Sites ranged in size from four to 40 ha. Plots were located at random and were dispersed throughout each site. In each plot, we estimated the abundance of *S. invicta* and those factors hypothesized to affect their abundance (Fig. 1). Plant abundance was estimated by collecting all plants and their roots to a depth of 5 cm from four 15 cm diameter circular areas (177 cm^2) randomly located within each plot. These samples were pooled within plots to result in a single overall sample per plot. Plants were sorted to species and whether or not they were host plants of honeydew-producing Hemiptera. A plant species was designated a host if Hemiptera were found on that species within any plot. Plants were inspected for Hemiptera in the field and again in the laboratory under 10× magnifications. All Hemiptera were then removed from host plants and the numbers in samples from each plot were counted, and then dried for 1 week at 50°C. Small, first instars (crawlers) of the mealybug *A. graminis* were excluded because this is the dispersal phase of the life cycle when honeydew is not produced. Hemiptera dry mass was measured from within only 32 of 37 plots where they occurred, so we used a common dry mass per individual over the 32 plots to estimate total mass per plot for all plots. Since the generation time of Hemiptera we found is short (approximately 60 days) and multiple

overlapping generations are produced per year (Chada and Wood 1960), the mean size across plots is a good estimate of their average size within plots at any particular point in time.

All plants were rinsed thoroughly with water to remove soil and debris, and then cut at their previous ground level in order to separate them into above and below-ground (root) material. Because our sites were grazed, we utilized below-ground dry mass as an estimate of plant abundance (e.g. McNaughton et al. 1998). Plant samples were dried for 1 week at 50°C. To estimate grazing intensity, we counted and recorded the number of individual dung deposits that appeared to result from a single deposition event of either cattle (four sites) or sheep (one site) in each plot. We estimate that such deposits ranged in age from at least a few months prior to our study to the time the study was conducted.

To assess the abundance of *S. invicta* and other ground-dwelling arthropods, we utilized four pitfall traps (9 cm in diameter, 12 cm in depth plastic cups) per plot. Traps were inserted into the soil flush with the surface in an equidistant grid at 3.3 m intervals within the interior of each plot, and then filled with soil. After acclimatizing arthropods to trap presence for 1 week, the soil was removed and the traps activated by adding propylene glycol to a depth of 3 cm. After 72 h, the traps were collected and the contents were sorted into taxonomic categories. All were rinsed thoroughly to remove propylene glycol, dried at 50°C for 1 week, and the dry mass of each category in each plot was measured. Because of collection in propylene glycol, our estimates of mass differ to some degree from their mass prior to immersion; however, the taxa of arthropods trapped appeared quite uniform between different plots and differed primarily in their abundance. Because our study analyzes only relative differences in arthropod abundance between plots, and makes no inferences regarding actual arthropod mass within plots, differences in mass attributable to propylene glycol immersion should not be of great importance. In seven of 50 plots, one (5 plots) or two (2 plots) traps were disturbed by animals and their data were excluded from analysis. Thus, data from traps were pooled within each plot and arthropod abundance was divided by the number of undisturbed traps to result in per trap per plot estimates. Because our a priori hypothesis was that available food would be one

important factor in affecting the abundance of *S. invicta*, and since this ant preys upon live arthropods and scavenges the remains of those that are dead, we combined the mass of the all arthropod groups in traps (other than *S. invicta*) into a single estimate of food available from arthropods. The assumptions of this procedure are addressed further in the discussion section.

Differences among sites in precipitation could result in different levels of arthropod activity and thus different pitfall trap capture rates between sites. To determine if this could be important in our results, we tested whether soil moisture differed among locations during trapping with gypsum sensors connected to a Delmhorst KS-D1, digital soil moisture meter (Delmhorst Instrument Company, Towaco, NJ). Sensors were buried in the center of each plot to a depth of 5 cm 5 days prior to trap activation, and measurements were made on the day of activation and on the day that traps were collected. Soil moisture in plots did not differ significantly among sites, either at the beginning ($F = 0.88$, $P > 0.40$, One-way ANOVA) or the end of trapping ($F = 0.54$, $P > 0.70$, One-way ANOVA).

