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Indirect Effects of a Fungal Entomopathogen, *Lecanicillium lecanii* (Hypocreales: Clavicipitaceae), on a Coffee Agroecosystem Ant Community

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ABSTRACT Fungal entomopathogens are widely distributed across natural and managed systems, with numerous host species and likely a wide range of community impacts. While the potential for fungal pathogens to provide biological control has been explored in some detail, less is known about their community interactions. Here we investigate the effects of fungal epizootics of the entomopathogen *Lecanicillium lecanii* (Zimmerman) on a keystone mutualism between *Azteca instabilis* (F. Smith), a dominant arboreal ant, and the green coffee scale (*Coccus viridis* Green), as well as broader impacts on a coffee agroecosystem ant community. We hypothesized that seasonal epizootics cause shifts in the foraging ranges of *A. instabilis* as the ants adapt to the loss of the resource. We further hypothesized that the magnitude of these shifts depends on the availability of alternate resources located in neighboring shade trees. To test these hypotheses, we induced an epizootic in experimental sites, which were compared with control sites. Surveys of ant activity were undertaken pre- and post-epizootic. We found a decrease in foraging activity of *A. instabilis* and increase in activity of other ant species in the experimental sites post-epizootic. The decrease in abundance of *A. instabilis* foragers was greater on plants in which an epizootic was induced than in other plants. This relationship was modified by shade tree density where higher shade tree density was associated with larger decreases in *A. intabilis* foraging activity in coffee plants. These results demonstrate the potential for fungal entomopathogens to influence the structure and diversity of ecological communities.

KEY WORDS *Lecanicillium lecanii*, fungal epizootic, *Azteca instabilis*, self-organization, disturbance

Fungal entomopathogens have been widely studied as biological control agents in numerous systems and against numerous different pests (Shah and Pell 2003, Scholte et al. 2005, Pell et al. 2010). While the direct effects of the use of fungal biocontrol on pest species have been of primary interest to date, the important roles these organisms play as interacting members of ecological communities in complex food webs are poorly understood and little studied (Meyling and Hajek 2010). Population regulation and host-pathogen interactions are implicit in the treatment of fungal entomopathogens simply as agents of biological control, yet indirect effects with important implications for the structure and diversity of communities are omitted. Therefore, addressing the role of entomopathogenic fungi in complex interactions and processes that structure communities is an important avenue of research.

Community structure and patterns of diversity have been demonstrated to result from numerous processes and underlying biotic and abiotic conditions (Paine 1966, 1969; Connell 1978; Hastings 1988; Caley and St. John 1996; Chesson 2000; Hansen 2000; Bascompte and Jordano 2007; Perfecto and Vandermeer 2008). Of particular importance in our study system, spatial pattern has emerged as an important variable in the study of the distribution of biodiversity at numerous scales (Ritchie 2010). The spatial distribution of a population or community throughout a landscape has often been assumed to result from underlying habitat structure (Forman and Gordon 1987, Turner et al. 1990). However, there may be other factors contributing to observed spatial patterns, particularly when considering relatively uniform landscapes (Skarpe 1991, Rohani et al. 1997, Bascompte and Solé 1998, Alados et al. 2007, Scanlon et al. 2007, Solé 2007). For example, observed patterns and distributions in relatively uniform ecosystems, such as managed agroecosystems, may be the result of endogenous self-organization arising from species interactions rather than underlying habitat variables (Pascual et al. 2002, Solé and Bascompte

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2006, Perfecto and Vandermeer 2008, Rietkerk and van de Koppel 2008, Vandermeer et al. 2008).

Here we address the indirect effects of a fungal entomopathogen, *Lecanicillium lecanii* (Zimmerman), and its ecological role, in promoting spatial self-organization and pattern formation. In our study system, a tropical coffee agroecosystem, a keystone arboreal-nesting ant, *Azteca instabilis* (F. Smith), forms a mutualism with the green coffee scale, *Coccus viridis* (Green). The ant protects the scale from predators and parasites, and the scale provides a nutrient rich secretion in return (Vandermeer et al. 2002). The ant colonies nest in shade trees that are planted in an approximately uniform distribution throughout the farm, tending the scale insects that live on the coffee plants below. The spatial distribution of the *A. instabilis* ant nests, which occur in clusters whose size distribution can be described with a fat tail distribution, is thought to arise through a process of self-organization characterized by local expansion of clusters counteracted by density-dependent mortality (Perfecto and Vandermeer 2008, Vandermeer et al. 2008). A fungal entomopathogen, *L. lecanii*, attacks the local concentrations of scale insects that are associated with the clusters of ant nests, greatly reducing the scale populations and possibly causing density-dependent control of the *A. instabilis* colonies (Jackson et al. 2009), although other factors have been proposed (Vandermeer et al. 2008).

We hypothesize that seasonal epizootics of *L. lecanii* periodically disrupt ant foraging activity, causing dynamic shifts in the foraging ranges of *A. instabilis* as the ants attempt to adapt to the loss of a primary food source. We further hypothesize that the magnitude of these shifts depends on the availability of alternate resources located in neighboring shade trees (e.g., extrafloral nectaries or other scale insects). For the purposes of this study, availability of alternate resources is approximated by proximity of shade trees to *A. instabilis* nests, rather than directly measured because of the difficulties associated with sampling insects in tropical forest canopies. Thus, habitat complexity, in the form of variation in the local abundance of shade trees, may be modifying this interaction (Armbrecht et al. 2004, Bos et al. 2008). While these mechanisms—disturbance and habitat complexity—are typically studied separately, in our study system the local interaction of a biotic disturbance driven by a fungal entomopathogen and habitat complexity may be mediating the foraging activity and resource acquisition of *A. instabilis* (the keystone ant), which could have implications for observed, landscape-level spatial pattern (Perfecto and Vandermeer 2008, Vandermeer et al. 2008).

