Tropical and Subtropical Agroecosystems, 15 (2012): 389 - 401

OCCURRENCE IN THE SOIL AND DISPERSAL OF *Lecanicillium lecanii*, A FUNGAL PATHOGEN OF THE GREEN COFFEE SCALE (*Coccus viridis*) AND COFFEE RUST (*Hemileia vastatrix*)

[INCIDENCIA EN EL SUELO Y DISPERSIÓN DE *Lecanicillium lecanii*, UN HONGO PATÓGENO DE LA ESCAMA VERDE DEL CAFÉ (*Coccus viridis*) Y DE LA ROYA DEL CAFÉ (*Hemileia vastatrix*)]

Doug Jackson[1*], Kate Zemenick[1] and Graciela Huerta[2]

[1]University of Michigan, Department of Ecology and Evolutionary Biology, 2077 Kraus Natural Science, 830 North University, Ann Arbor, Michigan, U.S.A., 48109.
[2]El Colegio de La Frontera Sur, Departamento de Entomología Tropical, Carretera Antiguo Aeropuerto km 2.5, Tapachula, Chiapas, Mexico
E-mail: dougjack@umich.edu
** Corresponding author*

SUMMARY

The fungus *Lecanicillium lecanii* attacks the green scale (*Coccus viridis*), a pest of coffee, and is also a hyperparasite of coffee rust (*Hemileia vastatrix*). Knowledge of the epizootiology of this fungus is potentially important for conservation biological control in coffee agroecosystems. The presence of viable propagules of *L. lecanii* in the soil, a possible environmental reservoir, was assessed using two baiting methods: the standard *Galleria mellonella* bait method and a *C. viridis* bait method. Infectious propagules of *L. lecanii* were detected in soil samples taken from a 45 ha study plot, both nearby and far from recent epizootics of *L. lecanii*. To test the potential for the transmission of *L. lecanii* conidia from the soil via rain splash or wind, coffee seedlings with populations of *C. viridis* were placed near *L. lecanii*-inoculated soil and then subjected to artificial rain and wind treatments. Rain splash was shown to be a potential transmission mechanism. Dispersal of *L. lecanii* conidia by the ant *Azteca instabilis* was tested using field and laboratory ant-exclusion experiments. *Azteca instabilis* was shown to transport conidia of *L. lecanii*; however, dispersal by *A. instabilis* may not be important under field conditions.

Key words: *Lecanicillium lecanii*; *Azteca instabilis*; *Coccus viridis*; dispersal; fungal entomopathogen; epizootiology

RESUMEN

Dos importantes plagas del café, la escama verde (*Coccus viridis*) y la roya del café (*Hemileia vastatrix*) son atacadas por el hongo *Lecanicillium lecanii*. Por consiguiente, conocer la epizootiología de este patógeno es importante para un exitoso control natural de estas plagas en agroecosistemas de café. Con este objetivo, evaluamos la presencia de propágulos viables de *L. lecanii* en el suelo, que es un potencial reservorio natural para este hongo. Para ello utilizamos dos diferentes carnadas: *C. viridis* y la carnáda estándar, *Galleria mellonella*. A lo largo de una parcela de 45 hectáreas, detectamos propágulos infecciosos de *L. lecanii* en muestras de suelo tomadas de sitios cercanos y lejanos a centros epizoóticos de *L. lecanii*. Para determinar el potencial de la lluvia y el viento para transmitir conidias de *L. lecanii* desde el suelo, colocamos plántulas de café infestadas con *C. viridis* cerca a tierra inoculada con *L. lecanii*. Luego sometimos las plántulas a tratamientos de lluvia y viento artificiales. Encontramos que la lluvia es un posible mecanismo de transmisión de las conidias. Adicionalmente, por medio de experimentos de exclusión en el campo y el laboratorio, evaluamos la capacidad de la hormiga *Azteca instabilis* para dispersar conidias de *L. lecanii*. Encontramos que, aparentemente, *A. instabilis* no es un importante medio de dispersión de las conidias en el campo.

Palabras clave: *Lecanicillium lecanii*; *Azteca instabilis*; *Coccus viridis*; dispersion; hongo entomopatógenico; epizootiología.

INTRODUCTION

Conservation biological control, based on management practices that promote the survival and effectiveness of natural enemies of potential pest species, has attracted considerable attention for sustainable crop production (Barbosa, 1998; Gurr *et al*., 2000; Bale *et al*., 2008; Cullen *et al*., 2008; Fiedler *et al*., 2008; Jackson *et al*., 2009). Fungi are promising candidates for conservation biological control programs, as they are known to attack a variety of pest organisms (Butt *et al*., 2001), including arthropods (Shah and Pell, 2003; Cruz *et al*., 2006), plants (Hasan and Ayres, 1990; Te Beest *et al*., 1992; Charudattan and Dinoor, 2000; Sauerborn *et al*., 2007), and plant pathogens (Kiss, 2003; Fravel, 2005). However, effective conservation biological control using fungal pathogens requires a thorough knowledge of their ecology (Pell *et al*., 2010), which is still lacking, particularly in semi-natural habitats such as complex agroecosystems (Hesketh *et al*., 2010).

The fungal entomopathogen and mycoparasite *Lecanicillium lecanii* (Zimmerman) Zare and Gams is a promising candidate for use in conservation biological control in our study system – an organic, shade coffee agroecosystem in Chiapas, Mexico. *Lecanicillium lecanii* has been shown to be an important natural enemy of the green scale, *Coccus viridis* Green (Hemiptera: Coccidae) in coffee (Easwaramoorthy, 1978; Reddy and Bhat, 1989; Uno, 2007; Jackson *et al*., 2009). It also is known to attack the coffee rust, *Hemileia vastatrix* Berkeley and Broome (Shaw, 1988; Eskes, 1989; González *et al*., 1995; Vandermeer *et al*., 2009), and may suppress this potentially devastating coffee disease (McCook 2006, Suffert *et al.* 2009).

In addition to its direct, negative effects on potential coffee pests, *L. lecanii* may have an important influence on a keystone mutualism between an arboreal-nesting ant, *Azteca instabilis* F. Smith (Hymenoptera: Formicidae), and *C. viridis*. *Azteca instabilis* tends *C. viridis* in a typical ant-hemipteran mutualism, wherein the ants protect the scale insects, which are sedentary as adults, from predators and parasitoids. In exchange, the scales excrete a carbohydrate-rich honeydew that the ants consume. Recent studies have shown that this mutualism may play a key role in maintaining multiple natural pest control agents in this agroecosystem. Because the ants also inadvertently protect the larvae of the coccinellid scale predator *Azya orbigera* Mulsant (Coleoptera: Coccinellidae), the *A. instabilis*-*C. viridis* mutualism provides enemy-free space and high prey density for this important biological control agent (Liere and Perfecto, 2008). This mutualism also contributes to the management of the coffee berry borer, *Hypothenemus hampei* Ferrari (Coleoptera: Scolytidae) through the deterrent effect of *A. instabilis* foragers (Perfecto and Vandermeer, 2006).