Potential problems with pitfall trap data arising because of differences in habitat, weather, and time of year during trapping (Topping and Sunderland 1992; Southwood 1994) are also minimized because plots were in a single habitat type within a single region, and trapping was conducted during a single period of time. Nevertheless, we evaluated the accuracy of pitfall trap data for relative abundance by testing how well estimates of *S. invicta* abundance from trap data corresponded with an independent estimate. We measured the total volume of all active *S. invicta* mounds within plots at four sites (40 plots). We then employed a relationship between mound volume and total *S. invicta* biomass developed by Tschinkel (1993) to estimate the total biomass of *S. invicta* per plot. Those estimates were positively and significantly correlated with the data from pitfall traps (Pearson's correlation coefficient (r) = 0.731, $P < 0.001$).

Because populations of *S. invicta* with polygyne (multiple queens) colonies are often larger than those with monogyne (single queen) colonies, both in numbers of workers and in total biomass of ants per unit area (Macom and Porter 1996), we assessed whether plots differed in the frequency of the different social forms. To do so, thirty workers were collected at random from peanut butter baits located at the center of each plot and their head-widths measured using a dissection microscope fitted with an ocular micrometer. As measured by width of their heads, workers from polygyne colonies are significantly smaller on average than those from monogyne colonies (Greenberg et al. 1992; Goodisman and Ross 1996). Although we address data on the estimated frequency of polygyne and monogyne colonies as supplemental information, our statistical analysis does not assign workers within plots as coming from monogyne or polygyne colonies based on body size (e.g. Greenberg et al. 1992). Rather, we predict that if social form is an important determinant of *S. invicta* population size, that mean worker size within plots will vary systematically according to *S. invicta* abundance. Specifically, if the abundance of *S. invicta* within plots increases with an increasing proportion of polygyne colonies, mean worker head-width should decrease with increasing *S. invicta* abundance. As supplementary information on the overall frequency of polygyny and monogyny at our study sites, we excavated a single colony mound from five plots at each site, and placed them into buckets lined with talcum powder to prevent escape. We allowed the ants 2 days to reconstruct a nest, and then the ants were separated from the soil by dripping water into the bucket (Jouvenaz et al. 1977). They were then removed from the bucket and any queens were recovered. Queens were dissected and their spermathecae removed, placed on a microscope slide in insect Ringer's solution, and broken open to release any sperm. The presence or absence of sperm was determined for each queen by observation under a compound microscope. The presence of more than a single inseminated queen per mound indicates a polygynous colony.

Statistical analyses and model construction

All data were corrected for non-normal distributions with Box–Cox power (λ) transformations using the statistical software Minitab 15 (Minitab, Inc.). Path analyses were conducted using the maximum likelihood method provided in the structural equation modeling software EQS (Multivariate Software, Inc.). Alternative models were constructed based on a priori hypothesized direct and indirect relationships

between variables and the abundance of *S. invicta* (Fig. 1). Model development first included a test of this model, then non-significant variables were removed and a new model constructed from the significant variables. Two criteria were used to validate the model: (1) a likelihood ratio chi-square test that evaluates the null hypothesis that the model covariance matrix is equal to the covariance matrix developed from the data, and (2) Akaike's information criterion (AIC) values which determine which model in a set of models is most parsimonious (Burnham and Anderson 2002).

Results

Solenopsis invicta were often abundant in pitfall traps, although their abundance also varied substantially among study plots (Table 1). An independent estimate using mound volumes to estimate biomass (Tschinkel 1993) concurs; the mean estimated total biomass of *S. invicta* per plot ($\pm$SE) was 579 ± 113 g (range = 0–2,983 g), while the mean number of mounds per plot was 6.4 ± 0.76 (range = 0–20). Variables hypothesized to affect the abundance of *S. invicta* also varied substantially in abundance among study plots (Table 1).

