Trunk Perimeter Applications of *Beauveria bassiana* to Suppress Adult *Curculio caryae* (Coleoptera: Curculionidae)[1]

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Abstract The pecan weevil, *Curculio caryae* (Horn), is a key pest of pecans. Entomopathogenic fungi, such as *Beauveria bassiana* (Balsamo) Vuillemin, can infect this pest. Our objectives were to determine the potential of *B. bassiana,* to suppress emerging *C. caryae* adults when applied to soil around pecan tree-trunks, and to determine persistence of *B. bassiana* in the soil. In 2000 and 2001, *B. bassiana* was applied to soil in a 2 m band around pecan tree trunks. Naturally emerging *C. caryae* adults, caught after they crawled to the trunk, were brought to the laboratory to determine percentage mycosis. In Byron, GA, irrigation was applied to one-half of the plots, whereas, in Griffin, GA irrigation was not applied. In Byron, in both years, we observed greater fungus-induced mortality in plots that received *B. bassiana* without irrigation than in plots that received *B. bassiana* with irrigation or in the checks (with and without irrigation). In Griffin, in the year 2000, we observed higher *C. caryae* mortality in *B. bassiana* treated plots than in non-treated plots, whereas 2001 results showed no difference. Although we observed up to 95% *B. bassiana*-induced mortality within the first 3 d post-application, treatment effects did not persist beyond the first week post-application. Future research should focus on extending the persistence of *B. bassiana* suppression. Estimates of conidia production per *B. bassiana* infected insect yielded up to 4.2×10^9 suggesting some recycling might occur in the soil.

Key Words *Beauveria bassiana,* biological control, *Curculio caryae,* pecan

The pecan weevil, *Curculio caryae* (Horn), is a major pest of pecans throughout the southeastern U.S. as well as in portions of Texas and Oklahoma (Payne and Dutcher 1985). The insects have a 2 or 3 year life cycle (Harris 1985). Adults emerge from soil in late July-August, feed on, and oviposit in developing nuts (Harris 1985). Larval development is completed within the nut, and fourth instars drop to the soil (late summer to late fall) where they burrow to a depth of 8 to 25 cm, form a pupal cell, and over-winter. The following fall approximately 90% of the larvae pupate and spend the next 9 months in the soil as adults before emerging (Harris 1985). The remaining 10% of the population spend 2 years in the soil as larvae and emerge as adults in the third year (Harris 1985).

Current control recommendations for the *C. caryae* consist solely of above-ground applications of carbaryl to suppress adults (Harris 1999, Hudson et al. 2002). Due to problems associated with aphid and mite resurgence (Dutcher and Payne 1985), as

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well as other environmental and regulatory concerns, research on developing alternative control strategies is warranted. Entomopathogenic fungi are one of the potential alternatives.

The most studied and perhaps most promising, entomopathogenic fungus for *C. caryae* control to date is *Beauveria bassiana* (Balsamo) Vuillemin (Gottwald and Tedders 1983, 1984, Sikorowski 1985, Harrison et al. 1993, Fuxa et al. 1998). Hyphomycetes, such as *B. bassiana,* invade the insect host through the cuticle, replicate in the host hemocoel, and form external conidiophores to disperse their spores (Tanada and Kaya 1993). *Beauveria bassiana* is pathogenic to a variety of insects including a number of curculionid or other coleopteran pests (Tanada and Kaya 1993, Booth et al. 2000, McCoy et al. 2000).

Laboratory studies have indicated relatively high virulence of some *B. bassiana* strains to *C. caryae* larvae (Harrison et al. 1993, Shapiro-Ilan et al. 2002). Field studies targeting *C. caryae* larvae, however, have resulted in highly variable levels of control ranging from <30% (Gottwald and Tedders 1983, Harrison et al. 1993) to 62% (Tedders et al. 1973). Additionally, a potential drawback to larval control with *B. bassiana* is that larvae emerge from nuts over several months, e.g., October to December (Boethel and Eikenbary 1979, Harris and Ring 1979), and thus a lack of fungal persistence (Storey et al. 1987) could require multiple applications. In contrast, the bulk of *C. caryae* adults emerge from soil over a 4 to 6 week period (Harris 1976), and thus, may require fewer applications.