Lecanicillium lecanii may strongly influence the location and abundance of *A. instabilis* colonies, and hence may determine the extent of the aforementioned biological control effects of the ant-hemipteran mutualism. In this system, *L. lecanii* often becomes a local epizootic, killing nearly all of the *C. viridis* on a single coffee plant or a small group of neighboring plants. Therefore, *L. lecanii* reduces the amount of carbohydrate food available to an ant colony, which may have an indirect negative effect on colony survival. The potential for *L. lecanii* to cause the ant nest density-dependent mortality of *A. instabilis* colonies — one of the fundamental processes underlying the spatial self organization that generates the low-density, clustered spatial distribution of ant nests in this farm — has recently been demonstrated through a combination of field studies and computer modeling (Jackson *et al.*, 2009).

Although a substantial amount of research has been done on the systematics (Zare *et al*., 2000; Gams and Zare, 2001; Sung *et al*., 2001; Zare and Gams, 2001; Zare *et al*., 2001; Kouvelis *et al.*, 2008) and production (Feng *et al*., 2000; Kamp and Bidochka, 2002; Gao *et al*., 2007; Gao *et al*., 2009, Shi *et al.*, 2009) of *L. lecanii*, much less is known about its basic ecology and natural history, including in the context of coffee agroecosystems.

In the current study, we investigated mechanisms contributing to the development of local epizootics of *L. lecanii.* Epizootics in this system are strongly influenced by the pronounced seasonality in this region, which is characterized by a wet season and a dry season. During the dry season, scale populations, and hence the prevalence of *L. lecanii*, are drastically reduced. *Lecanicillium lecanii* is re-established every wet season following the resurgence of the scale populations. Therefore, the initiation and progression of epizootics depend on one or more initial infection events following the onset of the wet season (primary dispersal) and the subsequent spread of infection from infected *C. viridis* individuals to susceptible individuals (secondary dispersal).

Three fundamental questions follow from the basic epizootiology of this system: 1) where do the propagules of *L. lecanii* persist during the dry season, 2) what are the mechanisms of primary dispersal, i.e., how are propagules initially dispersed onto the coffee plants and the scale insects during the wet season, and 3) what are the mechanisms of secondary dispersal within and between coffee plants following

an initial infection? In this study, we investigate a subset of the mechanisms that may be operative in this system. We hypothesize that the soil provides an environmental reservoir for *L. lecanii*, and that propagules are transmitted from the soil to susceptible scale populations via rain splash or wind dispersal. We also explore the possibility that *A. instabilis* itself is primarily responsible for the dispersal of *L. lecanii* conidia within and between coffee plants.

MATERIALS AND METHODS

The study was performed in a 45 ha plot located at Finca Irlanda, an approximately 300 ha, organic coffee farm in the Soconusco region of Chiapas, Mexico (15° 11' N, 92° 20' W). The farm is a shade coffee plantation, with coffee plants growing beneath trees that have been planted in an approximately uniform distribution. The locations of every shade tree in the 45-hectare plot were obtained from biannual censuses; the locations of *A. instabilis* colonies, which nest in the shade trees, were also recorded during each census. All experiments were performed in the months of July and August, during the wet season (typically early May through November), which is within the peak season for the growth and spread of *L. lecanii* (unpublished data).

Soil sample baiting

Two independent soil sample baiting methods were performed to detect the presence of viable propagules of *L. lecanii* in soil samples. The first employed larvae of the wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae), which is a standard method for detecting entomopathogenic fungi in soil (Zimmermann, 1986). As an alternative method, we used populations of *C. viridis* on coffee leaves to detect the presence of *L. lecanii* propagules.

We obtained soil samples from a total of 40 locations: 10 locations far from *A. instabilis* nests, and therefore far from where epizootics of *L. lecanii* had occurred the previous year; 15 locations near the center of a previous epizootic, site A; and 15 locations near the center of another epizootic, site B (sites and locations indicated in Figures 1 and 2). The first 10 locations were chosen to determine the potential for *L. lecanii* propagules to persist in the soil even without a recent influx of propagules from a nearby epizootic. The other 30 locations were chosen to determine if the prevalence of propagules in the soil decreases with distance from the center of recent epizootics.

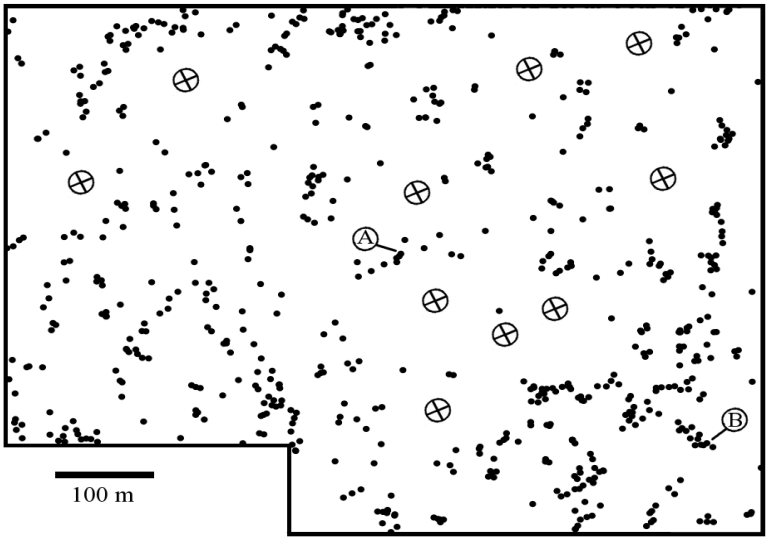

Figure 1. Location of *A. instabilis* ant nests (solid circles) in 45 ha plot: soil sample locations far from *A. instabilis* nests, and therefore far from recent epizootics of *L. lecanii* (circles with crosses); Site A; and Site B.