The path analysis of hypothesized interactions among variables showed that variation in the abundance of *S. invicta* was not significantly affected by site, grazing intensity as estimated by the number of dung deposits, or the frequency of different social forms as based on worker body size. All other factors were significant and were included in the final model. In that model, the abundance of *S. invicta* was positively affected by the abundance of the only honeydew-producing Hemiptera found, the invasive mealybug *A. graminis*, while the abundance of *A. graminis* was positively affected by the abundance of their host grasses (Fig. 2). The abundance of *S. invicta* was also positively affected by the abundance of other arthropods, while the abundance of these arthropods was positively affected by the abundance of both host and non-host plants (Fig. 2). There was also a negative correlation between the abundance of non-host plants and the abundance of *A. graminis* host plants (Fig. 2). Summary statistics from the path analysis are presented in Table 2.

The great majority of estimated plant abundance in study plots came from two non-native perennial grasses common in pastures of the southeastern United States, bermudagrass (*Cynodon dactylon*: 485.2 g, or 48.6% of total plant mass) and bahiagrass (*Paspalum notatum*: 402.5 g, or 40.2% of total plant

Table 1 Estimates of the abundance of *Solenopsis invicta* and factors hypothesized to be directly or indirectly important in determining their abundance

Variable	Mean number $\pm$ SE (range)	Mean dry mass (mg) $\pm$ SE (range)
Solenopsis invicta[a]	189.7 ± 39.5 (3.3–1,355.0)	28.01 ± 5.18 (0.54–154.47)
Antonina graminis[b]	11.10 ± 2.37 (0–79)	8.30 ± 1.76 mg (0–58.61)
Other arthropods[a]		
Crickets	5.30 ± 0.50 (1–19)	922 ± 104 (113–3,762)
Spiders	10.78 ± 1.41 (1–37)	52.24 ± 8.33 (2.00–294.25)
Beetles	No data	56.19 ± 8.82 (0–312.33)
Grasshoppers	0.25 ± 0.05 (0–1.50)	19.28 ± 4.18 (0–109.33)
All others	No data	26.28 ± 4.91 (0–154.50)
Belowground plant biomass[b]		
Host grasses	N/A	$10,298 \pm 1,188$ (0–38,910)
Non-host plants	N/A	$9,686 \pm 2,146$ (0–64,973)
Dung deposits[c]	19.36 ± 2.22 (0–70)	N/A

All estimates are the means (or counts) across all 50 plots

[a] Mean per pitfall trap per plot

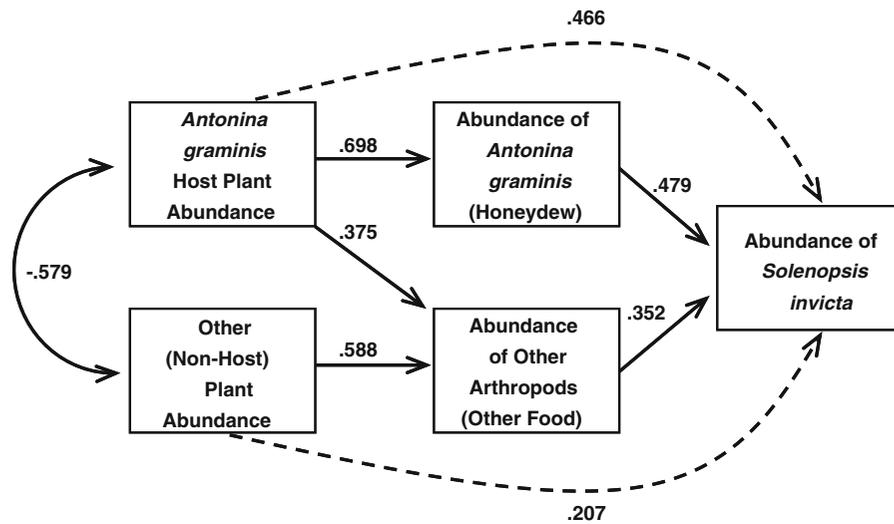[b] Mean per sample per plot

[c] Average per plot

Fig. 2 Path diagram of factors found to be significant in affecting the abundance of *Solenopsis invicta*. *Solid lines* indicate direct effects, while *dashed lines* indicate indirect effects. The path coefficients indicate the proportion of the standard deviation of the dependent variable for which the independent variable is responsible. The full path equations and error terms are presented in Table 2