One approach to controlling *C. caryae* adults with *B. bassiana* may be to apply the fungus to soil around the perimeter of each pecan tree. Prior research has indicated that a high proportion of emerging *C. caryae* crawl to the trunk (Raney and Eikenbary 1968, T.E.C, unpublished data). We hypothesized that a significant proportion of those *C. caryae* crawling to the trunk would become infected if the fungus were applied to soil around the trunk's perimeter. Infected *C. caryae* can infect other *C. caryae* in the canopy or in soil (Gottwald and Tedders 1983, 1984). Therefore, a significant proportion of the *C. caryae* population would be suppressed using this perimeter application approach. Additionally, treating only the perimeter of each tree, rather than treating the entire area of *C. caryae* emergence, i.e., within the dripline (Harris 1975), would result in significant savings in product and application costs.

Gottwald and Tedders (1983) demonstrated promise in this approach; when *B. bassiana* was applied around the base of pecan trees and adult *C. caryae* were released within 15 cm high enclosures near the point of inoculation, 78% (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) only tested immediate effects on artifically released *C. caryae,* and did not attempt to measure how long the effect of a perimeter *B. bassiana* application might last. Furthermore, effects of irrigation on this approach were not measured. To ensure the economic viability of this approach, residual treatment effects comparable to (or longer than) chemical insecticides may be required. Current control recommendations for *C. caryae* entail application of chemical insecticides every 7 to 10 d during peak *C. caryae* emergence (generally up to a 6 week period) (Hudson et al. 2002). Our primary objective was to determine the effects of a perimeter *B. bassiana* application on naturally emerging *C. caryae* over a 2 to 3 week period. Additionally, we investigated the effects of irrigation on the *B. bassiana* perimeter applications. Finally, we determined the average number of conidia produced per infected *C. caryae* in order to estimate the potential for *B. bassiana* recycling.

Materials and Methods

Field experiments to determine the ability of *B. bassiana* to suppress *C. caryae* using trunk perimeter applications were conducted in pecan orchards at two locations (Byron and Griffin, GA) in 2000 and 2001.

Fungi. *Beauveria bassiana* (GHA strain), i.e., Mycotrol®, which was used in all experiments was obtained from Emerald BioAgriculture Corporation (Butte, MT) as an emulsifiable oil formulation containing 2×10^{13} viable conidia per 946-ml container. The material was stored at approximately 4°C and used within 1 month of receipt. Prior to application, viability of conidia was verified according to percentage germination (on agar) as described by Goettel and Inglis (1997). For the Byron experiments, viability counts in 2000 and 2001 indicated approximately 77 and 75% viability, respectively. For the Griffin experiments, viability counts in 2000 and 2001 indicated approximately 73 and 48% viability, respectively.

Byron field tests. The orchard consisted of mature Stuart variety pecan trees approximately 60 years old with an average diameter of approximately 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 10 blocks of four treatments. The treatments were *B. bassiana* application and a check (no *B. bassiana* applied), each with and without irrigation. In treatments receiving irrigation, the water was applied via microjet (low volume application of water close to the soil surface through small microsprinklers) at a rate of approximately 1.3 liters per min for 30 min or until standing water began to be visible in the plots (if this occurred irrigation was immediately shut off to avoid runoff). Irrigation was applied following treatment application and thereafter on a daily basis for the duration of the experiment. In *B. bassiana* treatments, approximately 3.6×10^{11} conidia suspended in 8 liters tap water were applied by watering can to a band of soil of 2 m radius surrounding each trunk (approximately 3×10^{10} conidia per m^2). The rate of application might be considered high relative to some other studies (Booth et al. 2000), but it must be remembered that only a small proportion of the orchard was being treated (thus, the overall costs of application were reduced); we chose this higher rate with the idea that if the treatments would be effective at these rates then the optimum rate and area of application could be determined at a later time while taking into account economic considerations. The concentration of conidia to be applied was calculated based on the product labeled density of viable conidia. Check plots received 8 liters of tap water only around a similar area of each tree. Treatments were applied on 14 August 2000 and 20 August 2001 between approximately 0830 and 1130 hours.

Adult *C. caryae* were collected in Circle trunk traps attached to pecan trunks (Mulder et al. 1997, Cottrell and Mulder 2001). This is a passive trap that captures weevils crawling up the trunk. The traps were made of wire mesh (1.5 mm pore size) with an open area (approximately 44 cm wide) facing toward the soil and tapering up to a removable top. Traps were placed on the trunk low to the ground (30 cm above the soil surface) to maximize capture of *C. caryae* that crawled to the trunk, i.e., *C. caryae* that flew directly to the trunk or canopy were unlikely to be caught. The top of the trap (the removable one-way cone portion that actually captures the weevils) was placed on the trap approximately 24 h prior to each collection (sample date).

