Laboratory Mortality and Mycosis of Adult *Curculio caryae* (Coleoptera: Curculionidae) Following Application of *Metarhizium anisopliae* in the Laboratory or Field[1]

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Abstract The pecan weevil, *Curculio caryae* (Horn), is a key pest of pecans. The entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin are pathogenic to *C. caryae*. One approach to suppressing this pest may be to apply entomopathogenic fungi to adult *C. caryae* when they are emerging from the soil. However, thus far, laboratory screening of fungal isolates has been focused mostly on virulence to larval *C. caryae,* and published field trials on adult control have focused on application of *B. bassiana*. Our objective was to determine the potential of *M. anisopliae* to control emerging *C. caryae* adults. First, a laboratory test was conducted to compare 4 *B. bassiana* strains (Bb GA2, BbLA3, BbMS1, and GHA) and 3 *M. anisopliae* strains (F52, MaLA4, and MaLA7) for virulence to *C. caryae* adults. Virulence of the *M. anisopliae* strains was equal or greater than *B. bassiana* strains. Subsequently, a commercially available *M. anisopliae* strain (F52) was tested under field conditions when applied as a narrow fiber band that was impregnated with fungus and wrapped around the tree trunk, and/or when applied directly to the soil. In 2005, we applied *M. anisopliae* as trunk bands with or without additional application to the soil in the same plots. In 2006, we applied trunk bands or soil applications in separate plots. For 15 d posttreatment, weevils were trapped and transported to the laboratory to record mortality and mycosis. In 2005, weevil emergence was extremely low and statistical analysis was only feasible 3d posttreatment (at which time no treatment differences were detected), and 15 d posttreatment, at which time higher mortality and mycosis was observed in both the trunk band application and the trunk band + ground treatment compared with a nontreated control (and no difference between the two fungal treatments was detected). In 2006 overall average *C. caryae* mycosis was higher in trunk band and ground treatment compared with an untreated control, whereas average total mortality (from the fungus or other causes) was not different among treatments except at 8 d post treatment (in which only the band treatment was significantly greater than the control). It must be noted that our evaluation of efficacy was only an estimation of potential insect control (as opposed to actual field suppression of *C. caryae*) because our analysis was based on *C. caryae* mortality following transport to controlled environmental conditions. Nonetheless, our research indicates that trunk band or ground applications of *M. anisopliae* may have potential to cause significant infection in *C. caryae* populations.

Key Words biological control, *Beauveria bassiana, Curculio caryae*, *Metarhizium anisopliae*, pecan weevil

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Pecan, *Carya illinoinensis* (Wangenh.) K. Koch, is the most economically important native nut crop in North America (Wood 2003). The pecan weevil, *Curculio caryae* (Horn), is a major pest of pecans throughout the southeastern U.S. as well as portions of Texas and Oklahoma (Payne and Dutcher 1985). Adults emerge from soil in late July-August to feed on and oviposit in developing nuts (Harris 1985). Once larval development is completed within the nut, 4th instars drop to the soil and burrow to a depth of 8-25 cm, form a pupal cell, and overwinter. The following fall, approx. 90% of the larvae pupate and spend the next 9 mo in the soil as adults (Harris 1985). The remaining 10% of the population spend 2 yrs in the soil as larvae and emerge as adults in the 3rd year (Harris 1985). Thus, the insects have a two- or three-year life cycle (Harris 1985).

Current control recommendations for *C. caryae* consist mainly of above-ground applications of chemical insecticides (e.g., carbaryl) targeting adults in the canopy (Harris 1999, Hudson et al. 2006). Application of chemical insecticides is recommended every 7-10 d during peak *C. caryae* emergence (generally up to at least a 6-wk period) (Hudson et al. 2006). Due to problems associated with aphid and mite resurgence that often result from chemical applications (Dutcher and Payne 1985), as well as other environmental and regulatory concerns, research on developing alternative control strategies is warranted. Entomopathogenic fungi, such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin, are potential alternatives (Shapiro-Ilan 2003, Shapiro-Ilan et al. 2008).