Table 2 Summary statistics from the path analysis

Standardized solutions

Path equations

Abundance of *S. invicta* = 0.479, abundance of *A. graminis* +0.352, abundance of other arthropods +0.799, $E1$, $R^2 = 0.361$

Abundance of *A. graminis* = 0.698, *A. graminis* host plant abundance +0.716, $E2$, $R^2 = 0.487$

Abundance of other arthropods = 0.375, *A. graminis* host plant abundance +0.588, non-host plant abundance +0.588, E3, $R^2 = 0.231$

Indirect effects

Effect of *A. graminis* host plant abundance on the abundance of *S. invicta*: +0.466

Effect of non-host plant abundance on the abundance of *S. invicta*: +0.207

Correlations

Between *A. graminis* host plant abundance and non-host plant abundance: −0.579

Model fit

$\chi^2 = 2.923$, 4 *df*, $P = 0.571$

AIC = −5.077

All relationships shown are significant at the 0.05 level. *Note*: E in the path equations refers to the residual effects of unknown variables. The path coefficients between each independent and dependent variable indicates the proportion of the standard deviation of the dependent variable for which the independent variable is responsible. The paths are illustrated in Fig. 2

Note: A low χ^2 value and non-significant P indicate that the model is a good fit, as does a low AIC value

mass). The mealybug *Antonina graminis* occurred only on grasses. The great majority (467 of 555, or 84.1%) occurred on the most common grass, *C. dactylon*, with the remainder occurring on four additional grasses. The mealybug did not occur on the other common grass, *P. notatum*, or seven additional grass species. Wherever host plants

occurred, the mealybug was common or abundant (Table 1).

Solenopsis invicta, crickets (Orthoptera), and spiders (Araneae) were found in pitfall traps in all plots. Beetles (Coleoptera) and grasshoppers (Orthoptera) were less common, as were a wide variety of other arthropods that resulted in little of the biomass in traps

(Table 1). Other arthropods also included native ants, although they were very rare, with fewer than 50 individuals captured overall. Mean head width of *S. invicta* workers across all plots was 0.733 ± 0.009 mm (range of the mean within plots = 0.632–0.925 mm). Using the worker body size (head-width) criterion of Greenberg et al. (1992) for inferring social form, polygyne *S. invicta* were the dominant social form across all plots. The means from individual plots showed that 30 were consistent with polygyne colonies, two with monogyne colonies, and 18 with a mix of monogyne and polygyne colonies, or otherwise intermediate in size. Mound excavations resulted in the recovery of one or more inseminated queen from 13 of 25 mounds. Consistent with an overall large proportion of polygyne colonies at our study sites, more than a single inseminated queen was recovered from nine mounds.

Discussion

Few studies have quantitatively demonstrated a link between the abundance of invasive ants and the Hemiptera with which they associate in the field. Moreover, such Hemiptera often have a limited range of host plants on which they occur, and very little is known about how the abundance and distribution of those plants indirectly affects the abundance of invasive ants. Our results provide evidence from a field study that the availability of plant-based carbohydrates derived from honeydew could be important in promoting the abundance of an invasive ant. The association between abundance of the mealybug *A. graminis* and the abundance of *S. invicta* was positive and strong (Fig. 2). Moreover, *A. graminis* is also invasive, consistent with the hypothesis that trophic interactions with invasive Hemiptera can be important in promoting the abundance of invasive ants. Our results also provide what appears to be the first quantitative data consistent with the distribution and abundance of plants having a strong indirect affect on the abundance of invasive ants. The host plants of *A. graminis* had a positive indirect effect on *S. invicta* through their positive effect on *A. graminis* (Fig. 2). Moreover, the major host grass of *A. graminis*, *C. dactylon*, is also an invasive species. An earlier study showed that *S. invicta* cannot acquire significant carbohydrate resources directly from this

grass, but only through honeydew-producing Hemiptera feeding on the grass (Helms and Vinson 2008). Therefore, our results are consistent with the abundance of *S. invicta* being mediated by indirect as well as direct interactions among invasive species at three trophic levels. The abundance of *C. dactylon* as well as other host and non-host plants also had a positive indirect effect on *S. invicta* through their positive effect on other arthropods (Fig. 2). These results indicate two important pathways by which plants could influence the abundance of *S. invicta*: by serving as a food source for herbivorous arthropods and their predators which *S. invicta* may consume, and by serving as hosts for Hemiptera which extract plant phloem and excrete honeydew.