To explore these hypotheses, we examined the impact of *L. lecanii* epizootics on the foraging behavior of *A. instabilis.* We paid particular attention to *A. instabilis* foraging response with respect to shade tree density (a proxy for habitat complexity) and asked three specific questions: 1) How does *A. instabilis* respond to *L. lecanii* epizootics and the concomitant decrease in the food resource provided by the scale insects? 2) How does habitat complexity, in this case proximity of other shade trees to *A. instabilis* nest-sites, influence the response of *A. instabilis* to a local epizootic? and 3) How is the arboreal foraging ant community affected by the response of *A. instabilis*, the dominant ant competitor, to a local epizootic? We predicted that *A. instabilis* would abandon coffee bushes with large populations of infected scale insects and respond either by expanding their foraging activity to coffee bushes in the periphery of previously tended bushes, or by switching their foraging activity to shade trees, and the alternate resources therein, in close proximity to nests. Furthermore, we predicted that if *A. instabilis* foraging activity shifted to shade trees, it would impact the arboreal foraging ant community by promoting expansion of competitively subdominant arboreal foraging ant activity into coffee bushes previously tended by *A. instabilis*, thereby providing access to previously unavailable resources (i.e., low density scale insect populations). In contrast, we predicted that if *A. instabilis* were to expand foraging to peripheral coffee bushes, it would displace foraging activity of other arboreal foraging ants, forcing them to seek alternative resources.

Materials and Methods

The study site is located at Finca Irlanda, a 280 ha, organic coffee farm in the Soconusco region of Chiapas, Mexico (15° 11′ N, 92° 20′ W). The farm is a commercial polyculture (Moguel and Toledo 1999), with coffee bushes growing beneath shade trees that have been planted uniformly throughout the farm. As a result, shade trees as a whole are distributed in a statistically significant uniform manner, producing little underlying habitat variability (Vandermeer et al. 2008). The dominant shade trees are comprised of several *Inga* species, *Alchornea latifolia* (Swartz) and *Trema micrantha* (Blume) (Martinez and Peters 1996), some of which have extrafloral nectaries and many contain various species of scale insects and aphids (Livingston et al. 2008).

Site Selection and Data Collection. We selected experimental sites based on the following criteria. First, each site had to have one or more *A. instabilis* nest, with each nest cluster being independent from nests outside the study plot. That is, potential sites containing colonies with foragers traveling to and from nests outside of the proposed study site were rejected. This criterion was imposed to ensure that colonies could not simply respond to *L. lecanii* epizootics within treatment sites by increasing foraging on coffee bushes outside of the sites. Second, each site had to have a large number of healthy scales so that the epizootic-induced death of these scales would entail a significant reduction in the food resources available to the *A. instabilis* colonies. Third, we avoided sites in which the *A. instabilis* colonies were primarily foraging in shade trees, either tending other scale insects or foraging at extrafloral nectaries, as we wanted to focus on colonies whose primary carbohydrate source was *C. viridis.* Using these criteria, we

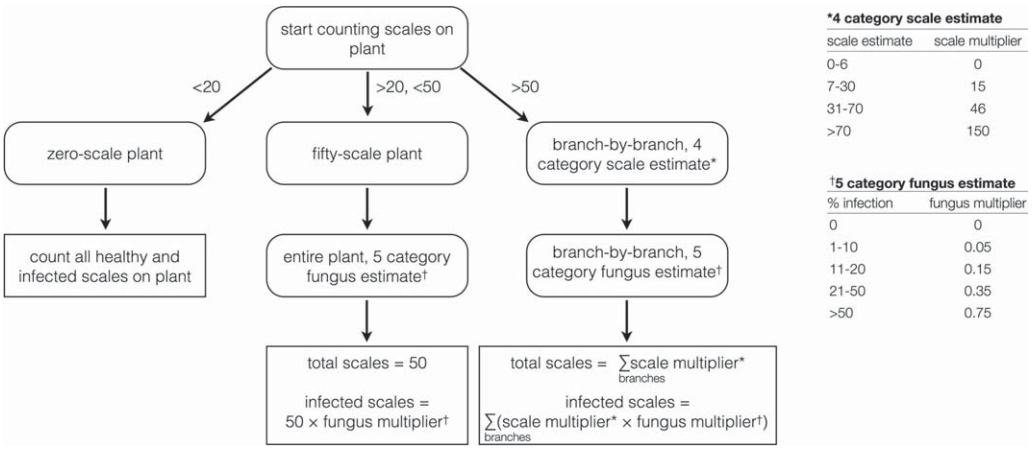

Fig. 1. Protocol for *C. viridis* and *L. lecanii* surveys. The coffee plant is assigned to one of the three pathways depending on how many scales are found in an initial count. If >50 scales are on the plant, the rightmost path is executed, which entails switching to a branch-by-branch estimate of the number of scales and the prevalence of *L. lecanii*. If <20 scales are encountered, the leftmost branch is followed, and the total number of infected scales is recorded. Otherwise, an entire-plant estimate of *L. lecanii* prevalence is used to estimate the number of infected scales, as specified by the center path.

selected four treatment sites and four control sites ranging in size from ≈200 to 350 m^2. The four treatment sites (Sites 1–4) and the first control site (Control 1) were surveyed in the summer of 2009. Because of logistical and time constraints, we were limited to a single control site during the 2009 field season, so, three additional control sites (Control 2–4) were added in the summer of 2010; to control for the effects of season, these sites were surveyed at the same time of the same months as the 2009 surveys.