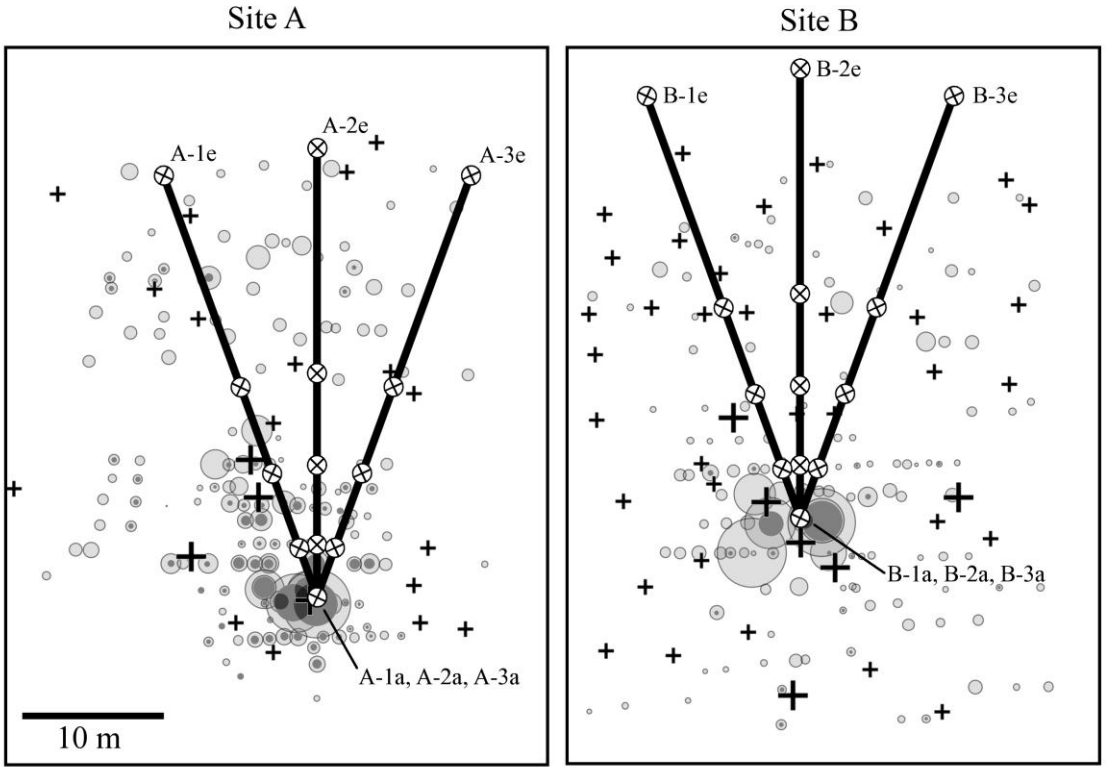

Figure 2. Locations of soil samples on transects leading away from foci of two *L. lecanii* epizootics. Small crosses indicate locations of shade trees. Large crosses indicate shade trees occupied by *A. instabilis* colonies. Light gray circles are proportional to the number of healthy *C. viridis* on individual coffee plants in the previous year, and dark gray circles are proportional to the number of *C. viridis* infected with *L. lecanii*. Circles with crosses show the locations of soil samples. Survey data are adapted from Jackson *et al.* (2009).

Soil samples were taken to a depth of 10 cm using a 2 cm-diameter, manual core sampler. The litter layer, when present, was included in the samples. At each location,10 samples from a 40 cm X 80 cm rectangular area were taken. The core sampler was thoroughly cleaned and rinsed with 100% ethyl alcohol between samples. The 10 samples from each location were combined in separate polyethylene bags. After collection, the soil was spread on paper under aseptic conditions to dry for 24 hours at ambient temperature in the dark. The soil was then homogenized by rolling and passing through a sieve (Niblack and Hussey, 1987).

After the soil was allowed to dry, 90 cc (approximately 80 g) from each sample were placed in a plastic container and moistened evenly with 20 mL of distilled water. Laboratory-reared *G. mellonella* larvae were prepared by placing them in 56 °C water for 7 seconds in order to reduce their activity and discourage them from producing silk webbing in the soil. Each sample was baited with 10 larvae. The plastic containers were then sealed with perforated lids and incubated at room temperature (26-28 °C) for 2 weeks. The larvae were inspected daily, and dead larvae were removed and placed in humidity chambers for later evaluation. In lieu of the usual step of inverting the containers to ensure that the larvae penetrate the soil evenly, the soil was thoroughly mixed during the daily inspection process. At the end of the incubation period, larvae exhibiting fungal growth were inspected using a stereomicroscope at 400x magnification to identify the fungi morphologically.

For the second soil sample baiting, we collected branches with uninfected *C. viridis* populations from three adjacent coffee plants located within the 45 ha plot; there were no scale insects with any visible signs of infection by *L. lecanii* on any of these three plants or the adjacent coffee plants. The average number of large (greater than approximately 0.7 mm in width) scales was 35.8 per leaf (s.d. = 14.3). We then divided the branches into sections of three leaves, selecting one section at random for each soil sample. Ten grams of soil from each sample were suspended in 10

mL of distilled water. The suspension was applied, using a small paintbrush, to inoculate the scale insects on a leaf. This procedure was immediately replicated for the other two leaves assigned to the soil sample, i.e., a separate suspension was prepared for each leaf. As a control, 10 groups of leaves with scale insects (30 leaves) were treated with distilled water. The leaves were placed in humidity chambers at 100% relative humidity and incubated for 2 weeks. Fungal infections were identified morphologically using a stereomicroscope (400x magnification).

Rain splash and wind dispersal

The potential for rain splash and wind dispersal of conidia from the soil was tested using coffee seedlings containing susceptible scale insect populations placed in four treatments: rain, rain-wind, wind, and control. The average number of scale insects per seedling was 112.6 (s.d. = 92.7). For this and all other experiments, only adult scales larger than approximately 0.7 mm in width were counted. The seedlings used in this and all other experiments were obtained from the farm's nursery, where they were planted and reared in 10 x 20 cm black polyethylene bags. Four seedlings were randomly assigned to each treatment, for a total of 16 seedlings. The seedlings were placed in the four corners of white 60 × 60 × 60 cm insect rearing tents (BugDorm-2, MegaView Science Co., Ltd., Taiwan). A plastic tray (26.5 × 17.5 × 6.0 cm) with soil that had been inoculated with an aqueous suspension of *L. lecanii* conidia was placed in the center of each group of four seedlings.

The inoculum was an aqueous suspension of *L. lecanii* conidia cultured from spores and hyphae acquired from an infected *C. viridis* obtained within the 45 ha plot. The *L. lecanii* isolate originated in a single *C. viridis* individual from a population affected by a severe epizootic, with nearly 100% prevalence of *L. lecanii*, and therefore was likely of average, or possibly above average (for our study site), pathogenicity to *C. viridis*. Following isolation of *L. lecanii* from the scale insect, conidia were mass-produced via solid-state fermentation using cooked rice as a substrate. We then suspended the resultant conidia in 5 L of 0.1% Tween 80 solution. Approximately 0.45 mL of suspension was added per cubic centimeter of soil at the start of the experiment. The conidial concentration, approximately 1.9 X 10^5 conidia/mL, was determined using a hemacytometer.

Seedlings in the rain and rain-wind treatments were removed from their tents once every 24 hours during the two week experiment to be exposed to artificial rain splash. During the rain treatment, the seedlings were placed around their respective plastic trays, with one seedling on each edge. Two minutes of simulated rain were created using a 2.5-gallon plastic bladder connected to a hose with a spray nozzle and filled with room-temperature tap water. Prior to the experiment, the volume and intensity of the simulated rain was compared and adjusted to qualitatively match rainfall typical of the study site, approximately 20 mm rain/day during the wet season (Richter, 2000). The simulated rain was focused on the center of the plastic tray such that the rain impinged primarily on the soil but also fell on the seedlings. After one minute, the plants were moved in a clockwise manner to an adjacent edge of the tray to account for the rectangular shape of the tray, i.e., so that each plant was exposed to equivalent rain splash intensity. The bottoms of the plastic trays were perforated to allow the water from the simulated rain to drain. To prevent any potential loss of conidia from the inoculated soil, we placed the rain-wind treatment tray underneath the rain treatment tray while the simulated rainfall was performed on the rain treatment, and vice versa. To balance the net washout of conidia, we alternated the order of the simulated rain treatment, i.e., every other day the same treatment was rained on first. The plants from all of the treatments were taken out of their cages and left outside while the simulated rain was being applied so that each plant spent the same amount of time outside of the tents. The seedlings were always returned to the same corners of the tents in order to avoid cross contamination between plants.