One approach to controlling *C. caryae* with fungi may be to expose the adult insects to entomopathogenic fungi when they are emerging, i.e., before they enter the canopy to feed and oviposit. However, unlike comparisons made on virulence to the larval stage (Harrison et al. 1993, Shapiro-Ilan et al. 2003), laboratory comparisons among *B. bassiana* and *M. anisopliae* strains for baseline virulence to adult *C. caryae* are lacking. Nonetheless, potential to control emerging *C. caryae* adults with entomopathogenic fungi has been demonstrated under field conditions using *B. bassiana* (Gottwald and Tedders 1983, Shapiro-Ilan et al. 2004, 2008). In contrast, relatively limited attention has been devoted to field-testing *M. anisopliae*. A single field test using *M. anisopliae* to target *C. caryae* adults was reported by Gottwald and Tedders (1983). Conidia were applied around the base of pecan trees, and adult *C. caryae* were released within 15 cm high enclosures near the point of inoculation; 49.8% (corrected with Abbott's formula, Abbott 1925) of the *C. caryae* that crawled to the trunk were killed by the fungus (Gottwald and Tedders 1983). Gottwald and Tedders (1983), however, only tested immediate effects on artificially-released *C. caryae* when the fungus was applied to the soil. Thus, studies on long-term effects of *M. anisopliae* applications on natural *C. caryae* populations are lacking. In this study, we compared 7 *B. bassiana* and *M. anisopliae* strains for virulence to *C. caryae* adults in the laboratory, and subsequently, in 2 field trials (each extending over a 15-d period) we examined the effects of 1 of the *M. anisopliae* strains (F52) on naturally-emerging *C. caryae* adults.

An important consideration in approaching control of *C. caryae* adults with entomopathogenic fungi is the method or placement of application. Prior research has indicated that a high proportion of emerging *C. caryae* either crawl or fly to the trunk (Raney and Eikenbary 1968, Cottrell and Wood 2008). By exploiting this behavior, significant control might be achieved by applying the fungus to the trunk or to soil under the canopy where the insects are emerging. Indeed, recently, Shapiro-Ilan et al. (2008) reported that *C. caryae* suppression was affected by the method of applying *B. bassiana*, i.e., to the trunk versus application to soil with or without cultivation or addition of a cover crop. Results indicated that trunk applications can cause higher *C. caryae* mortality relative to direct application to the soil (Shapiro-Ilan et al. 2008). Trunk-based applications may also be efficacious for application of *M. anisopliae.*

An innovation for controlling insects on the trunk has been developed in the form of a nonwoven fiber band that is impregnated with the entomopathogenic fungus and wrapped around the tree. Using *M. anisopliae* or *Beauveria brongniartii* (Sacc.) Petch, the fiber bands, which can remain viable for 30 d or longer, have provided control of various cerambycid beetles (Shimazu et al. 1995, Dubois et al. 2004, Hajek et al. 2006). Additionally, *B. bassiana* fungal bands caused significant mortality when applied for control of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) (Liu and Bauer 2008). We hypothesized that the fungus band approach may also be efficacious for control of *C. caryae*. Thus, in this study we determined *C. caryae* mortality following treatments of *M. anisopliae* when applied as a band of fungus wrapped around the tree trunk and or when applied directly to the soil.

Materials and Methods

Laboratory assays. Laboratory virulence assays were conducted based on procedures described by Shapiro-Ilan (2001) and Shapiro-Ilan et al. (2003). The experiments included 2 fungal strains that are commercially available, i.e., the GHA strain of *B. bassiana* and the F52 strain of *M. anisopliae*, as well as 3 additional strains of *B. bassiana* (BbGA2, BbLA3, and BbMS1), and 2 additional strains of *M. anisopliae* (MaLA4 and MaLA7). The noncommercial strains were originally collected in pecan orchards in the southeastern United States (Shapiro-Ilan et al. 2003). Prior to experimentation all fungi were cultured in parallel on Sabouraudís dextrose agar (Becton Dickson, Sparks, MD) with yeast extract (Sigma Chemical, St. Louis, MO) according to Goettel and Inglis (1997), and stored at 4°C for < 2 wk. Experiments were conducted in plastic cups (3-4 cm i.d., 3.5 cm deep, Bioserv Inc., Frenchtown, NJ) containing oven-dried soil from a USDA-ARS pecan orchard (Byron, GA) and 1 adult *C. caryae* each. The weevils had been collected on the day of experimentation from Circle traps (Shapiro-Ilan 2001). Cups held 10 g of soil, which was a loamy sand with the percentage sand:silt:clay = 84:10:6, pH = 6.1, and organic matter = 2.8% by weight.