Curculio caryae were collected in traps 1, 2, 3, 7, 8 and 9 d after treatment.

Additionally, in 2001 only, *C. caryae* were also collected 14, 15, and 16 d after treatment. Collection was terminated earlier in 2000 because the *C. caryae* emergence was already substantially diminished by the 9 d post-treatment. Furthermore, to increase the number of *C. caryae* trapped per tree, 6 traps were placed on each tree in 2001 relative to 3 traps in 2000. To avoid contamination among plots, we placed plastic bags over our shoes (held with rubber bands) just prior to entering plots treated with *B. bassiana* and removed the bags upon exiting.

On each day that *C. caryae* were trapped in the field, the insects captured in each trap top were placed in separate plastic bags and brought to the laboratory to determine levels of fungal infection. All *C. caryae* were placed individually in 30-ml plastic cups (3 to 4 cm i.d., 3.5 cm deep) with a 3 cm cotton wick moistened with approximately 2.1 ml of tap water, and a slice of apple (~ 1 cm × 0.5 cm) for food. Cups were placed in plastic boxes (28 × 15 × 9.5 cm deep) organized by block and incubated in darkness at 25°C. After 7 and 14 d of incubation, the percentage *C. caryae* mycosis per plot was estimated by examining the cadavers for signs of fungal infection (Goettel and Inglis 1997). The percentage of total *C. caryae* mortality (mycosis plus other causes) was also recorded.

Griffin field tests. The orchard consisted of a mixture of Stuart and Ellis variety pecan trees approximately 35 years old with an average diameter of approximately 0.8 m, and spacing at least 25 m between trees used in the study. The soil was a sandy loam (90:6:4, sand:silt:clay; pH = 6.1). Because irrigation was not available, only two treatments were applied: *B. bassiana* application and a check. In 2000, *B. bassiana* was applied on 25 August, and *C. caryae* were collected 2 d later and every other day thereafter until 20 d post-application. In 2001, *B. bassiana* was applied on Friday 31 August, and *C. caryae* were collected each Monday, Wednesday and Friday thereafter until 23 d post-application. The percentage of *C. caryae* mycosis was determined 14 d after collection as described above (7 d observations were not made). All other experimental methods were identical to those described for the Byron experiments.

Determination of conidia produced per insect. The number of conidia produced per infected *C. caryae* cadaver was estimated in insects from *B. bassiana* treated plots (year 2000) that received irrigation. Conidia production was estimated in these insect cadavers following efficacy evaluation described above, i.e., after the insects had been incubated for 2 weeks at 25°C. The number of conidia per insect was estimated using a haemocytometer (Goettel and Inglis 1997). Each cadaver was placed on a rotary shaker at 250 rpm in 25 ml Erlenmeyer flasks for 2 h with 10 ml of 0.01% Silwet L-77 (Setre Chemical Co., Memphis, TN). The cadavers were then subjected to sonication for 4 min (Branson B-12, SmithKline Co., Shelton, CT; 177 V, 80 Watts), and homogenized (in a glass tissue grinder). Each sample was vortexed prior to counting. Estimates of conidia production were based on five replicates (insects).

Data analysis. In field trials (Byron and Griffin) differences among treatment effects compounded throughout the experimental periods (all days after treatment) were analyzed using repeated measures analysis and LSMEANS (Proc Mixed, SAS 1996). Additionally, treatment effects were analyzed by day (for each sampling date separately) using ANOVA and Student-Newman-Keuls test (SAS 1996). All percentage data were transformed by arcsine of the square root prior to analysis. The alpha level for all statistical tests was 0.05.

Results

Byron field tests. In 2000, after 7 d of incubation, the *B. bassiana* treatment without irrigation caused greater estimated mycosis than other treatments (including controls) in *C. caryae* collected 2 d after application ($F = 4.4$; df = 3,26; $P = 0.014$) (Table 1). No other differences among treatments for any of individual sampling dates were detected ($P > 0.05$) (Table 1). When the treatment effects were averaged over the entire experimental period (and analyzed using repeated measures), the *B. bassiana* treatment without irrigation caused a greater level of fungal-induced mortality than other treatments ($F = 5.41$; df = 3,26; $P = 0.005$) (Table 1).