After all plants were returned to their tents, the wind and rain-wind treatments were exposed to simulated wind that was created by small electric fans (one fan per tent). The fans were run for 30 minutes at maximum speed, which is qualitatively similar to the typical maximum daily wind speed at the study site (3-4 on the Beaufort Scale). The orientation of each fan was changed daily by rotating the fan 90 degrees clockwise; this was done to vary the direction of the airflow impinging on the plants.

Seedlings were inspected daily for scale individuals exhibiting the white halo of mycelia characteristic of infection by *L. lecanii*. A final count of infected and healthy *C. viridis* adults was performed after two weeks, at the conclusion of the experiment.

Ant exclusion

Two ant exclusion experiments were performed: a laboratory experiment, in which most potential conidia dispersal mechanisms were eliminated, and a field experiment, which included the full complement of potential conidia dispersal pathways (e.g., wind, rain splash, arthropods, and other animals).

For the laboratory ant exclusion experiment, eighteen small coffee seedlings inhabited by populations of *C. viridis* were obtained from the farm's nursery. The *C. viridis* populations on six of the seedlings showed signs of being infected with *L. lecanii*, with some of the scales surrounded by the white halo of mycelia indicative of *L. lecanii* infection. The scales on the other 12 seedlings showed no signs of infection. The average number of scales on these 12 seedlings was 99.8 per plant (s.d. = 38.5). The six seedlings harboring infected scales were set aside as sources of fungal conidia, and the 12 infection-free seedlings were designated for use in the treatments.

For each replicate, three plastic flower pots were attached in a line to a wooden board, with approximately five cm separating the pots. An infected seedling was planted in the center pot and then covered with an enclosure of clear plastic in order to prevent transmission of fungal conidia by air currents or flying insects. The top of the plastic enclosure was rolled up and sealed with metal clips to allow for periodic access to the seedling. A small opening covered with mosquito netting was included on one side at the top of the enclosure as a vent to prevent condensation from accumulating inside. Two fungus-free seedlings were then planted in the two adjacent pots. These seedlings were also covered with plastic enclosures, with the vents on both of these enclosures facing in the opposite direction from the infected seedling's vent. To allow the passage of ants from the center seedling to the ant inclusion treatment seedling, an approximately 2.5 cm-diameter clear plastic tube penetrating the enclosures was routed between the two seedlings. An identical tube was routed between the center seedling and the ant exclusion treatment, with the exception that one end of the tube was covered with mosquito netting to prevent ants from entering the tube. Hot glue was used to thoroughly seal the enclosures to ensure that ants could not escape and that other arthropods could not enter the enclosures. Six identical replicates were constructed.

At the beginning of the experiment, a single coffee leaf with scales heavily infected by *L. lecanii* was tied to the base of each infected seedling in order to increase the amount of conidia available for the ants to spread. The coffee leaves were collected from the site of a severe epizootic, with nearly 100% prevalence of *L. lecanii*, and therefore it is probable that the pathogenicity of the strain(s) of *L. lecanii* used as inoculum were of at least average (for our study site) pathogenicity to *C. viridis*. Approximately 150 *A. instabilis* ants were then placed in the enclosures with the seedlings and leaves harboring infected scales. After three weeks, the scales on the seedlings were counted and the number of scales showing signs of infection by *L. lecanii* was noted.

For the field ant exclusion experiment, twenty coffee seedlings inhabited by *C. viridis* populations, with a mean of 202.1 scales per plant (s.d. = 136.9), were placed in plastic pots and arranged in a circle around a shade tree containing an active *A. instabilis* colony. Since the purpose of the *A. instabilis* colony was simply to provide a source of ant foragers, all of the field ant exclusion replicates were located near a single, vigorous colony. The plants were placed two meters from the base of the shade tree, with 20 cm separating each pot. To encourage discovery of the seedlings by the ants, bridges of plastic twine were tied between the shade tree and the bases of the seedlings.

The seedlings were assigned in an alternating manner to either the ant exclusion treatment or the ant inclusion treatment, i.e., 10 seedlings were assigned to each treatment type. A piece of a coffee leaf covered with approximately 10 *C. viridis* that had been infected by *L. lecanii*, obtained from a site subject to a severe epizootic, was tied around the stem at the base of each coffee seedling to provide a source of conidia. An approximately eight centimeter wide strip of flagging tape was wrapped around the base of the seedlings, just beneath the infected coffee leaf; Tanglefoot® (Tanglefoot Co., Grand Rapids, Michigan, USA) was applied to the flagging tape on the ant exclusion seedlings. Surrounding vegetation was cleared to ensure that no bridges were available whereby the ants could access the seedlings from neighboring vegetation. All ants were removed from the ant exclusion seedlings by hand, using a small paintbrush, following the application of Tanglefoot®. The seedlings were left in the field from 15 July to 4 August. They were inspected daily to ensure that no ants had gained access to the ant exclusion seedlings. To encourage a more typical number of ants to discover and tend the scale insects on the ant inclusion seedlings, small pieces of tuna were placed at the bottom of all seedlings on 18 July. The leaves with fungus that had been tied to the base of the seedlings were beginning to show signs of decomposition by 27 July, so a single coffee berry with approximately five fungus-infected scales from the location of a major epizootic was attached with a wire-tie to the base of each seedling to provide a fresh source of inoculum. Following the experiment, prevalence of *L. lecanii* was assessed.

Statistical analyses were performed following the resampling, or bootstrapping with permutation, method described in Liere and Perfecto (2008). In this method, synthetic treatment and control populations are created by resampling without replacement from

the original observations. The relevant statistical measure (e.g., the mean number of infections) is then calculated for these synthetic treatment and control populations. Next, the difference between the values of this metric for the two synthetic populations is compared to the difference between the values of this metric for the actual treatment and control populations. This procedure is repeated many times, and a *P*-value is calculated based on the proportion of repeats for which the difference between synthetic populations is as extreme or more extreme than the difference between the actual populations. The result is an estimate of the probability that the treatment and control populations could be as different as they are by chance alone. Data were resampled 10,000 times. The rain splash and wind dispersal data were resampled using a custom script in Matlab, while the ant exclusion data were analyzed using the Resampling Stats Excel add-in version 3.2 (Resampling, 2006).