We used an aqueous suspension of *L. lecanii* conidia cultured from an infected *C. viridis* obtained from within the farm to inoculate scale insects, thereby creating an artificial epizootic (Easwaramoorthy 1978). *L. lecanii* was identified as the prominent fungal pathogen affecting *C. viridis* in this system based on both morphological identification (Zare and Gams 2001) and DNA sequencing of infected scale insects (D.J., unpublished data). After isolation from the scale insect and culturing of conidia, conidia were mass produced via solid-state fermentation using cooked rice and sorghum as substrates. We then suspended the resultant conidia in water, added Tween 80 surfactant (0.1%) to the suspension, and applied it directly to the scale insects using a handheld, manual pump sprayer. Each coffee plant with scale insects and *A. instabilis* foragers was sprayed until the surfaces of all leaves were thoroughly wet to the point of dripping; an average of 0.25 liters of suspension was applied to each plant. Each site was sprayed twice to maximize *L. lecanii*-induced scale mortality: once on the morning of 4 July 2009 and again the morning of 18 July 2009. The spore concentrations, $\approx 1.9 \times 10^5$ spores/ml for the first spraying, and 2.2×10^6 spores/ml for the second application, were determined using a hemocytometer.

We censused the scale insects at each site before (between 25 June and 3 July) and after (between 19 July and 23 July) inoculation using the following protocol, adapted from Vandermeer and Perfecto (2006). Each plant was rapidly surveyed to determine scale insect abundance. If plants had fewer than 20 scales, we categorized plants as "zero scales," as virtually every coffee plant in the farm has at least a few scale insects and *A. instabilis* foraging activity is primarily restricted to plants with much larger scale populations. For plants with between 20 and 50 scales, we categorized them as "50 scales." For plants with >50 scales, a four-category protocol was applied to each branch of the plant. Branches with 0–6, 7–30, 31–70, and in excess of 70 scales were placed in categories 1, 2, 3, and 4, respectively. We then calculated an estimated total scale count for the entire plant as (0 × branches in category 1) + (15 × branches in category 2) + (46 × branches in category 3) + (150 × branches in category 4) (Perfecto and Vandermeer 2006). A flowchart of the survey protocol is shown in Fig. 1.

We estimated the prevalence of *L. lecanii* while performing the scale censuses (Fig. 1). For plants with between 20 and 50 scales, we visually estimated the percentage of scales infected with *L. lecanii*. Based on this estimate, the plant was placed in one of five fungal prevalence categories: 0% = category 0, 1–10% = category 1, 11–20% = category 2, 21–50% = category 3, and >50% = category 4. For plants assessed using the four-category scale counting protocol (i.e., those plants with >50 scale insects), the same five fungal prevalence categories were applied to each branch individually. The number of infected scales was estimated as 0, 0.05, 0.15, 0.35, or 0.75 times the total number of scales for fungal categories 0, 1, 2, 3, and 4, respectively.

The abundance of *A. instabilis* and the identity of the most abundant arboreal foraging ant species were noted for each coffee plant. Abundance was estimated by counting number and species of ant in a five minute period on each coffee plant censused—between 14 and 66 plants per site depending on the site. Abun-

dance was then assessed using a four category protocol: < 10 foragers = category 1, 10–25 = category 2, 26–50 = category 3, and >50 = category 4. The ants were censused twice before and three times after the experimental inoculation. Each census was conducted at the same time of day at each site to control for possible effects of time of day on foraging activity. Additionally, censuses were conducted under similar conditions (e.g., censuses were not conducted and compared between cool, overcast days and hot, sunny days) to the greatest extent possible. Only data from the first and last censuses were used in the analysis to ensure that our "post-epizootic" census was not influenced by transient dynamics taking place during the epizootic.

Data Analysis. We used a bootstrapping with permutation (resampling) approach to determine if the increase in the number of scales infected at the treatment sites was significantly greater than at the control sites; that is, to confirm that the *L. lecanii* inoculation significantly increased infection. Resampling (bootstrapping) approaches are not dependent on assumptions about normality, which is useful in many ecological systems, and are becoming increasingly accepted as the preferred method for a wide range of analyses given the availability of powerful computers (Chihara and Hesterberg 2011). The method involves the creation of synthetic control and treatment groups by combining the data from the treatment sites and the control sites into a single pool and resampling randomly from this pool to assemble synthetic populations of the same sizes as the observed populations. The difference between the increase in the number of infected scales at the synthetic control site and the increase at the synthetic treatment site was then compared with the observed difference between the control and treatment sites. This procedure was repeated 10,000 times, with the *P* value being the fraction of times the difference between the simulated treatment and control sites was as great, or greater than, the observed difference. This resampling approach determines the probability that we would see, by chance alone, as large of a difference in the change at the two sites as was observed.

We used a similar resampling approach to determine if the changes in the abundances of *A. instabilis* foragers in the treatment sites were significantly different from the changes observed in the control sites, using the percent change in the number of *A. instabilis* foragers on an entire-site basis.