In 2000, after 14 d of incubation, the *B. bassiana* treatment without irrigation yielded greater fungal-induced mortality than other treatments in samples taken 2 d after application ($F = 4.84$; df = 3,22; $P = 0.01$), than the controls at 7 d after application ($F = 3.73$; df = 3,29; $P = 0.02$), and when the treatment effects were averaged over the entire experimental period ($F = 5.95$; df = 3,26; $P = 0.003$) (Table 1). No other differences were detected among treatments ($P > 0.05$). In 2000 a mean (SE) of 115.8 (35.4) *C. caryae* adults were captured per sample date.

Table 1. Mean percentage (±SE) of adult *Curculio caryae* exhibiting signs of mycosis following *Beauveria bassiana* application in field trials (Byron, GA 2000)

Incubation period	DAT	Treatment			
		Bb	Bb + H_2O	Control	Control + H_2O
7 days	1	44.3 (5.6) a	41.6 (20) a	0 a	25.0 (25.0) a
	2	30.5 (16.3) a	0 b	0 b	0 b
	3	31.2 (23.6) a	10.7 (7.4) a	0 a	6.2 (6.3) a
	7	4.7 (3.7) a	0 a	0 a	1.8 (1.8) a
	8	5.0 (2.5) a	12.0 (10.0) a	4.1 (2.7) a	5.8 (5.0) a
	9	7.2 (2.8) a	0 a	4.6 (2.4) a	13.3 (10.2) a
	Average*	15.1 (4.0) a	9.3 (3.8) b	2.9 (1.1) b	8.0 (3.5) b
14 days	1	89.0 (11.0) a	58.3 (20.0) a	58.3 (25.0) a	50.0 (28.9) a
	2	94.5 (5.5) a	44.8 (15.3) b	27.8 (18.1) b	16.7 (16.7) b
	3	66.8 (23.5) a	33.3 (12.2) a	56.6 (19.5) a	33.8 (15.6) a
	7	30.2 (6.6) a	19.3 (7.7) ab	4.4 (2.9) b	11.7 (6.3) b
	8	21.8 (3.6) a	15.4 (10.2) a	10.0 (4.0) a	9.8 (5.6) a
	9	28.1 (8.7) a	29.7 (12.3) a	21.2 (11.2) a	28.7 (11.4) a
	Average	46.2 (5.7) a	31.6 (5.3) b	24.2 (5.6) b	22.9 (5.3) b

Curculio caryae were collected on various days after treatment (DAT), and incubated in the laboratory for 7 and 14 d before the percentage exhibiting mycosis was determined, Bb, *B. bassiana;* +H_2O, irrigation was applied. Different letters following numbers indicate statistical significance within each row ($\alpha = 0.05$).

* Percentage of weevils exhibiting signs of mycosis averaged over the entire experiment, (all DAT).

Overall, results from the experiment conducted in 2001 (Table 2) were similar to those obtained in 2000 (Table 1). After 7 d of incubation, for samples taken one d after treatment, *B. bassiana* application with irrigation caused greater fungal-induced mortality than the controls, but was not different from the other *B. bassiana* treatment (F = 4.32; df = 3,36; P = 0.01) (Table 2). Applications of *B. bassiana* without irrigation caused greater mycosis than other treatments for samples taken 2 d after treatment (F = 6.18; df = 3,35; P = 0.002), and for the entire experimental period (F = 4.94; df = 3,27; P = 0.0073) (Table 2). No other differences were detected among treatments after 7 d of incubation (P > 0.05) (Table 2).

Table 2. Mean percentage (±SE) of adult *Curculio caryae* exhibiting signs of mycosis following *Beauveria bassiana* application in field trials (Byron, GA 2001)