Fungi were pipetted onto the soil surface of each cup in 0.5 ml of water and 0.05% Tween 80 (Fisher Scientific, Pittsburgh, PA) so the final moisture was standardized at field capacity (14%). Approximately 3.75×10^6 conidia (ca. 3×10^5 per cm^2) were applied to each cup; control cups received 0.5 ml of water only. After inoculation, cups were incubated at 25°C, and mortality due to fungi, i.e., visible signs of mycosis (Goettel and Inglis 1997, Shapiro-Ilan et al. 2004), was recorded at 5, 6, 7, 8, 9, and 13 d posttreatment. The percentage of total *C. caryae* mortality (mycosis plus other causes) also was recorded on the same dates. The experiment was organized as a completely randomized design with 4 replicates of 7 weevils per treatment, and the experiment was repeated once in time (56 total weevils per treatment).

Field experiments. Field experiments to estimate efficacy of *M. anisopliae* for adult *C. caryae* suppression were conducted in a pecan orchard at the USDA-ARS research farm in Byron, GA, in 2005 and 2006. Methods were based on those described by Shapiro-Ilan et al. (2004, 2008). The orchard consisted of mature 'Stuart' variety pecan trees approx. 65 years old with an average diam. of approx. 2 m (at about 1 m height) and spacing 20 m apart. Soil type was a loamy-sand (80:16:4, sand:silt:clay; pH = 6.1). The experiments were conducted in a randomized complete block design with 4 blocks (tree rows) of 2 treatments and an untreated control. Each plot consisted of a single tree (thus there were 4 rows of 3 trees in the experiment).

Metarhizium anisopliae (F52 strain) used in all field experiments was produced at the USDA-ARS Research Station in Stoneville, MS, and shipped to Byron, GA, under refrigeration (i.e., using ice packs). Conidia for soil applications were produced through a biphasic system (liquid blastospore suspension poured into sterile rice medium in bags) based on Leland et al. (2005) and trunk bands were produced based on procedures described by Hajek et al. (2006). The material was stored at approx. 4°C and used within 1 month of receipt.

Treatments were applied on 17 August 2005 and 22 August 2006 and consisted of fungal application via trunk bands and/or to soil surrounding the trunk. All soil treatments were applied within a 5-m radius of the tree trunk at a rate of 5×10^{12} conidia per plot (hence, the rate per unit area was approx. 6.4×10^{10} conidia per m^2); *M. anisopliae* for each tree was mixed with 30.3 L of water and 0.01% silwet (Silwet L-77, Loveland Industries, Inc. Greeley, CO), and applied using water cans. The fungus bands (ca. 45 × 3 cm) were attached horizontally around the trunk's circumference by stapling the ends and middle of each; it took 6 or 7 bands to go around the trunk. In 2005, one treatment (Band+ground) received soil application along with 2 rings of bands around the tree, 1 approx. 123 cm above the ground and the other 135 cm above the ground. A second treatment (Band only) received one ring of trunk bands placed 135 cm above the ground. In 2006, one treatment (Ground) received a soil treatment (with no bands), and a second treatment received two rings of bands on the trunk (123 and 135 cm). In 2005 and 2006, control plots received 30.3 L of water applied to the soil as in the ground treatments.

Adult *C. caryae* were collected in Circle traps attached to pecan trunks (Mulder et al. 2003). This is a passive trap that captures weevils crawling up the trunk. The traps were made of wire mesh (1.5 mm mesh) with an open area (approx. 61 cm wide) facing toward the soil (to collect ascending weevils) and tapering up to a removable top. Traps were placed on the trunk so that the bottom of the trap was approx. 140 cm above the soil surface. The top of the trap (the removable one-way cone portion where weevils accumulate) was placed on the trap approx. 24 h prior to each collection (sample date). Six traps were placed on each tree so that weevils were collected from around the entire tree. *Curculio caryae* were collected in traps 1, 3, 8, 10 and 15 d after treatment. To avoid contamination among plots, we placed plastic bags over our shoes just prior to entering plots treated with *M. anisopliae* and removed the bags upon exiting. Daily maximum and minimum temperatures for soil (5 cm below the surface) and air temperatures were recorded during the 15 d experimental periods; these data were collected from a weather station located on the USDA-ARS research farm approx. 0.65 km from the application site.

On each day that *C. caryae* were trapped in the field, the insects captured in each trap were placed in separate plastic bags and transported to the laboratory to determine levels of fungal infection. All *C. caryae* were placed individually in 30-ml plastic cups (3-4 cm i.d., 3.5 cm deep) with a 3 cm cotton wick moistened with approx. 2.1 ml of tap water. Cups were placed in plastic boxes (28 × 15 × 9.5 cm deep) organized by block and incubated in darkness at 25°C. After 14 d of incubation, the percentage *C. caryae* mycosis per plot was estimated by examining the cadavers for visible signs of fungal infection (Goettel and Inglis 1997, Shapiro-Ilan et al. 2004). The percentage of total *C. caryae* mortality (mycosis plus other causes) also was recorded.