To more directly test the effect of inoculation, we also tested whether the absolute decrease in the number of *A. instabilis* foragers was greater on sprayed plants than on unsprayed plants, that is, comparing only those plants in the treatment sites that had *C. viridis* and *A. instabilis* in the preinoculation census to control site plants with *C. viridis* and foraging *A. instabilis* present in the preinoculation census.

The *A. instabilis* abundance resampling analyses were performed as follows. First, for both the treatment and control sites, we calculated the number of *A. instabilis* foragers on each coffee plant before inoculation and summed these to determine the total number of foragers in the entire site. Using these preinoculation totals and the total number of foragers in each site present in the final ant census, we calculated the percentage change in foragers. From these values we obtained the difference in the percentage change at the control and treatment sites. We then combined the coffee plants from both the treatment and control sites into a single pool for resampling.

From this combined pool, coffee plants were randomly assigned to either the control or treatment site, resulting in simulated control and treatment groups with the same numbers of plants as the actual control and treatment sites. The difference in the percentage change in foragers at these two simulated sites was then compared with the actual, observed difference. This procedure was repeated 10,000 times. The *P* value was then calculated as the fraction of resamples resulting in a difference in percentage change that was as extreme or more extreme than the observed difference.

To estimate the food resources in shade trees (extrafloral nectaries, other scale insects, and so forth) available in each site, we defined a shade tree resource index. We made the simplifying assumption that the accessibility of shade tree resources to an ant nest would fall off linearly with distance from the nest. Therefore, the total shade tree resources available to a nest are approximated by: $\sum_{i=1}^{n} \frac{1}{d_i}$ = Shade Tree Resource Index (STRI), where n is the number of shade trees in the neighborhood of the nest and d_i is the distance between the nest and the shade tree. We used an analysis of covariance (ANCOVA) in the statistical computing program R (R Core Team 2010) to determine the effect of the STRI on the absolute change in the number of *A. instabilis* foragers per plant.

Results

The treatment sites experienced nearly complete infection in the final survey, with prevalence increasing to an average of 92.1% from an initial baseline of 36.8%. In the control sites, the peak prevalence was much lower, increasing to 45.2% from an initial value of 11.8%. This increase in infected scales at the treatment sites translated into an 87.5% reduction in the fraction of healthy scales as opposed to the 37.8% background reduction observed at the control sites. The mean per-site increase in scales infected by *L. lecanii* from the first (1 wk before treatment) to the final (2 wk after treatment) scale insect survey was 374.5 (SD = 295.6) for Sites 1–4 and 55.9 (SD = 71.0) for the control sites, respectively. The increase was significantly greater at the treatment sites, in which a fungal epizootic was induced, compared with the control sites ($P < 0.001$). Individual plants varied widely in the number of scale insects infesting them (from 0 to over 25,000), which contributes to the large SDs reported above.