Incubation Period	DAT	Treatment			
		Bb	Bb + H_2O	Control	Control + H_2O
7 days	1	14.0 (3.8) ab	15.8 (3.4) a	5.1 (2.9) b	4.3 (2.3) b
	2	30.4 (9.9) a	11.7 (4.0) b	10.5 (3.2) b	2.0 (1.3) b
	3	18.9 (6.2) a	10.0 (3.3) a	15.3 (6.2) a	9.3 (3.5) a
	7	28.1 (5.4) a	8.3 (2.9) a	15.1 (7.0) a	12.2 (5.5) a
	8	6.5 (2.3) a	10.0 (3.2) a	7.7 (3.1) a	4.3 (1.8) a
	9	14.5 (5.7) a	12.0 (3.3) a	9.6 (4.5) a	5.4 (4.0) a
	14	26.5 (6.3) a	20.9 (5.1) a	21.5 (4.3) a	35.1 (6.5) a
	15	21.6 (4.7) a	21.6 (4.3) a	23.3 (5.4) a	20.3 (4.1) a
	16	13.1 (4.0) a	10.5 (3.7) a	13.3 (3.0) a	7.3 (2.6) a
	Average*	19.2 (2.0) a	13.4 (1.3) b	13.5 (1.6) b	10.8 (1.6) b
14 days	1	56.3 (7.8) a	41.4 (6.3) ab	36.1 (10.1) ab	22.6 (6.8) b
	2	71.6 (5.7) a	29.5 (5.5) b	28.6 (4.8) b	14.7 (3.7) b
	3	68.4 (4.8) a	34.0 (5.6) b	37.2 (6.1) b	29.2 (4.0) b
	7	71.0 (8.9) a	37.7 (6.6) b	52.8 (9.0) ab	48.9 (5.6) ab
	8	37.7 (5.2) a	31.8 (4.5) a	28.9 (7.3) a	22.7 (4.0) a
	9	28.7 (7.7) a	30.5 (5.4) a	19.8 (4.7) a	21.3 (4.0) a
	14	42.2 (6.8) a	49.4 (6.9) a	46.2 (5.5) a	55.4 (6.1) a
	15	36.7 (8.0) a	42.1 (4.1) a	37.7 (6.1) a	32.4 (4.9) a
	16	29.9 (4.5) a	26.9 (4.1) a	26.9 (3.8) a	22.3 (3.9) a
	Average	48.9 (2.8) a	35.9 (1.9) b	34.9 (2.4) bc	29.5 (2.1) c

Curculio caryae were collected on various days after treatment (DAT), and incubated in the laboratory for 7 and 14 d before the percentage exhibiting mycosis was determined, Bb, *B. bassiana;* +H_2O, irrigation was applied. Different letters following numbers indicate statistical significance within each row (α = 0.05).

* Percentage of weevils exhibiting signs of mycosis averaged over the entire experiment, (all DAT).

After 14 d of incubation, *B. bassiana* applications without irrigation caused greater fungal-induced mortality than the following: the control with irrigation when sampled 1 d after application ($F = 3.15$; df = 3,36; $P = 0.037$), *B. bassiana* without irrigation 7 d after application ($F = 3.13$; df = 3,35; $P = 0.038$), all other treatments at 2 ($F = 15.52$; df = 3,35; $P = 0.0001$) and 3 ($F = 8.52$; df = 3,36; $P = 0.0002$) d after application, and when averaged over the experimental period ($F = 12.33$; df = 3,27; $P = 0.0001$) (Table 2). Also, after 14 d of incubation averaged over the experimental period, a greater percentage of fungal infections were observed from the *B. bassiana* application with irrigation than the control with irrigation (but not than the control without irrigation) ($F = 12.33$; df = 3,27; $P = 0.0001$). The highest level of estimated mycosis observed both years was in plots where *B. bassiana* was applied without irrigation at 2 d post inoculation (approximately 95% and 72% mycosis in 2000 and 2001, respectively). In the both years, control mortality due to *B. bassiana* was occasionally observed to be >50% (Tables 1, 2). In 2001 a mean (SE) of 328.3 (8.97) *C. caryae* adults were captured per sample date.

Trends observed in total mortality data from the Byron experiments (Tables 3, 4) were essentially the same as those observed for mycosis data (Tables 1, 2). When averaged over the entire experimental period, the *B. bassiana* treatment without irrigation caused greater mortality than all other treatments in 2000 after 7 d of incubation, and in 2001 after 7 and 14 d of incubation ($F = 3.4$; df = 3,26; $P = 0.034$, $F = 6.6$; df = 3,27; $P = 0.002$, $F = 13.24$; df = 3,27; $P = 0.0001$, respectively), though not in 2000 after 14 d incubation ($F = 2.4$; df = 3,26; $P = 0.09$) (Tables 3, 4). Additionally, the *B. bassiana* treatment without irrigation occasionally caused higher mortality than other treatments on particular sample dates ($P > 0.05$) (Tables 3, 4).