In 2005, weevil emergence was extremely low. Therefore, to bolster the number of weevils in the analysis, 20 *C. caryae* adults (captured in untreated plots earlier that day) were released at the base of each tree in all plots. The release was made 14 d posttreatment (i.e., 1 day before the final sampling date on d 15).

Statistical analyses. For the laboratory virulence experiment, treatment effects averaged over the sampling period (5-13 d posttreatment) were analyzed using repeated measures analysis and LSMEANS (Proc Mixed, SAS 2002). The data from the 2 laboratory trials repeated in time were combined and variation among trials was accounted for as a block effect. For field experiments, differences among treatments in mortality and mycosis were analyzed by averaging effects over the entire experimental period as well as (separately) on each sampling date, i.e., similar to the approach of McCoy et al. (2000) and Shapiro-Ilan et al. (2004, 2008). Treatment effects averaged over the 15-d sampling period were analyzed using repeated measures analysis and LSMEANS (Proc Mixed, SAS 2002). Additionally, treatment effects were analyzed by day (for each sampling date separately) using ANOVA and Student-Newman-Keuls test (SAS 2002). All percentage data were transformed by arcsine of the square root prior to analysis (nontransformed means are presented in the Results section). The alpha level for all statistical tests was 0.05.

Results and Discussion

In the laboratory virulence experiment, the level of mycosis was higher in all fungal treatments relative to the control except in BbGA2 and BbMS1 (Table 1; Fig. 1). Additionally, the levels of mycosis varied among strains, i.e., MaLA7, BbGHA, and BbLA3 had higher mycosis than BbGA2, and MaLA7 had higher mycosis than BbMS1 (Fig. 1). When total weevil mortality was measured (disregarding signs of mycosis), all fungal strains exhibited a higher percentage kill compared with the control (Table 1; Fig. 1). The strains exhibited varying levels of total mortality, i.e., all *M. anisopliae* strains caused greater mortality than BbGA2, and MaLA7 caused greater mortality than all *B. bassiana* strains. The levels of mycosis and mortality observed in the control were not considered unusual given that these species of fungi are endemic in pecan orchards (Shapiro-Ilan et al. 2003, 2004, 2008).

Overall, the *M. anisopliae* showed equal or superior virulence compared with *B. bassiana*; none of the *B. bassiana* strains exhibited higher mortality or mycosis than *M. anisopliae* strains. Therefore, the laboratory data bolstered the justification to test *M. anisopliae* under field conditions for potential to suppress *C. caryae* adults. From the 3 *M. anisopliae* strains producing the highest levels of mortality (MaLA7, MaLA4, and F52), we chose the F52 strain of *M. anisopliae* for additional testing under field conditions because it is already under commercial development (and thus a large investment has already been made in terms of production technology and registration).

In field experiments, during the 15 d experimental periods, average daily minimum and maximum ambient temperatures were (± SD) 22.3 ± 1.5°C and 33.1 ± 1.9°C in 2005, and 21.70 ± 1.0°C and 32.3 ± 1.8°C in 2006. Average daily minimum and maximum soil temperatures at 5 cm below the surface were (± SD) 26.3 ± 1.4°C and 32.2 ± 1.5°C in 2005, and 26.3 ± 0.5°C and 31.8 ± 1.4°C in 2006.

In field experiments, the total number of *C. caryae* captured during the 2005 field trial was 104, with captures of 9, 12, 19, 8, and 56 weevils 1, 3, 8, 10, and 15 d posttreatment,

Table 1. Statistics from experiments measuring *Beauveria bassiana* and Metarhizium anisopliae suppression of adult *Curculio caryae*