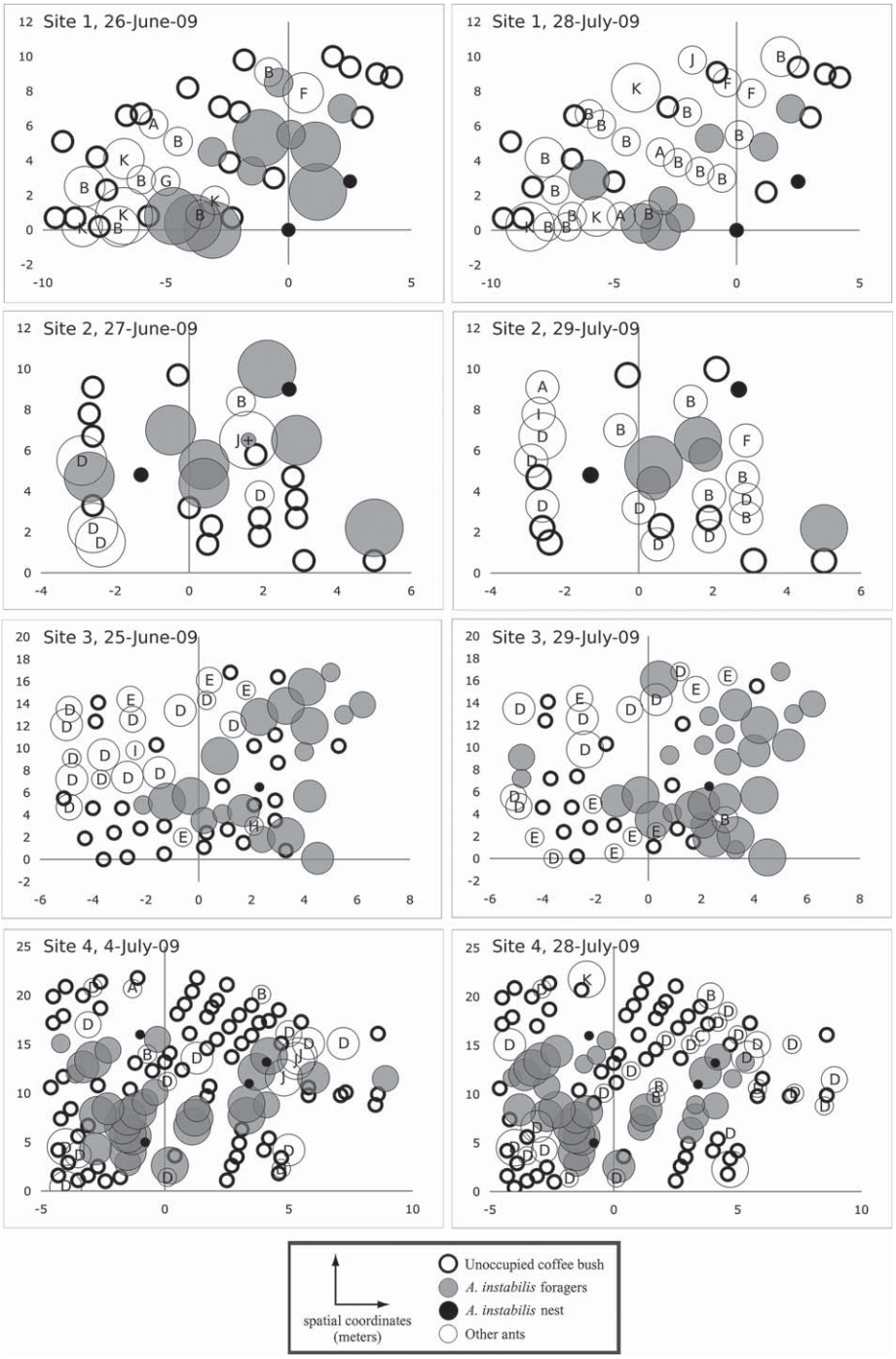

Fig. 2. Spatial distributions of arboreal foraging ants in coffee bushes at four experimental sites before (left column) and after (right column) *L. lecanii* inoculation. Gray circles represent numbers of *A. instabilis* foragers. Labeled white circles represent numbers of foragers of other ant species, as follows: (A) *Brachymyrmex* sp. 1, (B) *Brachymyrmex* sp. 2, (C) *Crematogaster carinata*, (D) *Pheidole synanthropica*, (E) *Pheidole* sp. 2, (F) *Procryptocerus hylaeus*, (G) *Pseudomyrmex ejectus*, (H) *Pseudomyrmex gracilis*, (I) *Pseudomyrmex simplex*, (J) *Solenopsis geminata*, (K) *Technomyrmex* sp., (L) *Azteca* sp., (M) *Solenopsis* sp. 2, (N) *Pachycondyla* sp., and (O) *Camponotus* sp. The sizes of the circles in each site correspond to one of four different abundance classes, from smallest to largest circles: < 10, 10–25, 26–50, and >50 foragers. Unlabeled white circles represent coffee bushes without ants. Solid black circles indicate nests of *A. instabilis* in shade trees. The gray circle with a cross in Site B indicates a nest of the ground-nesting ant *Solenopsis geminata*. Dimensions are in meters. Note that the axes are scaled differently to maximize separation between data points for visual clarity.

The median total number of scale insects per plant (excluding plants with zero scales) at the treatment and control sites before inoculation was 200 and 21 scale insects, respectively (mean values: 1,085 and 337). Median post inoculation was 50 scale insects in both the treatment and control sites (mean values: 1,051 and 275). Because of the presence of five plants in the treatment sites that were heavily infested with scale insects (between 10,000 and 25,000 on a single plant) the median rather than the mean more accurately reflects conditions in the field.

There was a contraction in the *A. instabilis* foraging range at Sites 1 and 2 after the *L. lecanii* inoculation (Fig. 2). The number of coffee bushes occupied by non-*A. instabilis* arboreal-foraging ants also increased in both sites between the first and last censuses: from 13 to 23 occupied bushes in Site 1 and from 6 to 15 in Site 2. The trends were less apparent in Sites 3 and 4. In Site 3, there was an increase in the number of coffee bushes tended by *A. instabilis*, from 19 to 28, while the number of bushes occupied by other ant species was relatively unchanged, decreasing from 19 to 18. However, three bushes in this site were destroyed by a falling tree trunk just before the final census. The portion of the tree trunk that fell contained part of an *A. instabilis* nest. The foragers found on the two newly colonized bushes located at the left edge of the site came from this fallen nest. In Site 4, there was a slight increase in the number of coffee bushes tended by *A. instabilis*, from 29 to 31. However, the spatial extent of the *A. instabilis* foraging range appears to have decreased slightly (Fig. 2). The number of bushes occupied by other ant species increased markedly, from 19 to 28. In the control sites (Control 1–4), the qualitative picture is one of relative stasis, although there was some change in the number of occupied plants (Fig. 3).

On a per-site basis, the decrease in *A. instabilis* was greater in the treatment sites, but not significantly so ($P = 0.153$). However, if the site where the shade tree housing the *A. instabilis* nest fell (Site 3) is excluded from the analysis, the percentage decrease in *A. instabilis* foraging is significant ($P = 0.011$). The decrease in the abundance of *A. instabilis* foragers was significantly greater on sprayed plants than in the unsprayed plants ($P = 0.02$). This relationship was significantly modified by the STRI (ANCOVA; $F_{3,112} = 8.1159$; $P = 0.005$), with a larger decrease in the number of foragers on sprayed plants with increasing STRI (Fig. 4).

The absolute changes in the number of *A. instabilis* foragers for Sites 1–4 was −360, −255, 225, and −388, respectively; the changes in the control sites (1–4) were −35, 65, −55, and −18 (Fig. 5). The change in the number of coffee plants occupied by other arboreal-foraging ant species was negatively correlated with the change in the number of *A. instabilis* foragers ($R^2 = 0.86$; $P = 0.0004$; Fig. 5). That is, sites with a larger decrease in the number of *A. instabilis* foragers tended to have a larger increase in the number of coffee plants tended by another ant species.