While both arthropod tissues and honeydew could be derived from a single plant, the resources themselves differ greatly from the ants' perspective. The majority of the nutritional value of arthropod tissues to omnivorous ants such as *S. invicta* and other invasive ants are in the form of proteins (amino acids), which are necessary for constructing new tissue during the development of ant larvae. Honeydew and other plant exudates, however, are rich in carbohydrates (sugars) and are important in fueling the activity of workers, which also cannot consume solid foods (Abbott 1978; Stradling 1978). Consistent with these differences, proteins are fed primarily to developing brood and carbohydrates are consumed primarily by adults (Abbott 1978; Sorensen and Vinson 1981). Recent studies show that honeydew in addition to arthropod tissue substantially increases the growth of *S. invicta* colonies over that possible on a diet of only arthropods (Helms and Vinson 2008). In the invasive Argentine ant, *Linepithema humile*, carbohydrates are similarly important, and enable increased colony defense and foraging activity, enabling a positive feedback loop that can increases resource acquisition (Grover et al. 2007). Animal tissues are limited relative to those of plants, and ants with extremely large populations such as invasive species could achieve such populations by acting as primary consumers, making extensive use of plant exudates and honeydew (e.g. Tennant and Porter 1991; Tobin 1994). Substantial energy acquired from plant carbohydrates could support extensive activity, facilitating the location and defense of more limited protein-rich foods (Davidson 1997; Grover et al. 2007). Thus, the importance of carbohydrates to

colony growth and maintenance could be greater than their simple caloric contribution to a colony's energy budget.

Our path analysis does not provide a quantitative estimate of the potential importance of *A. graminis* to the population size of *S. invicta*. The standardized path analysis coefficient (Fig. 2, Table 2) describes the proportion of the standard deviation in *S. invicta* abundance that could be attributed to the abundance of *A. graminis*. Moreover, this estimate is based on a sample of *S. invicta* captured in pitfall traps, and only relative and not absolute abundance between plots can be estimated from the trap data. The abundance of *A. graminis* is also based on samples within plots rather than overall abundance within plots. We can, however make a quantitative estimate of the potential importance of *A. graminis* to *S. invicta* within plots by using our estimates of total *S. invicta* dry mass in plots at four sites where mound volumes were measured (Tschinkel 1993). For those plots, we can also estimate the total number of *A. graminis* by multiplying the numbers found in the four sample areas per plot (four 177 cm^2 samples) by that area divided by the total plot area (100 m^2). Doing so results in an estimate of the mean number of *A. graminis* per plot (±SEM) of 22,470 ± 4,966, with their numbers ranging among plots from zero to 150,035. The total dry mass of *A. graminis* within plots can then be estimated by multiplying the total estimated numbers per plot by an average dry mass per *A. graminis* of 0.75 mg. A regression analysis of these data results in an estimate that *S. invicta* dry mass increases in our study plots by 13.5 mg per individual *A. graminis*, or by 17.9 mg per mg dry mass of *A. graminis* (Table 3). Data available to evaluate whether this estimate is consistent with an important role for *A. graminis* in the abundance of *S. invicta* comes from a greenhouse experiment by Helms and Vinson (2008). That study tested for the effect of insect prey, *A. graminis*, and the *A. graminis* host plant *C. dactylon* on the growth of small *S. invicta* colonies over a two month period. There was a significant and substantial (approximately 50%) increase in the mass of colonies with access to insect prey as well as *A. graminis* and their host plant over colonies with access only to insect prey or colonies with access to insect prey with the host plant in the absence of *A. graminis* (Helms and Vinson 2008). The growth of *S. invicta* colonies in the

Table 3 Comparison of regression analysis estimates of the abundance of *Solenopsis invicta* as a function of the abundance of the mealybug *Antonina graminis*