Test	F	df	P
Laboratory test measuring mycosis	7.01	7, 21	0.0002
Laboratory test measuring total mortality	3.31	7, 21	0.0156
Mycosis, field experiment 2005, 3 DAT*	0.36	2, 2	0.7334
Total mortality, field experiment 2005, 3 DAT	0.06	2, 2	0.9441
Mycosis, field test 2005, 15 DAT	12.10	2, 5	0.0121
Total mortality, field test 2005, 15 DAT	12.55	2, 5	0.0113
Mycosis, field test 2006, repeated measures	8.12	2, 6	0.0196
Total mortality, field test 2006, repeated measures	2.69	2, 6	0.1466
Mycosis, field test 2006, 1 DAT	2.64	2, 4	0.1854
Mycosis, field test 2006, 3 DAT	0.57	2, 5	0.60
Mycosis, field test 2006, 8 DAT	1.75	2, 3	0.3136
Mycosis, field test 2006, 10 DAT	∞	2, 3	<0.0001
Mycosis, field test 2006, 15 DAT	2.41	2, 1	0.4148
Total mortality, field test 2006, 1 DAT	0.83	2, 4	0.4997
Total mortality, field test 2006, 3 DAT	3.40	2, 5	0.1169
Total mortality, field test 2006, 8 DAT	13.53	2, 3	0.0315
Total mortality, field test 2006, 10 DAT	0.73	2, 3	0.3818
Total mortality, field test 2006, 15 DAT	2.41	2, 1	0.4148

* DAT = Days after treatment.

respectively. The higher number caught 15 d posttreatment reflect the artificial release of weevils. Due to the low and variable numbers of weevils captured in 2005, statistical comparisons among means could not be estimated when data were averaged over the 15 d sampling period (using Proc Mixed), nor on any of the specific sampling dates except 3 d and 15 d posttreatment. No differences in overall mortality or mycosis were detected 3 d posttreatment (Table 1), whereas 15 d posttreatment *C. caryae* mortality and signs of mycosis were higher in both treatments than in the nontreated control, and no difference was detected between fungal treatments (Table 1; Fig. 2). Weevil mycosis (± SE) from samples taken 3 d posttreatment was 0%, 20.0 ± 20.0%, and 16.7 ± 16.7%; and overall mortality (± SE) was 50 ± 50%, 50 ± 28.9%, and 66 ± 33.3% for the band only, band+ground treatments, and the control, respectively. Weevil mortality in both *M. anisopliae* treatments 15 d posttreatment was > 79% and reached 86% in the band+ground treatment (Fig. 2).

Prior to our research, we did not know whether *M. anisopliae* applied as fungal bands alone, or as a ground treatment, could cause significant *C. caryae* mortality. We were limited in the number of treatments we could apply (due to availability of space, labor, and fungus) and thus could not include all combinations of treatments.

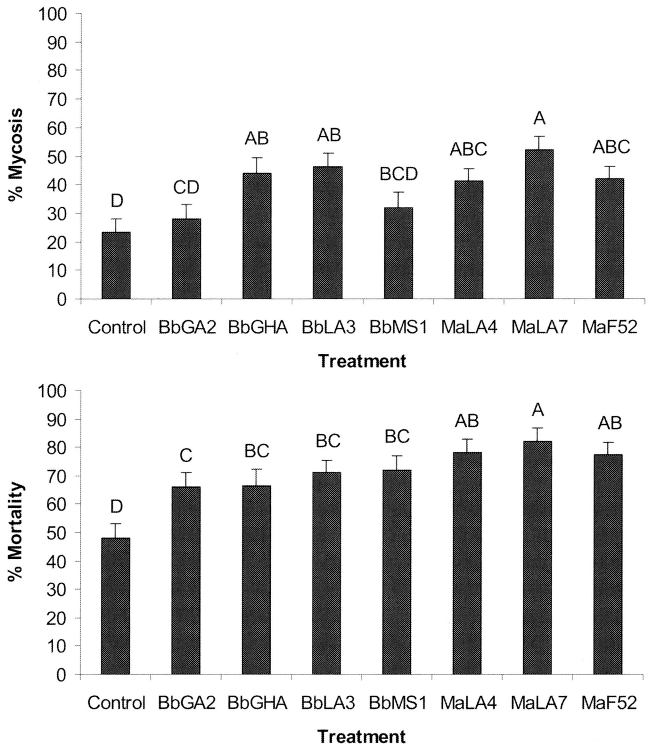

Fig. 1. Percentage adult *Curculio caryae* mortality and mycosis following applications of *Beauveria bassiana* or *Metarhizium anisopliae* strains in the laboratory. Bars represent mean percentages (± SE) averaged over 5-13 d postinoculation. Approximately, 3.75×10^6 conidia were applied per weevil. Different letters above bars indicate statistically significant differences (LSMEANS test, $\alpha = 0.05$)

Therefore, we included a treatment that would encompass a combined effect, i.e., the band+ground treatment. We also included the band treatment alone for comparison. The data from 2005 indicated that the ground treatment did not add any benefit when combined with the trunk treatment in comparison with the trunk treatment alone. Thus, in the second year of the study, we chose to explore whether the ground treatment when applied alone has any suppressive abilities. Hence in 2006 we compared the trunk and ground treatments each applied separately.