Discussion

Our results show that the foraging activity of a dominant arboreal ant, *A. instabilis*, is significantly reduced by a fungal epizootic of *L. lecanii* infecting the ant's mutualist partner, the green coffee scale. This direct effect of the fungal pathogen on an important food resource for the dominant ant in this system may have indirect effects on the larger ant community through the observed reduction in foraging activity of *A. instabilis.* While there exist qualitative differences in the associated subdominant ant species between each of the sites, both across space (e.g., between control Sites 2 and 3; between treatment Sites 1 and 2) and over time (e.g., between Control 1 in 2009, and Controls 3 and 4 in 2010; between the first and last surveys at treatment Site 2), there was a clear effect of the experimentally induced fungal epizootic on the foraging activity of the dominant arboreal ant *A. instabilis.* It is possible that other unmeasured factors (e.g., differences in temperature or precipitation between years in the control plots; differences in competitive hierarchies of the associated subdominant ant species in each plot; competitive exclusion of *A. instabilis* from an alternate resource) could have influenced these results, but we could not identify any other factors that differed systematically between the control and treatment sites or between any given individual sites. What is clear is that *A. instabilis* foraging activity changed very little from the first to the last survey in all of the control sites as compared with the treatment sites, suggesting the induced epizootic is the most parsimonious explanation for the change in foraging activity.

The magnitude of this shift in foraging activity was significantly modified by the STRI—or amount of alternative resources located in nearby shade trees—and was negatively correlated with foraging activity of other species of ants. These results suggest that *A. instabilis* colonies are able to adapt to *L. lecanii* epizootics by shifting their foraging activity, but only if sufficient alternative resources are available. In the absence of alternative resources, or foraging refugia, as illustrated in the shade tree resource index, colonies are forced to continue tending the original, decimated scale populations. Thus, colonies without foraging refugia almost certainly experience a substantial reduction in food intake as a result of an epizootic. In the long-term, this could lead to the forced migration or mortality of these colonies, thereby contributing to the density-dependent control that gives rise to the spatial self-organization of *A. instabilis* nests in this system.

These results are counter to the hypothesis that *A. instabilis* can respond to epizootics of *L. lecanii* by expanding its foraging activity to coffee bushes on the periphery of the original foraging range. Had *A. instabilis* responded by expanding foraging activity to the small populations of uninfected scales in peripheral coffee bushes, the negative implications of epizootics for the survival of the colony would likely be mitigated. Taking advantage of these small aggre-