RESULTS

Soil sample baiting

Of the 400 larvae used in the *G. mellonella* larvae baiting (10 larvae/sample X 40 samples), 202 were infected by one or more entomopathogenic fungi. Of these, six were infected with *L. lecanii*, based on morphological identification using the characteristic conidia and diagnostic phialides (Zare and Gams, 2001). Two of the *L. lecanii*-infected larvae were from samples taken from the points nearest to the focus of the *L. lecanii* epizootic at Site B (B-1a and B-2a); one was from a sample taken at one of the fourth-furthest transect points at Site A (A-2d); and the other three larvae were from samples taken far from *A. instabilis* nests (Table 1). In no case was there more than one larva per soil sample infected by *L. lecanii.*

The *C. viridis* baiting method yielded eight positive identifications of *L. lecanii* from the 40 soil samples, at the following locations: the fourth-furthest point at Site A (A-1d); all five distances at Site B (B-2a through B-2e); and two locations far removed from *A. instabilis* nests (Table 1). All of the positive samples from Site B were taken from the middle transect. All three replicates from the third-furthest point at Site B were positive, and two of the replicates from the fifth-furthest point were positive, meaning that a total of 11 of the 120 assays (3 replicates per sample X 40 soil samples) were positive. None of the scale insects on the control leaves were infected.

Of the 14 sampling locations that tested positive for the presence of *L. lecanii*, only one location – a point nearest to the center of the epizootic at Site A – tested positive using both methods. That is, a total of 13 of the 40 sampling locations tested positive for *L. lecanii* using one or the other of the two methods.

Table 1. Locations of positive *G. mellonella* and *C. viridis* baiting results. Three locations near the centers of previous *L. lecanii* epizootics yielded positive results with the *G. mellonella* method; six were positive with the *C. viridis* method. Three positives were obtained from locations far from previous epizootics using *G. mellonella*, while the *C. viridis* method yielded two. See Figures 1 and 2 for location information.

Location	*G. mellonella*	*C. viridis*
A-1a		
A-1b		
A-1c		
A-1d		X
A-1e		
A-2a		
A-2b		
A-2c		
A-2d	X	
A-2e		
A-3a		
A-3b		
A-3c		
A-3d		
A-3e		
B-1a	X	
B-1b		
B-1c		
B-1d		
B-1e		
B-2a	X	X
B-2b		X
B-2c		X
B-2d		X
B-2e		X
B-3a		
B-3b		
B-3c		
B-3d		
B-3e		
Far from nests	3	2

Rain splash and wind dispersal

At the end of the experiment, three of the four rain treatment seedlings had scales infected by *L. lecanii*; one of the rain-wind treatment seedlings had infected scales; and none of the wind or control treatment seedlings had infected scales. The mean percentages of scales infected with *L. lecanii* were 0.0%, 3.2 ±

2.6% (SE), 0.0%, and 0.3 ± 0.3% for the control, rain, wind, and rain-wind treatments, respectively. The difference in the number of scales infected in the rain treatments compared to the control, wind, and rain-wind treatments was significantly greater than the random expectation ($P < 0.0001$, $P < 0.0001$, and $P < 0.01$, respectively). The difference in the number of scales infected in the rain-wind and the control treatments, however, was not greater than expected by chance ($P = 0.27$). There was no significant linear relationship between the number of scales per plant and the rate of infection ($P = 0.28$).

Ant exclusion

In the laboratory ant exclusion experiment, scales on five of the six ant inclusion seedlings exhibited the white mycelial mat characteristic of *L. lecanii* infection, while only one scale on the ant exclusion seedlings showed signs of possibly being infected. On the ant inclusion seedlings with *L. lecanii*-infected scales, the percentage of infected scales ranged from 1.8% to 12.5%. The mean percentage of scales killed by *L. lecanii* was significantly greater for the ant inclusion seedlings than for the ant exclusion seedlings (0.1 ± 0.2% [SE] without ants, 4.3 ± 1.8% with ants, $P < 0.01$).
In the field ant exclusion experiment, the percentage of infected scales on the ant exclusion seedlings ranged from 3.0% to 46.5%, while on the ant inclusion seedlings the range was 3.6% to 42.2%. The mean percentages of scales killed by *L. lecanii* with or without ants were not significantly different (17.4 ± 4.6% [SE] without ants, 18.2 ± 4.3% with ants, $P = 0.44$).

There was no significant linear relationship between the average number of scales per plant and the rate of infection in either the lab experiment ($P = 0.80$) or the field experiment ($P = 0.84$)

DISCUSSION

These results suggest the following scenario for the development of epizootics in this coffee agroecosystem. During the dry season, the populations of *C. viridis* are markedly smaller than during the wet season. Therefore, individual populations of scale insects are below the epizootic threshold density, and *L. lecanii* persists primarily in the environmental reservoir provided by the soil. As the scale populations increase following the onset of the wet season, they are exposed to *L. lecanii* propagules splashed up from the soil, which provide the inocula necessary to initiate epizootics. Further development of an epizootic almost certainly requires transmission of conidia between individuals in the scale population, which can be effected by *A. instabilis* and other, as yet unknown, vectors. These processes lead to a rapid increase in the prevalence of *L. lecanii* shortly after the start of the wet season, which has been observed in our study site (unpublished data) and others (Reimer and Beardsley, 1992).

The baiting results demonstrate that viable propagules of *L. lecanii* can be found in locations that are as far removed as possible in this system (up to approximately 50 m) from recent *L. lecanii* epizootics. This suggests that either 1) *L. lecanii* can persist in the soil for multiple seasons or 2) *L. lecanii* is not dispersal limited in this system.

The fact that the soil can act as an environmental reservoir for *L. lecanii* in this system has important implications for the epizootiology of this fungus. The temporal dynamics of diseases have been shown to be strongly influenced by the presence of a pathogen reservoir: Hochberg (1989) showed that intermediate levels of translocation of a pathogen from a reservoir result in damped oscillations and relative stability of an otherwise oscillatory system. While the results of the rain splash experiment demonstrate that translocation of *L. lecanii* from the soil is possible, further study will be necessary to determine the actual level of translocation under field conditions. In particular, the concentration of *L. lecanii* in the soil in the field, and how this concentration varies spatially and temporally, are unknowns that could significantly affect the realized translocation rate.

The spatial dynamics of this system will also be strongly affected by the apparent ubiquity of infectious propagules in the soil. Transmission of *L. lecanii* upwards from infected soil widely distributed within the farm would likely result in much more rapid and widespread infection at the onset of the wet season compared to transmission from multiple point sources, e.g., from isolated cadavers left over from epizootics that occurred in the previous wet season. The potential for *C. viridis* to escape foci of previous epizootics by dispersing is also likely to be greatly reduced by the widespread occurrence of *L. lecanii* propagules in the soil.

The results of the two soil sample baitings are also interesting from a methodological perspective. In none of the samples were multiple replicates of the *G. mellonella* larvae infected by *L. lecanii*, which suggests that there is a large element of chance with this method, i.e., the presence of infectious material in a sample will not necessarily result in infection of the larvae. This may be due to the larvae failing to come into contact with the infectious material, possibly due to a very low density of infectious material in the sample; resistance of the larvae to

infection; mortality due to other causes that occurs before the larvae can become infected; or *L. lecanii* being outcompeted within a single larva by another entomopathogenic fungus. Negative results of this method, therefore, should be treated with caution. Results from the *C. viridis* baiting were similarly subject to chance. However, the issue of other entomopathogenic fungi outcompeting *L. lecanii* was not a concern with this method, as *C. viridis* did not become infected by any fungi other than *L. lecanii*, perhaps because it is not susceptible to the broad range of entomopathogenic fungi that infected the *G. mellonella* larvae.