The total number of *C. caryae* captured in 2006 was 101, with captures of 36, 24, 17, 12 and 12 weevils 1, 3, 8, 10, and 15 d posttreatment, respectively. When averaged over the 15 d sampling period, higher levels of mycosis were observed in both *M. anisopliae* treatments relative to the control (with no difference detected between treatments) (Table 1; Fig. 3). Overall *C. caryae* mortality (when averaged over the 15-d period) was not different among the treatments and control (Table 1; Fig. 3).

In 2006, when mycosis was analyzed on each sample date, a higher percentage of *C. caryae* mortality was observed in the band treatment compared with the control

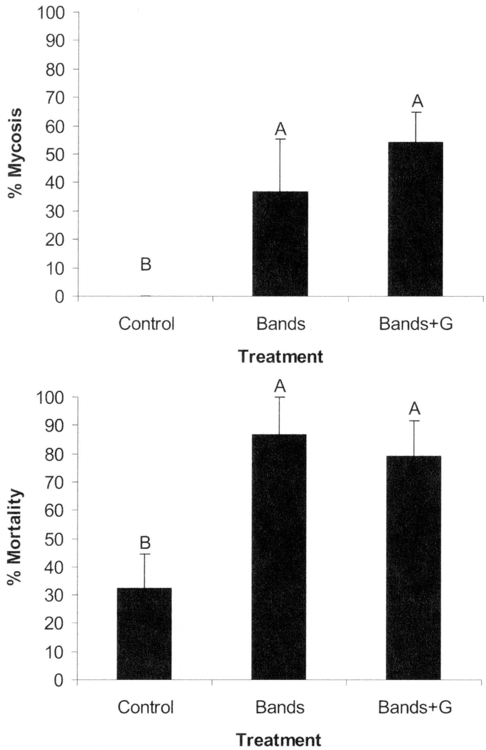

Fig. 2. Mean (± SE) percentage adult *Curculio caryae* mortality and mycosis following applications of *Metarhizium anisopliae* in a pecan orchard in Byron, GA, 2005. Treatments were applied as fungal impregnated fiber bands attached to the tree trunk (Bands), or the Band treatment combined with an aqueous suspension of conidia application to the ground (Bands+G). The ground treatment consisted of approximately 5 × 10^{12} conidia applied per tree; a control received water only. Weevils were collected 15 d postapplication. Different letters above bars indicate statistically significant differences (SNK test, α = 0.05)

and ground treatment 10 d after treatment; no other differences between fungal treatments and the control were observed (Table 1; Fig. 4). When overall weevil mortality was analyzed on each sample date, a higher percentage of *C. caryae* mortality was observed in the band treatment compared with the control 8 d after treatment; no other differences between fungal treatments and the control were observed (Table 1; Fig. 5). Overall weevil mortality in the trunk band treatment was maximum at 10 d posttreatment reaching 100% (Fig. 5).

Our data indicate that *M. anisopliae* (F52) applied as a trunk band can cause significant infection and mortality in emerging *C. caryae* adults. The trunk band applications, when applied alone, caused significant suppression of *C. caryae* in both years of the study. These results are consistent with other studies that have demonstrated efficacy in suppressing other Coleoptera pests using fungal fiber bands (Shimazu

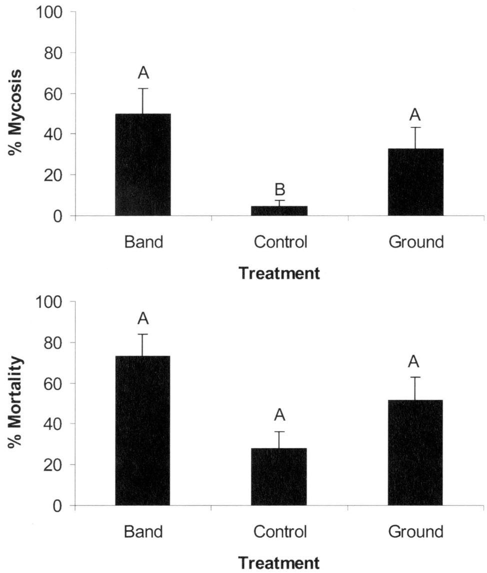

Fig. 3. Percentage adult *Curculio caryae* mortality and mycosis following applications of *Metarhizium anisopliae* in a pecan orchard in Byron, GA. Bars represent mean percentages (± SE) averaged over a 15 d sampling period in 2006. Treatments were applied directly to the ground (Ground) at approximately 5×10^{12} conidia per tree, or as fungal impregnated fiber bands attached to the tree trunk (Band); a control received water only applied to the ground. Different letters above bars indicate statistically significant differences (LSMEANS test, $\alpha = 0.05$)