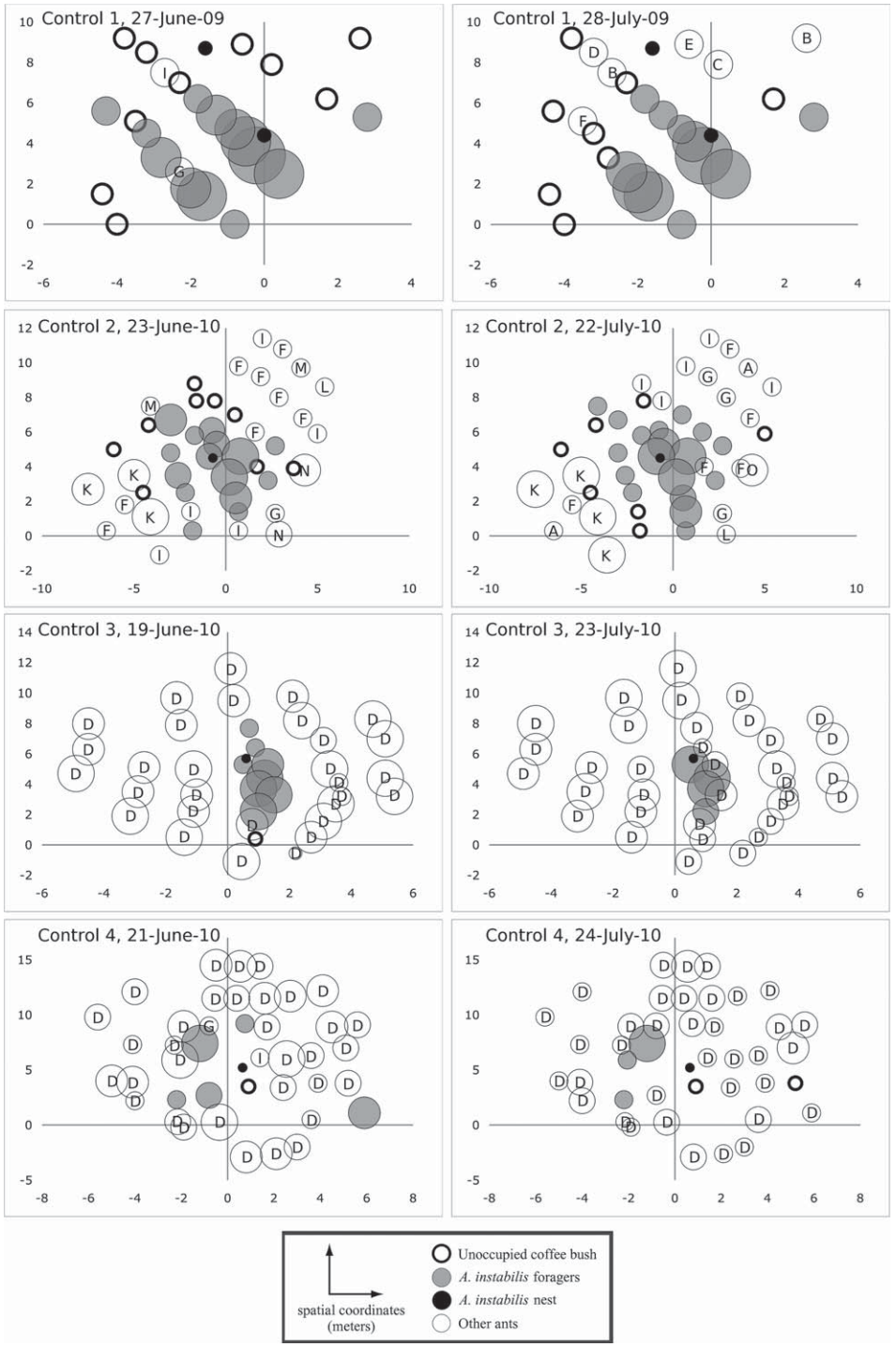

Fig. 3. Spatial distributions of arboreal foraging ants in coffee bushes at the control sites. Gray circles represent numbers of *A. instabilis* foragers. Labeled white circles represent numbers of foragers of other ant species, as follows: (A) *Brachymyrmex* sp. 1, (B) *Brachymyrmex* sp. 2, (C) *Crematogaster carinata*, (D) *Pheidole synanthropica*, (E) *Pheidole* sp. 2, (F) *Procryptocerus hylaeus*, (G) *Pseudomyrmex ejectus*, (H) *Pseudomyrmex gracilis*, (I) *Pseudomyrmex simplex*, (J) *Solenopsis geminata*, (K) *Technomyrmex* sp., (L) *Azteca sp.*, (M) *Solenopsis* sp. 2, (N) *Pachycondyla* sp., and (O) *Camponotus* sp. The sizes of the circles correspond to one of four different abundance classes, from smallest to largest circles: < 10, 10–25, 26–50, and >50 foragers. Unlabeled white circles represent coffee bushes without ants. Solid black circles indicate nests of *A. instabilis* in shade trees. Dimensions are in meters. Note that the axes are scaled differently to maximize separation between data points for visual clarity.

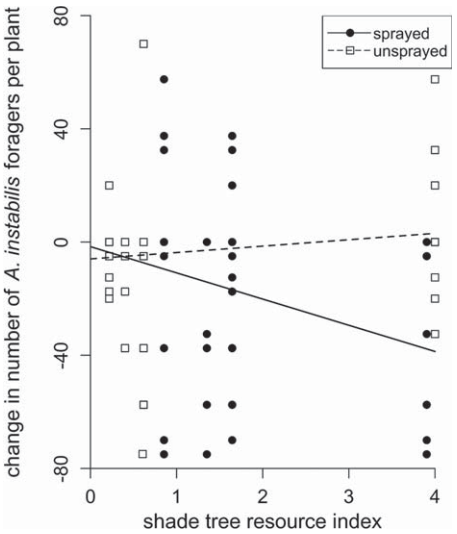

Fig. 4. Scattergraph of ANCOVA results. The change in the number of *A. instabilis* foragers per coffee plant (post-inoculation minus pre-inoculation) versus the shade tree resource index was significantly different for coffee plants sprayed and unsprayed with the *L. lecanii* suspension, as indicated by the significantly different slopes of the regression lines. The shade tree resource index is the sum of the inverse of the distances to the neighboring shade trees, $\sum_{i=1}^{n} \frac{1}{d_i}$.

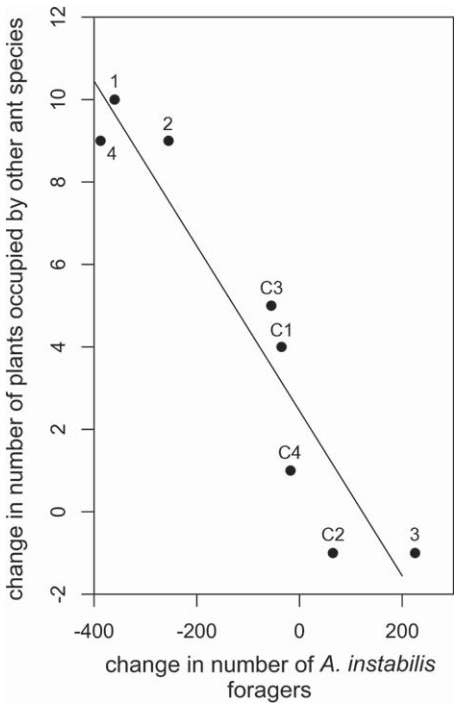

Fig. 5. Change in the number of plants occupied by other arboreal-foraging ant species versus change in number of *A. instabilis* foragers ($R^2 = 0.86$; $P = 0.0004$). Points are labeled with site numbers (C = control).

gations of unaffected scale insects would allow *A. instabilis* to begin to build up and tend substantial populations of *C. viridis* within range of the colony, possibly leading to the formation of new satellite nests in shade trees or large coffee bushes adjacent to the expanded foraging range.

However, in contrast with these expectations, we observed a contraction in foraging activity and resource switching in sites with the highest shade tree resources and increased intensity of foraging on a reduced number of healthy scales in sites with lower shade tree resources. The decline in *A. instabilis* foraging activity on coffee plants in areas with abundant alternate resources indicates that there is a significant reduction in the quantity of scale resources on coffee plants impacted by *L. lecanii.* In the absence of sufficient alternative resources, this reduction may lead to either a weakening of colonies and eventual nest movement to escape the fungal pathogen, or nest mortality, either of which would contribute to the self-organization process and promote the emergence of the spatial pattern of *A. instabilis* nest clusters observed at the landscape scale (Jackson et al. 2009).