Another consideration raised by our study is that using a bait species known to be a target of the entomopathogen of interest may be a more powerful detection strategy than using a non-target bait species. Although there was not a significant difference in the total number of positive samples obtained using the two bait species employed in our study, Klingen *et al*. (2002) reported that using a pathogen-specific host species as a bait yielded significantly more positives than using *G. mellonella*. Therefore, when considering the apparent rarity of *L. lecanii* in our study system (15% and 20% positive samples with the *G. mellonella* and *C. viridis* methods, respectively) and other agroecosystems [e.g., 0.4-2.6% in a study by Meyling and Eilenberg (2007)], the potential influence of the sensitivity of the bait species should be kept in mind. An understanding of the role of the soil as an environmental reservoir for fungal entomopathogens in a given system would likely benefit from a combination of standard baiting methods (e.g., the *G. mellonella* bait method), baiting methods that are specifically tailored to the system (e.g., the *C. viridis* method used here), and molecular approaches (Enkerli and Widmer, 2010), including those that allow for quantitative assessments. A quantitative assessment of the abundance of *L. lecanii* propagules may reveal a dispersal kernel dependent on the distance from recent epizootics, which we were unable to detect using our experimental methods.

In the rain splash and wind dispersal experiment, the lower infection rate in the rain-wind treatment relative to the rain treatment suggests that there may be an important interaction between rain splash and wind in this agroecosystem. Wind increases the rate of evaporation of rain splash from the surface of the scale insects, and therefore may decrease infection rates due to desiccation of conidia. Airflow may also remove rain splash-dispersed conidia from the scale insects before they are able to germinate. This potential interplay between rain splash and wind may have important implications for management of shade levels in coffee agroecosystems. As shade level increases, the intensity of rain splash and wind will both decrease, which may serve to simultaneously decrease dispersal of conidia from the soil while increasing the probability of success of the conidia that are dispersed. Therefore, prevalence of *L. lecanii* may be maximized at an intermediate shade level. To our knowledge, although the effect of shade on prevalence following artificial inoculation has been studied (Easwaramoorthy and Jayaraj, 1977), the effects of shade level on the occurrence of natural epizootics of *L. lecanii* has not been investigated.

Rain splash dispersal of fungal entomopathogens has not been studied extensively, but has been previously noted by other researchers, including dispersal of *Beauveria bassiana* from the soil onto leaves of corn plants (Bruck and Lewis, 2002) and of the mealybug pathogen *Hirsutella cryptosclerotium* (Fernandez-Garcia and Fitt, 1993). Fitt *et al*. (1989) identify characteristics of fungi that tend to be rain splash dispersed, such as mucilaginous conidia; Heale (1988) notes that *Verticillium lecanii* conidia are produced in mucilaginous heads and dispersed by water splash or insects. There is also a substantial literature on rain splash dispersal of fungal pathogens of plants (for example, Madden, 1997; Geagea *et al.*, 2000; Ahimera *et al.*, 2004; Huber *et al*., 2006).

The results from the laboratory ant exclusion experiment suggest that *A. instabilis* is capable of transporting conidia of *L. lecanii*, and hence may play a role in dispersing the fungus throughout populations of *C. viridis*. This would seem to indicate that transmission of conidia via ants between branches in a coffee plant, or perhaps between coffee plants themselves, is possible. However, the proportion of scale insects infected by the fungus was very low in the laboratory experiment relative to the field experiment, so the ants appear to be relatively poor dispersal agents. It is important to consider, however, that differences in pathogenicity of the inocula used in the two experiments could be partially responsible for the disparity in infection rates.

These results are consistent with a previous study that showed that the common black ant, *Lasius niger* (Hymenoptera: Formicidae), was capable of retaining conidia of an entomopathogenic fungus previously grouped in the *V. lecanii* species complex (Sitch and Jackson, 1997; Bird *et al.*, 2004) and that by transporting conidia to tended aphids, it can serve as a vector. Bird *et al*. (2004) demonstrated that *L. niger* workers artificially inoculated with *Lecanicillium longisporum* (Zimmerman) Zare and Gams [*Verticillium lecanii* (Zimmerman) Viégas] conidia could infect aphid populations, causing significant mortality under laboratory, semi-field, and field conditions. Aphid mortality due to *L. longisporum*

was greatest under laboratory conditions and least under field conditions, which contrasts with the observations of this study. However, relative mortality under laboratory and field conditions depends heavily on the specific attributes of the methodologies and the laboratory and field environments (e.g., microclimate, presence of other potential vectors, etc.), so it is not possible to draw any general conclusions from this discrepancy.

The coffee seedlings used in the field ant exclusion experiment were most representative of smaller coffee plants and the lowest branches of larger plants. Based on the results from this study, other dispersal mechanisms besides *A. instabilis*-vectored dispersal from one scale insect to another dominate in these locations. There are a number of dispersal agents that could disperse *L. lecanii* conidia, such as rain splash from the soil or between *C. viridis* individuals, or any of the sundry flying and crawling arthropods that visit the coffee plants.

Roditakis *et al.* (2000) showed that aphids are capable of transporting conidia of *L. lecanii*, so it is likely that other arthropods in this system are also capable of spreading conidia of *L. lecanii*. Sitch and Jackson (1997) demonstrated that resistant arthropods from a variety of orders are capable of retaining *Verticillium lecanii* conidia, albeit at lower rates than target aphid species. A particularly intriguing possibility is that the predatory beetle *A. orbigera*, a key predator of scale insects in this system that is positively associated with the presence of the *A. instabilis*-*C. viridis* mutualism (Liere and Perfecto, 2008), may be a primary vector of *L. lecanii*. Such a phenomenon would not be unprecedented, as the coccinellid aphid predator *Coccinella septempunctata* (Coleoptera: Coccinellidae) has been shown to be a potential vector of an entomopathogenic fungus when artificially inoculated, causing significant aphid mortality due to fungal infection (Roy *et al.*, 2001). Whatever the dominant dispersal agents are, previous work showing a signal of dispersal-limited spread between coffee plants (Jackson *et al.*, 2009) suggests that these mechanisms are primarily transmitting the fungus between adjacent plants.

It is important to note that *A. instabilis* very likely plays a central role in the dynamics of *L. lecanii* infection of *C. viridis* even if it is not primarily responsible for dispersal of conidia. There appears to be a minimum abundance and density of *C. viridis* that are necessary for an outbreak of *L. lecanii* to occur, i.e., an epizootic threshold density (unpublished data). When such an outbreak occurs, the fungus kills the vast majority of scales on entire coffee plants. Without *A. instabilis* tending the scales and providing protection from predators and parasitoids, the scale population is unlikely to reach a sufficient size for a fungal outbreak to occur (Reimer *et al.*, 1993; Uno, 2007). Therefore, *A. instabilis* is likely an important factor in determining the local prevalence of *L. lecanii*.