et al. 1995, Dubois et al. 2004, Hajek et al. 2006, Liu and Bauer 2008). For example, Hajek et al. (2006) observed reduced insect longevity and oviposition when applying *M. anisopliae* and *B. brogniartii* for control of the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Hajek et al. 2006), and in a single year field trial Liu and Bauer (2008) observed 31.6% infection of *A. planipennis* after applying *B. bassiana* fungal bands to ash trees (*Fraxinus* spp.). Given that the efficacy of the fiber bands can vary with fungal species or strain (Dubois et al. 2004, Hajek et al. 2006) it may be beneficial to test other strains or species of *Metarhizium* and *Beauveria* for their efficacy versus *C. caryae* when applied as trunk bands.

Our data also provide evidence that ground applications of *M. anisopliae* can cause significant *C. caryae* infection. Given that some weevils fly directly into the pecan canopy after emerging from soil (Cottrell and Wood 2008), the ground application is important to overall weevil suppression. However, our results suggest a combination of band and ground applications does not appear to be beneficial relative to band applications alone that affect those weevils climbing the trunk. Unlike the band-only treatments, we did not repeat the band+ground or ground only treatments in 2 consecutive years. Hence, additional research on these approaches (e.g., additional replication through more than 1 season) may be warranted.

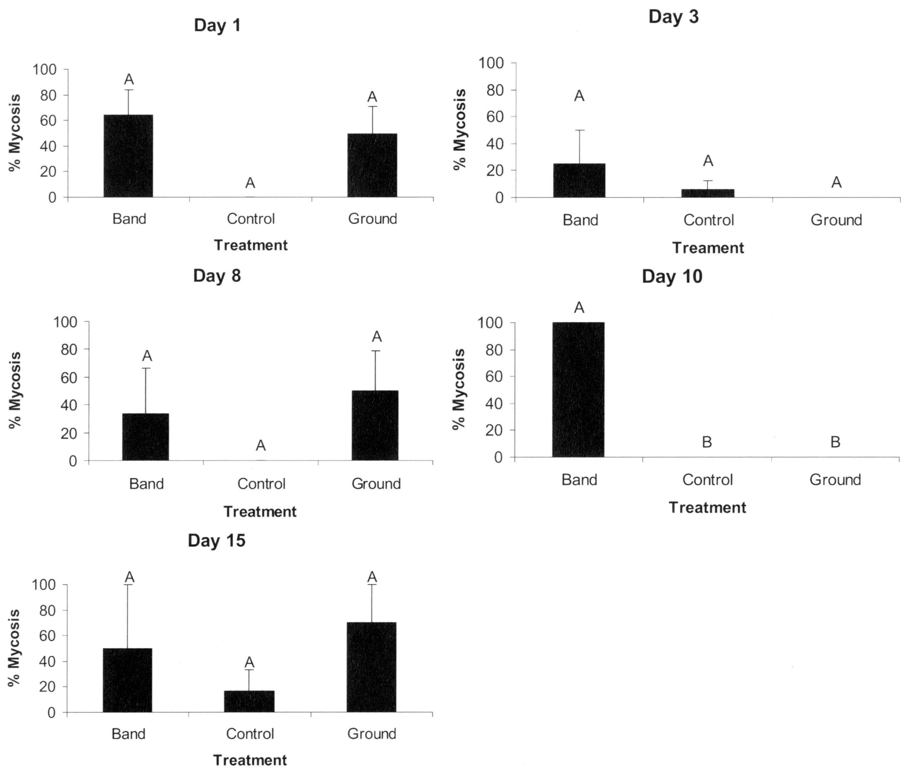

Fig. 4. Percentage adult *Curculio caryae* exhibiting signs of mycosis following applications of *Metarhizium anisopliae* in a pecan orchard in Byron, GA, 2006. Bars represent mean percentages (± SE) 1, 3, 8, 10 and 15 days after treatment. Treatments were applied directly to the ground (Ground) at approximately 5 × 10^{12} conidia per tree, or as fungal impregnated fiber bands attached to the tree trunk (Band); a control received water only applied to the ground. Different letters above bars indicate statistically significant differences (SNK test, α = 0.05)

In both years, relatively low numbers of weevils were captured. For example, in a recently published study by Shapiro-Ilan et al. (2008) that used an experimental and sampling design identical to this study, more than three times as many weevils were captured per tree. The low number of weevil captured in this study caused some of the statistical analyses (ANOVAs) to fail and thus some treatment effects may have been masked. Nonetheless, the treatment effects that we observed were consistent over the 2 years of study and thus support our conclusions.