These results highlight how habitat complexity may interact with resource availability to affect colony survival. *A. instabilis* did not respond equally at all sites to the fungal epizootic and loss of resources. With increasing shade tree density, and thus more readily available alternative resources, including extrafloral nectaries and other species of scale insects, foraging contracted in coffee bushes. In contrast, *A. instabilis* foraging shifted much less in response to the artificial epizootic at sites with low shade tree density. Sufficiently high densities of shade trees surrounding *A. instabilis* nest-sites appear to provide alternative resources, as well as potential nest-site locations, allowing adaptation to the loss of scale insect resources in coffee bushes. This finding highlights the possible importance of local habitat complexity in facilitating pattern formation.

Furthermore, in the absence of readily available alternative resources provided by adjacent shade trees (Sites 3 and 4), *A. instabilis* increased intensity of scale-tending activities in coffee bushes affected by the fungal epizootic. Based on observations made at these two particular sites, the number of foragers tending the few remaining healthy scales drastically increased after inducing the artificial epizootic. This suggests that a localized epizootic could cause persistent stress because of deficient resource availability for colonies located in areas lacking sufficient shade tree resources. This habitat complexity may also feed back to the self-organization process: in the absence of alternate resources, *A. instabilis* colonies are more negatively impacted by *L. lecanii* epizootics, implying that the density-dependent mortality of nests is modulated by habitat complexity.

The spatial pattern and distribution of *A. instabilis* nests resulting from this dynamic interaction have been shown to promote biological control of coffee and ecosystem function in numerous other ways (Liere and Perfecto 2008, Perfecto and Vandermeer

2008, Vandermeer et al. 2008, Jackson et al. 2009). For example, *A. instabilis*-tended patches provide an enemy free space for the development of the coccinellid beetle larvae of *Azya orbigera* (Mulsant) (Coleoptera: Coccinellidae), which are immune to *A. instabilis* attack (Liere and Perfecto 2008). In tending the green coffee scale, *A. instabilis* also inadvertently protects the *A. orbigera* larvae from predators and parasitoids (Liere and Perfecto 2008). This protection allows *A. orbigera*, an important predator of the green coffee scale, to develop into a mobile adult coccinellid and provide biological control of the scale in areas of the farm that are unprotected by *A. instabilis*. Therefore, the patchy distribution of *A. instabilis* nest clusters provides not only an enemy free space for larval development, but also untended areas in which *A. orbigera* acts as an effective control agent for the green coffee scale (Liere and Perfecto 2008, Perfecto and Vandermeer 2008, Vandermeer et al. 2008, Jackson et al. 2009).

In addition to the implications for self-organization in this system, our results may signal that variability in the number and accessibility of neighboring shade trees, that is, habitat complexity, could have ramifications for ant diversity in the farm because of the role of the *A. instabilis–C. viridis* association as a keystone mutualism in this system. *A. instabilis* hemipteran-tending activity creates patches of resources, such as scale insects and various other associated arthropods, which are aggregated around individual colonies. Overflow of these resources attracts numerous other species of insects including many other arboreal foraging ants. The dynamic expansion and contraction of *A. instabilis* foraging activity mediated by *L. lecanii* epizootics and habitat complexity (availability of shade tree resources) may be promoting the aggregation of diversity through disruption of the competitive exclusion that would be expected to occur in a static system. Therefore, the local contraction of the foraging range of *A. instabilis*, caused by the *L. lecanii* epizootic and mediated by the availability of foraging refugia, may promote a local increase in the foraging activity of other arboreal-foraging ant species, including twig-nesting and ground-nesting ants. Without this periodic disturbance, diversity of arboreal foraging ants would be expected to be much lower in the farm as a whole. Thus, *L. lecanii* epizootics, in concert with the variable availability of foraging refugia, may contribute to both the generation of spatial structure through self-organization and the maintenance of ant biodiversity in this system. While our results are suggestive of such a pattern, further research is necessary to conclude that ant diversity is in fact increased by this periodic disturbance.

In summary, our results suggest that the localized dynamic interaction between a biotic disturbance (*L. lecanii* epizootics) and habitat complexity (density of shade trees) may promote pattern formation at the landscape scale. This interaction has important implications for the spatial distribution of nest clusters of the keystone species, *A. instabilis*, and provides further evidence that the self-organization of nests may be the result of *L. lecanii* epizootics. By promoting self-organization of *A. instabilis* nests, this interaction between disturbance and habitat complexity may be promoting diversity of arboreal foraging ants, which has been shown to be crucial for maintaining biological control in coffee through autonomous ecosystem function (Larsen and Philpott 2009, Philpott and Armbrecht 2006).

These findings have potentially significant implications for coffee management. This study has demonstrated that local habitat complexity can act to dampen the effect of *L. lecanii* epizootics on *A. instabilis* colonies, maintaining *A. instabilis*, and its important biocontrol services, in the system. Management of shade tree densities in coffee agroecosystems could potentially be used to promote landscape-level pattern formation and local-level maintenance of diversity to maximize ant-derived biological control of coffee pests. Furthermore, our treatment of a fungal entomopathogen as a potentially important actor in driving spatial pattern formation and community interactions is novel and could inform future study of these important organisms.

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