Griffin field tests. In experiments conducted in Griffin, GA, up to 74% infection was observed in *B. bassiana*-treated plots and up to 33% in control plots (Table 5). In 2000, fungal-induced mortality was greater in plots receiving *B. bassiana* than control plots 2 ($F = 74.68$; df = 1,13; $P = 0.0001$), 4 ($F = 31.3$; df = 1,13; $P = 0.0001$), and 10 ($F = 6.76$; df = 1,13; $P = 0.0220$) d after treatment as well as over the entire experimental period ($F = 37.9$; df = 1,9; $P = 0.0002$). In 2001, no differences among treatments were detected on any sample date ($P > 0.05$), or over the entire experimental period ($P > 0.05$); *B. bassiana* induced mortality did not exceed 35% during the experiment. The number of insects that died but did not exhibit signs of mycosis was minimal, i.e., for the entire experimental period a mean (SE) of 1.3 (0.34), 1.3 (0.42), per plot in the *B. bassiana* and control 2000, respectively, and 2.1 (0.51), and 1.9 (0.53) in 2001. Total mortality for each treatment at each sample date was not recorded (as in the Byron experiments). Mean (SE) capture of *C. caryae* adults per sample date in 2000 and 2001 was 39.6 (7.8), and 311.6 (28.6), respectively.

Determination of conidia produced per insect. Mean (SE) conidia production per insect was 4.17×10^9 (5.67×10^8).

Discussion

During the first week after treatment, we observed high levels of *B. bassiana* infection (up to 95%) in adult *C. caryae* collected from plots receiving the fungus without irrigation. The level of infection we observed in adults is substantially higher than what has been observed previously in applications of entomopathogenic fungi to control the larval stage of *C. caryae* (Tedders et al. 1973, Gottwald and Tedders 1983, Harrison et al. 1993). Our results are consistent with those of Gottwald and Tedders

Table 3. Mean percentage (±SE) of adult *Curculio caryae* mortality following application of *Beauveria bassiana* in field trials conducted in Byron, GA 2000

Incubation period	DAT	Treatment			
		Bb	Bb + H_2O	Control	Control + H_2O
7 days	1	89.0 (11.0) a	50.0 (18) a	33.3 (23.6) a	25.0 (25.0) a
	2	44.5 (20.5) a	18.8 (13.1) a	11.9 (7.9) a	33.3 (21.1) a
	3	56.3 (25.8) a	31.0 (15.6) a	40.0 (24.5) a	31.3 (16.2) a
	7	15.1 (5.0) a	9.4 (4.6) a	23.9 (9.3) a	22.2 (8.2) a
	8	51.4 (8.5) a	32.9 (8.8) a	22.4 (5.1) a	27.2 (11.2) a
	9	26.8 (5.4) a	26.7 (6.7) a	20.7 (5.9) a	21.9 (10.9) a
	Average*	39.7 (5.5) a	27.3 (4.6) b	23.7 (4.5) b	26.7 (5.6) b
14 days	1	100.0 a	66.7 (21.1) a	91.8 (8.3) a	100.0 a
	2	100.0 a	57.3 (15.2) b	44.5 (20.5) b	58.3 (20.1) b
	3	66.8 (23.6) a	56.3 (15.1) a	80.0 (20.0) a	33.8 (15.6) a
	7	42.2 (7.7) a	40.6 (9.2) ab	36.7 (8.9) b	60.3 (14.2) b
	8	66.3 (8.4) a	52.3 (7.8) a	46.3 (4.0) a	38.2 (9.8) a
	9	61.0 (8.5) a	56.0 (10.8) a	40.2 (10.7) as	56.3 (11.4) a
	Average	67.5 (4.9) a	54.3 (5.1) a	51.1 (5.6) a	53.0 (5.8) a

Curculio caryae were collected on various days after treatment (DAT), and incubated in the laboratory for 7 and 14 d before percentage mortality was determined. Bb, *B. bassiana;* +H_2O, irrigation was applied. Different letters following numbers indicate statistical significance within each row ($\alpha = 0.05$).

* Percentage total weevil mortality averaged over the entire experiment, (all DAT).

(1983) who also observed relatively high levels of adult *C. caryae* infection from *B. bassiana* (72%) immediately following trunk perimeter applications.

Our evaluation was an estimation of the potential for suppression of *C. caryae* with *B. bassiana* under field conditions. Because our evaluation was based on *C. caryae* mycosis following transport to controlled environmental conditions, we cannot know how many of those weevils might have died if they remained in the field. Additional research is necessary to determine the correlation between our approach (of moving field-collected weevils to the laboratory for evaluation) and the number of weevils that actually would become infected and die if they remained under field conditions.