CONCLUSIONS

Our results suggest that a complete understanding of the epizootiology of *L. lecanii* will require knowledge of multiple phases of transmission and persistence: persistence in the soil, particularly during the dry season; translocation of propagules from the soil via rain splash; secondary dispersal between coffee plants, branches, and *C. viridis* individuals; and subsequent replenishment of the environmental reservoir in the soil. The spatial extent, phenology, and dynamics of epizootics in this system are all influenced by the details of these processes.

Understanding the development of *L. lecanii* epizootics in this system is crucial because of the role *L. lecanii* may play in the biological control of important coffee pests: directly, by attacking *C. viridis* and the coffee rust *H. vastatrix*, and indirectly, via its potential to influence the spatial distribution of the *A. instabilis*-*C. viridis* keystone mutualism. Consequently, enhanced understanding of the mechanisms controlling the occurrence of *L. lecanii* epizootics in this system, and appropriate management practices informed by this knowledge (e.g., coffee plant height and planting density, shade levels, etc.), appear to have an enormous potential benefit in terms of improved conservation biological control in this and other similar coffee agroecosystems.

ACKNOWLEDGEMENTS

We thank the Peters family for permission to work on their farm. Ricardo Alberto Toledo Hernández and Juan Cisneros Hernández at El Colegio de La Frontera Sur provided valuable assistance with the laboratory work. John Vandermeer and two anonymous reviewers provided helpful comments on earlier versions of this manuscript. We thank Heidi Liere for help with translation. This work was supported by the International Institute of the University of Michigan and NSF Grant DEB-0349388 to Ivette Perfecto and John Vandermeer

REFERENCES

Ahimera, N., Gisler, S., Morgan, D., Michailides, T. 2004. Effects of single-drop impactions and natural and simulated rains on the dispersal of *Botryosphaeria dothidea* conidia. Phytopathology 94:1189-1197.

Bale, J., Van Lenteren, J., Bigler, F. 2008. Biological control and sustainable food production. Philosophical Transactions B 363:761-776.

Barbosa, P. 1998. Conservation biological control. Academic Press, San Diego, California.

Bird, A.E., Hesketh, H., Cross, J.V., Copland, M. 2004. The common black ant, *Lasius niger* (Hymenoptera : Formicidae), as a vector of the entomopathogen *Lecanicillium longisporum* to rosy apple aphid, *Dysaphis plantaginea* (Homoptera : Aphididae). Biocontrol Science and Technology 14:757-767.

Bruck, D., Lewis, L. 2002. Rainfall and crop residue effects on soil dispersion and *Beauveria bassiana* spread to corn. Applied Soil Ecology 20:183-190.

Butt, T. M., Jackson, C., Magan, N. 2001. Fungi as biocontrol agents: progress, problems, and potential. CABI Publishing, Wallingford.

Charudattan, R., Dinoor, A. 2000. Biological control of weeds using plant pathogens: accomplishments and limitations. Crop Protection 19:691-695.

Cruz, L., Gaitan, A., Gongora, C. 2006. Exploiting the genetic diversity of *Beauveria bassiana* for improving the biological control of the coffee berry borer through the use of strain mixtures. Applied Microbiology and Biotechnology 71:918-926.

Cullen, R., Warner, K., Jonsson, M., Wratten, S. 2008. Economics and adoption of conservation biological control. Biological Control 45:272-280.

Easwaramoorthy, S. 1978. Effectiveness of the white halo fungus, *Cephalosporium lecanii*, against field populations of coffee green bug, *Coccus viridis*. Journal of Invertebrate Pathology 32:88-96.

Easwaramoorthy, S., Jayaraj, S. 1977. The effect of shade on the coffee green bug, *Coccus viridis* (Green) and its entomopathogenic fungus, *Cephalosporium lecanii* Zimm. Journal of Coffee Research 7:111-113.

Enkerli, J., Widmer, F. 2010. Molecular ecology of fungal entomopathogens: molecular genetic tools and their applications in population and fate studies. Biocontrol 55:17-37.

Eskes, A. B. 1989. Natural enemies and biological control. In: Kushalappa, A.C., and Eskes, A.B. (eds.). Coffee Rust: Epidemiology, Resistance, and Management. CRC Press, Boca Raton, FL. pp. 162-168.

Feng, K., Liu, B., Tzeng, Y. 2000. *Verticillium lecanii* spore production in solid-state and liquid-state fermentations. Bioprocess and Biosystems Engineering 23:25-29.

Fernandez-Garcia, E., Fitt, B. 1993. Dispersal of the entomopathogen *Hirsutella cryptosclerotium* by simulated rain. Journal of Invertebrate Pathology 61:39-43.

Fiedler, A., Landis, D., Wratten, S. 2008. Maximizing ecosystem services from conservation biological control: the role of habitat management. Biological Control 45:254-271.

Fitt, B., McCartney, H., Walklate, P. 1989. The role of rain in dispersal of pathogen inoculum. Annual Review Phytopathology 27:241-270.

Fravel, D. 2005. Commercialization and implementation of biocontrol 1. Annual Review Phytopathology 43:337-359.

Gams, W., Zare, R. 2001. A revision of *Verticillium* sect. Prostrata. III. Generic classification. Nova Hedwigia 72:329-337.

Gao, L., Liu, X., Sun, M., Li, S., Wang, J. 2009. Use of a novel two-stage cultivation method to determine the effects of environmental factors on the growth and sporulation of several biocontrol fungi. Mycoscience 50:317-321.

Gao, L., Sun, M., Liu, X., Che, Y. 2007. Effects of carbon concentration and carbon to nitrogen ratio on the growth and sporulation of several biocontrol fungi. Mycological Research 111:87-92.

Geagea, L., Huber, L., Sache, I., Flura, D., McCartney, H., Fitt, B. 2000. Influence of simulated rain on dispersal of rust spores from infected wheat seedlings. Agricultural and Forest Meteorology 101:53-66.

González, E., Bravo, N., Carone, M. 1995. Caracterización de *Verticillium lecanii* (Zimm.) Viegas hiperparasitando *Hemileia vastatrix* Berk y Br y *Coccus viridis* Green. Revista de Protección Vegetal 10:169-171.

Gurr, G.M., Wratten, S.D., Barbosa, P. 2000. Success in conservation biological control of arthropods. In: Gurr, G.M. and Wratten, S.D. (eds.). Biological control: measures of success. Kluwer Academic Publishers, London, UK. pp. 105-132.

Hasan, S. and Ayres, P. 1990. The control of weeds through fungi: principles and prospects. New Phytologist 115:201-222.

Heale, J. B. 1988. The potential impact of fungal genetics and molecular biology on biological control, with particular reference to entomopathogens. In: M. N. Burge, (ed.). Fungi in biological control systems. Manchester University Press, Manchester, U.K. pp. 211-234.