Current Study
Dry mass of *S. invicta* (mg) = 17.94. Dry mass of *A. graminis* (mg) +276.7
$F = 20.20$, $P < 0.001$, $r^2 = 0.347$, $N = 40$
95% confidence interval of the regression coefficient, 17.94: 10.01, 25.80
Helms and Vinson (2008)[a]
Dry mass of *S. invicta* (mg) = 20.22. Dry mass of *A. graminis* (mg) +11.28
$F = 4.52$, $P = 0.049$, $r^2 = 0.210$, $N = 19$
95% confidence interval of the regression coefficient, 20.22: 0.16, 40.24

[a] Data are from the $P + I$ treatment of Helms and Vinson (2008) summarized in the text. That study determined the increase in size of *S. invicta* colonies over the course of an experiment as increase in live mass from an initial starting point. Increase in live mass is converted here to increase in dry mass by multiplying live mass by 0.329 (Macom and Porter 1995)

treatment with insect prey, *A. graminis*, and the host plant did not increase with increasing numbers of *A. graminis*, apparently because *A. graminis* were always present in excess of the ability of the colonies to use the honeydew they produced (Helms and Vinson 2008). However, in some replicates where *S. invicta* had access to only insect prey and the host plant, the host plants were accidentally colonized by relatively small numbers of *A. graminis* over the course of the experiment, and honeydew was not available in excess of their ability to utilize it. When this occurred, a regression analysis of those data showed that the growth of *S. invicta* colonies increased by 15.2 mg dry mass per individual *A. graminis*, or by 20.2 mg dry mass of *S. invicta* per mg dry mass of *A. graminis* (Table 3). This result is very similar to and not significantly different from the field estimate in this current study of an increase of 13.5 mg per individual *A. graminis*, or 17.9 mg per mg dry mass of *A. graminis*. This suggests a similar substantial effect of *A. graminis* on the growth and maintenance of *S. invicta* colonies in the field to that found in the earlier greenhouse experiment (Table 3).

That *A. graminis* was the most abundant honeydew-producer found in our study is not surprising because, while they do not utilize all grass species as hosts, they utilize a number of common grasses, and

the mealybug is often abundant wherever those grasses occur in the southeastern United States (Chada and Wood 1960; Helms and Vinson 2000, 2002; Chantos et al. 2009). That they were the only honeydew-producer found was unexpected. The likely explanation for this result is that our study was conducted in the fall (September). Many of the honeydew-producing Hemiptera in this system occur on broadleaf plants, and are most common during the spring (e.g. Helms and Vinson 2002). *A. graminis*, however, can also be abundant throughout the summer and fall (Chada and Wood 1960; Helms and Vinson 2002), perhaps one reason why it can be a particularly important resource for *S. invicta* (Helms and Vinson 2008).

The result that arthropod tissues also appear important to *S. invicta* abundance has also been supported in laboratory experiments (Porter 1989; Helms and Vinson 2008). However, we note that our interpretation that the arthropods captured in pitfall traps are representative of the abundance of arthropod food available for *S. invicta* rests upon two assumptions: first, that the abundance of arthropods captured in traps is representative of overall arthropod abundance in plots, and second, that the fraction of arthropods that were trapped that *S. invicta* can consume does not differ systematically among plots. These assumptions are likely to be valid because our study was conducted in a single habitat over a relatively short time interval. Moreover, most arthropods co-occurring with *S. invicta*, whether predator or prey, are likely to be consumed at some point in time because scavenging dead arthropods in a primary means by which *S. invicta* acquires arthropod prey (e.g. Tschinkel 2006).

While earlier studies have shown that multiple queen (polygyne) populations of *S. invicta* have substantially smaller workers and significantly larger populations than single queen (monogyne) populations (e.g. Macom and Porter 1996), our study did not find that mean worker body size within plots was a significant predictor of *S. invicta* abundance. One possible reason is that monogyne colonies were too rare at our study sites to result in a strong test of the role of social form in determining population size. The mean body size (head-width) over all plots was consistent with polygyne *S. invicta*, as were measurements for a great majority of the individual plots. Moreover, a majority of excavated mounds contained

more than a single inseminated queen, and the polygyne form of *S. invicta* appears exceptionally common in Texas (e.g. Porter et al. 1991). Thus, while our study found no significant effect of social form on population size, such an effect is evident in studies with a greater mix of the two forms (Macom and Porter 1996).