Although high levels of *B. bassiana* infection were observed initially, for the most part, no differences were detected between control and treated plots after the first week post-application. This loss of activity may have been caused by decreased conidia survival due to ultraviolet radiation (Goettel and Inglis 1997), which can be a factor despite the fact that applications were made under the tree canopy (Smits et al. 1996), or microbial degradation by antagonists (Lingg and Donaldson 1981, Fargues et al. 1983), or other physical factors, e.g., temperature or moisture (Lingg and Donaldson 1981, Studdert et al. 1990). Drastic reductions in conidial survival in soil have

Table 4. Mean percentage (±SE) of adult *Curculio caryae* mortality following application of *Beauveria bassiana* in field trials conducted in Byron, GA 2001

Incubation Period	DAT	Treatment			
		Bb	Bb + H_2O	Control	Control + H_2O
7 days	1	28.8 (4.1) a	20.8 (3.8) a	17.6 (5.1) a	13.83 (4.3) a
	2	40.7 (8.2) a	24.3 (6.2) b	15.8 (4.1) bc	5.0 (2.2) c
	3	41.6 (7.0) a	23.0 (3.3) a	29.6 (7.9) a	24.3 (4.4) a
	7	44.5 (8.2) a	22.6 (4.3) a	36.7 (7.5) a	28.9 (6.1) a
	8	27.6 (3.7) a	25.2 (4.9) a	20.6 (6.0) a	14.6 (3.2) a
	9	29.7 (6.8) a	34.5 (6.5) a	35.3 (9.5) a	22.8 (3.6) a
	14	48.1 (6.5) a	42.7 (5.9) a	48.8 (5.3) a	48.2 (7.3) a
	15	39.3 (6.9) a	39.1 (4.7) a	44.5 (4.7) a	35.9 (5.6) a
	16	30.6 (4.9) a	37.1 (2.7) a	29.2 (4.8) a	24.6 (2.6) a
	Average*	36.7 (2.2) a	29.9 (1.8) b	30.9 (2.3) b	23.9 (2.0) c
14 days	1	70.0 (5.5) a	51.0 (6.0) ab	43.9 (10.4) ab	32.6 (6.5) b
	2	73.8 (5.8) a	44.1 (5.3) b	40.8 (6.5) b	24.8 (3.5) b
	3	71.8 (5.3) a	53.0 (6.0) b	43.6 (6.6) b	40.6 (5.1) b
	7	82.4 (6.8) a	49.8 (6.1) b	68.3 (10.4) ab	56.7 (6.5) a
	8	59.0 (5.0) a	46.9 (5.6) a	38.4 (9.6) a	32.2 (4.6) a
	9	54.0 (9.3) a	50.9 (8.3) a	58.0 (7.9) a	44.4 (5.8) a
	14	65.9 (7.0) a	65.2 (8.7) a	69.7 (4.4) a	77.4 (3.8) a
	15	53.4 (5.6) a	60.5 (4.4) a	50.5 (4.4) a	42.0 (6.9) a
	16	50.8 (4.2) a	53.3 (4.9) a	46.7 (2.9) a	44.2 (5.6) a
	Average	64.5 (2.2) a	52.7 (2.1) b	51.1 (2.7) b	43.3 (2.3) c

Curculio caryae were collected on various days after treatment (DAT), and incubated in the laboratory for 7 and 14 d before percentage mortality was determined. Bb, *B. bassiana;* +H_2O, irrigation was applied. Different letters following numbers indicate statistical significance within each row ($\alpha = 0.05$).

* Percentage total weevil mortality averaged over the entire experiment, (all DAT).

been recorded in other studies (Storey et al. 1987, 1989, Krueger et al. 1991). For example, Storey et al. (1987) reported no recovery of conidia 7 d post-treatment when applied to soil via irrigation, and Storey et al. (1989) observed a decrease of more than 85% in conidia survival in soil 12 d after application.

Irrigation was detrimental to *B. bassiana* infection of *C. caryae* in perimeter applications. This phenomenon may also have been due to poor survival or accelerated degradation of conidia. Krueger et al. (1991) observed a negative relationship between soil moisture and *B. bassiana* infection of the chinch bug, *Blissus leucopterus leucopterus* (Say), or persistence. Lingg and Donaldson (1981) also demonstrated

Table 5. Mean percentage (±SE) of adult *Curculio caryae* exhibiting signs of mycosis following application of *Beauveria bassiana* in field trials conducted in Griffin, GA