Hesketh, H., Roy, H.E., Eilenberg, J., Pell, J.K., Hails, R.S. 2010. Challenges in modelling complexity of fungal entomopathogens in semi-natural populations of insects. Biocontrol 55:55-73.

Hochberg, M. 1989. The potential role of pathogens in biological control. Nature 337:262-265.

Huber, L., Madden, L., Fitt, B.D. 2006. Environmental biophysics applied to the dispersal of fungal spores by rain-splash. In: Cooke, B.M., Gareth Jones, D., and Kaye, B. (eds.). The Epidemiology of Plant Diseases. Springer, Netherlands. pp. 417-444.

Jackson, D., Vandermeer, J., Perfecto, I. 2009. Spatial and temporal dynamics of a fungal pathogen promote pattern formation in a tropical agroecosystem. The Open Ecology Journal 2:62-73.

Kamp, A., Bidochka, M. 2002. Conidium production by insect pathogenic fungi on commercially available agars. Letters in Applied Microbiology 35:74-77.

Kiss, L. 2003. A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. Pest Management Science 59:475-483.

Klingen, I., Eilenberg, J., Meadow, R. 2002. Effects of farming system, field margins and bait insect on the occurrence of insect pathogenic fungi in soils. Agriculture, Ecosystems & Environment 91:191-198.

Kouvelis, V., Sialakouma, A., Typas, M. 2008. Mitochondrial gene sequences alone or combined with ITS region sequences provide firm molecular criteria for the classification of *Lecanicillium* species. Mycological Research 112:829-844.

Liere, H., Perfecto, I. 2008. Cheating on a mutualism: indirect benefits of ant attendance to a coccidophagous coccinellid. Environmental Entomology 37:143-149.

Madden, L. 1997. Effects of rain on splash dispersal of fungal pathogens. Canadian Journal of Plant Pathology 19:225-230.

McCook, S. 2006. Global rust belt: *Hemileia vastatrix* and the ecological integration of world coffee production since 1850. Journal of Global History 1:177-195.

Meyling, N., Eilenberg, J. 2007. Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control. Biological Control 43:145-155.

Niblack, L.T., Hussey, S.R. 1987. Extracción de nematodes del suelo y de tejidos vegetales. In: Zuckerman, B.M., Mai, W.F., and Harrison, M.B. (eds.). Fitonematologia: manual de laboratorio. Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba, Costa Rica. pp. 235-242.

Pell, J., Hannam, J., Steinkraus, D. 2010. Conservation biological control using fungal entomopathogens. Biocontrol 55:187-198.

Perfecto, I., Vandermeer, J. 2006. The effect of an ant-hemipteran mutualism on the coffee berry borer (*Hypothenemus hampei*) in southern Mexico. Agriculture Ecosystems & Environment 117:218-221.

Reddy, K.B., Bhat, P.K. 1989. Effect of relative humidity and temperature on the biotic agents of green scale *Coccus viridis* (Green). Journal of Coffee Research 19:82-87.

Reimer, N.J., Beardsley, J.W. 1992. Epizootic of white halo fungus, *Verticillium lecanii* (Zimmerman), and effectiveness of insecticides on *Coccus viridis* (Green) (Homoptera: Coccidae) on coffee at Kona, Hawaii. Proceedings, Hawaiian Entomological Society 31:73-81.

Reimer, N.J., Cope, M., Yasuda, G. 1993. Interference of *Pheidole megacephala* (Hymenoptera: Formicidae) with biological control of *Coccus viridis* (Homoptera: Coccidae) in coffee. Environmental Entomology 22:483-488.

Resampling. 2006. Resampling stats for Excel user's guide version 3. Resampling Stats.

Richter, M. 2000. The ecological crisis in Chiapas: a case study from Central America. BioOne 20:332-339.

Roditakis, E., Couzin, I., Balrow, K., Franks, N. 2000. Improving secondary pick up of insect fungal pathogen conidia by manipulating host behaviour. Annals of Applied Biology 137:329-335.

Roy, H.E., Pell, J.K., Alderson, P.G. 2001. Targeted dispersal of the aphid pathogenic fungus *Erynia neoaphidis* by the aphid predator *Coccinella septempunctata*. Biocontrol Science and Technology 11:99-110.

Sauerborn, J., Müller-Stöver, D., Hershenhorn, J. 2007. The role of biological control in managing parasitic weeds. Crop Protection 26:246-254.

Shah, P., Pell, J. 2003. Entomopathogenic fungi as biological control agents. Applied Microbiology and Biotechnology 61:413-423.

Shaw, D. E. 1988. *Verticillium lecanii* a hyperparasite on the coffee rust pathogen in Papua New Guinea. Australasian Plant Pathology 17:2-3.

Shi, Y., Xu, X., Zhu, Y. 2009. Optimization of *Verticillium lecanii* spore production in solid-state fermentation on sugarcane bagasse. Applied Microbiology and Biotechnology 82:921-927.

Sitch, J.C. and Jackson, C.W. 1997. Pre-penetration events affecting host specificity of *Verticillium lecanii*. Mycological Research 101:535-541.

Suffert, F., Latxague, É., Sache, I. 2009. Plant pathogens as agroterrorist weapons: assessment of the threat for European agriculture and forestry. Food Security 1:221-232.

Sung, G.H., Spatafora, J.W., Zare, R., Hodge, K.T., Gams, W. 2001. A revision of *Verticillium* sect. Prostrata. II. Phylogenetic analyses of SSU and LSU nuclear rDNA sequences from anamorphs and teleomorphs of the Clavicipitaceae. Nova Hedwigia 72:311-328.

Te Beest, D., Yang, X., Cisar, C. 1992. The status of biological control of weeds with fungal pathogens. Annual Review Phytopathology 30:637-657.

Uno, S. 2007. Effects of management intensification on coccids and parasitic hymenopterans in coffee agroecosystems in Mexico. University of Michigan, Ann Arbor, MI.

Vandermeer, J., Perfecto, I., Liere, H. 2009. Evidence for hyperparasitism of coffee rust (*Hemileia vastatrix*) by the entomogenous fungus, *Lecanicillium lecanii*, through a complex ecological web. Plant Pathology 58:636-641.

Zare, R., Gams, W. 2001. A revision of *Verticillium* section Prostrata. IV. The genera *Lecanicillium* and *Simplicillium* gen. nov. Nova Hedwigia 73:1-50.

Zare, R., Gams, W., Culham, A. 2000. A revision of *Verticillium* sect. Prostrata - I. Phylogenetic studies using ITS sequences. Nova Hedwigia 71:465-480.

Zare, R., Gams, W., Evans, H.C. 2001. A revision of *Verticillium* section Prostrata. V. The genus *Pochonia*, with notes on *Rotiferophthora*. Nova Hedwigia 73:51-86.

Zimmermann, G. 1986. The *Galleria* bait method for detection of entomopathogenic fungi in soil. Journal of Applied Entomology 102:213-215.

Submitted November 22, 2010 – Accepted March 30, 2012
Revised received July 18, 2012