Our study adds new evidence to previous research suggesting that trophic interactions among invasive species can be important in promoting their success (e.g. Simberloff and Von Holle 1999; Helms and Vinson 2002, 2008; O'Dowd et al. 2003; Grosholz 2005; Simberloff 2006). While the path analytic approach we employ is a powerful technique for testing hypotheses of cause and effect relationships among variables, as noted, the procedure cannot determine causation between significantly correlated variables. At present, we know that *S. invicta* is widely associated with *A. graminis* in the southeastern United States and that *A. graminis* and other Hemiptera are likely to contribute greatly to the energy available to *S. invicta* colonies (Helms and Vinson 2002). Moreover, the results of an experiment showed that *A. graminis* honeydew substantially increases the growth and size of *S. invicta* colonies over that possible from the consumption of only arthropod prey (Helms and Vinson 2008). This current study shows that larger populations of *S. invicta* occur where there are larger populations of *A. graminis* and their host plants. Moreover, the magnitude of the positive correlation between *A. graminis* and *S. invicta* abundance estimated by our current study is consistent with that predicted by an experimental study of the effect of *A. graminis* on *S. invicta* colony growth (Helms and Vinson 2008). Available evidence is thus consistent with the importance of *A. graminis* and other honeydew-producing Hemiptera and their host plants in promoting the abundance of *S. invicta* in the southeastern United States.

Our study does not directly address the broadly applicable hypothesis that *S. invicta* and other invasive species are facilitated by an escape from natural enemies, but it does address the possibility that they may have access to novel important resources in the introduced range; honeydew provided by an abundant invasive mealybug. Moreover, our study could provide insight into the hypothesis that invasive species such as *S. invicta* are promoted

by disturbance. We did not find that differences in grazing intensity at different locations within pastures had an effect on the abundance of *S. invicta*, suggesting that increased disturbance by grazing mammals on small scales may not be an important determinant of success in this study system. In any case, we test only whether increased local disturbance within a disturbed habitat is important. Indeed, certain characteristics of disturbed habitats could be important in promoting *S. invicta*. The most abundant grass in our study and the major host for *A. graminis*, *C. dactylon*, is an invasive grass originating from Africa (Holm et al. 1977). This grass also appears to be an important host for *A. graminis* throughout the range of *S. invicta* in the southeastern United States (Chada and Wood 1960; Helms and Vinson 2008; Chantos et al. 2009). Ironically, *C. dactylon* is a human-preferred and commonly planted grass in grazing pastures, lawns, and recreation areas within the southern United States, habitats that could clearly be described as disturbed systems. This grass also readily escapes cultivation into other disturbed habitats (e.g. Holm et al. 1977). In our study system, humans encourage the abundance of this invasive grass to promote livestock and other uses, coincidentally promoting the abundance of an invasive mealybug, which could then promote the abundance of an invasive ant (Fig. 2). Moreover, this system is likely to involve interactions among additional introduced species. *S. invicta* not only gains honeydew resources from *A. graminis*, but it could also protect the mealybug from natural enemies such as *Neodusmetia sangwani*, a parasitic wasp introduced from Asia to control *A. graminis* in the United States (Schuster et al. 1971; Helms and Vinson 2003; Chantos et al. 2009). *S. invicta* could have a negative indirect effect on *C. dactylon* by protecting *A. graminis*, or an overall positive effect on the grass by eliminating herbivorous arthropods that consume *C. dactylon* (Styrsky and Eubanks 2007). These additional factors outline a system more complex than our current model (Fig. 2). That model is simply a first step in understanding the role that trophic interactions are likely to play in promoting invasive ants. Further research on this and similar systems is likely to provide substantial new insights into the importance of interactions among invasive species in promoting their abundance and into how such species act to structure ecological communities in human mediated systems.

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