Based on the findings of this study, there may be potential to use *M. anisopliae* as a biocontrol tactic to suppress emerging *C. caryae* adults. Additional research should be conducted on varying methods of application. For example, Shapiro-Ilan et al. (2008) reported that spray applications of *B. bassiana* made directly to the tree trunk

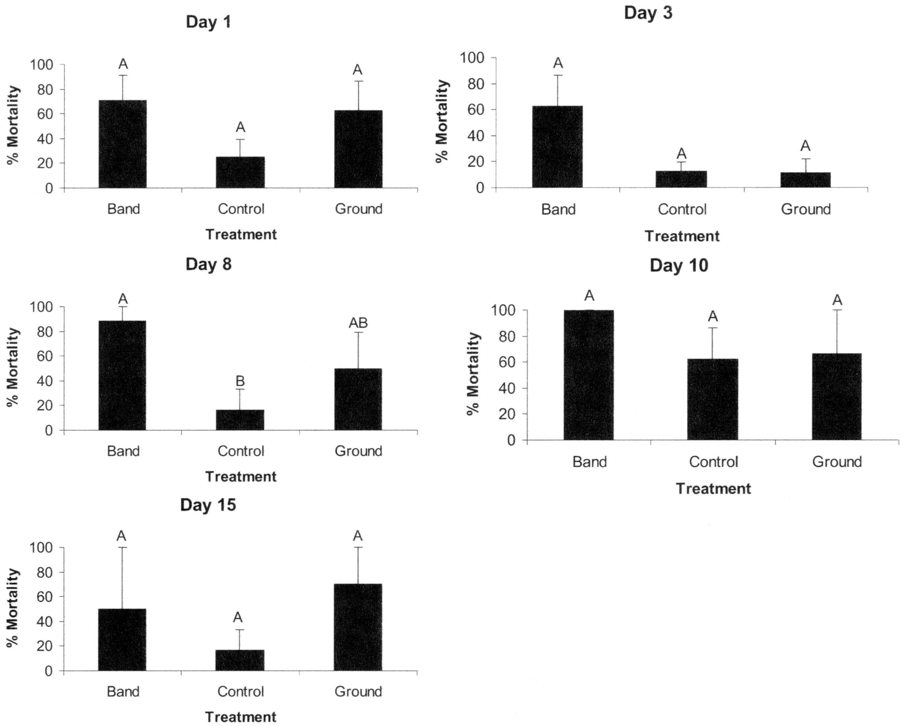

Fig. 5. Percentage adult *Curculio caryae* mortality following applications of *Metarhizium anisopliae* in a pecan orchard in Byron, GA, 2006. Bars represent mean percentages (± SE) 1, 3, 8, 10 and 15 days after treatment. Treatments were applied directly to the ground (Ground) at approximately 5 × 10^{12} conidia per tree, or as fungal impregnated fiber bands attached to the tree trunk (Band); a control received water only applied to the ground. Different letters above bars indicate statistically significant differences (SNK test, α = 0.05)

or addition of a cover crop to *B. bassiana* ground applications appeared to be beneficial relative to ground applications alone. Possibly, trunk sprays or cover crops would also be advantageous for *M. anisopliae* and could be compared with ground and trunk band applications. Additionally, it must be noted that our evaluation was only an estimation of potential insect control (as opposed to actual field suppression of *C. caryae*). This is because our analysis was based on *C. caryae* mortality following transport to controlled environmental conditions; thus, we cannot know how many of those weevils might have survived if they had remained in the field. Furthermore, we do not know if, or how much, crop damage infected weevils may inflict before they succumb to the fungus; *C. caryae* has an average 6.5 d pre ovipositional phase upon emergence (Criswell et al. 1975) though they can start feeding immediately after emergence. Therefore, additional research is required to determine the ability of *M. anisopliae* applications to protect pecans from *C. caryae* damage.

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