2000			2001		
DAT	Bb	Control	DAT	Bb	Control
2	73.7 (8.4) a	4.1 (4.1) b	3	34.7 (7.9) a	24.1 (3.5) a
4	59.4 (9.9) a	7.1 (7.1) b	5	24.4 (5.9) a	18.4 (5.3) a
6	72.5 (11.5) a	33.3 (33.3) a	7	25.5 (1.1) a	18.2 (11.0) a
8	69.4 (13.4) a	28.6 (14.9) a	10	13.2 (5.8) a	23.4 (4.3) a
10	27.8 (8.4) a	4.8 (4.8) b	12	12.3 (5.5) a	24.0 (6.5) a
12	4.2 (4.2) a	0 a	14	11.5 (5.0) a	14.7 (3.1) a
14	8.2 (4.2) a	5.6 (5.6) a	17	6.8 (3.2) a	8.4 (2.3) a
16	14.8 (11.3) a	0 a	19	12.7 (12.5) a	4.0 (2.1) a
18	18.8 (16.4) a	0 a	21	12.3 (7.3) a	2.4 (1.1) a
20	0 a	4.0 (4.0) a	24	5.1 (4.1) a	9.1 (3.5) a
Average*	37.9 (4.6) a	7.9 (2.8) b	Average	16.3 (2.3) a	14.8 (1.8) a

Curculio caryae were collected on various days after treatment (DAT), and incubated in the laboratory for 14 d before the percentage exhibiting mycosis was determined, Bb, *B. bassiana*. Different letters following numbers indicate statistical significance within each row and year of study ($\alpha = 0.05$).

* Percentage of weevils exhibiting signs of mycosis averaged over the entire experiment, (all DAT).

decreased *B. bassiana* persistence with increased soil moisture. The detrimental moisture effect on *B. bassiana* infections may have been due to increased microbial activity (Paul and Clark 1989) and subsequent degradation by antagonistic microbes (Lingg and Donaldson 1981, Fargues et al. 1983), or premature germination. One may also speculate that the irrigation caused leaching of conidia making them unavailable to infect *C. caryae* as they crawled across the treated soil surface. In a 235 d study following surface application of *B. bassiana,* however, less than 10% of conidia penetrated below 5.0 cm from the soil surface (Storey et al. 1989).

Unlike the other three experiments, one of the experiments (conducted in Griffin, GA) failed to show any differences in *C. caryae* mortality in treated and non-treated plots. This was unexpected because the experiment conducted in Byron, GA, at approximately the same time, used *B. bassiana* produced in the same lot, yet the results were quite different. The *B. bassiana* used in all experiments was handled similarly to avoid damage to spores, e.g., avoiding excessive heat, or undue exposure to ultraviolet radiation prior to application (Goettel et al. 2000). Thus, we do not know the reason for the inconsistency in results. Conceivably, the container of *B. bassiana* used in the 2001 Griffin experiment was mishandled prior to the time we received it. This hypothesis is supported by the fact that viability estimates were lower in the container used in the Griffin 2001 experiment.

The estimates for *B. bassiana* conidia produced in *C. caryae* adults were higher than reported in some insects, e.g., infected *B. leucopterus* adults yielded 4.4×10^6

conidia per insect (Ramoska 1984), and *Rhodnius prolixus* (Stål) yielded 1.7×10^8 (Luz and Fargues 1998). Yet due to variation in method of extraction, comparison of conidia production among different insect host species is difficult, and is probably best accomplished based on yield per g of insect and using the identical *B. bassiana* strain(s).

Estimates of *B. bassiana* conidia production per *C. caryae* adult indicate potential for recycling. Using the estimate of 4.2×10^9 conidia per insect 86 infected *C. caryae* would be required to replenish the 3.6×10^{11} conidia initially applied per tree (to approximately 12 m^2 of surface area). *Curculio caryae* infestations can be more than 1×10^3 per tree (Cocke et al. 1984). Thus, conceivably a 10% infection rate among the *C. caryae* entering each tree could return an equal number of conidia to the soil as was initially applied. Additionally, a proportion of *C. caryae* coming in contact with infected ones (prior to death) may themselves become infected; Gottwald and Tedders (1983) reported 44% infection of healthy *C. caryae* that were caged with infected insects. Future research will be required to determine the potential of recycling and horizontal transmission under field conditions.

The utility of the *B. bassiana* perimeter application approach, as a management tool for *C. caryae* control will require additional research. A residual time of 1 week or less may be insufficient because of the potential need for multiple applications, which may cause the approach to be uneconomical. Perhaps persistence of *B. bassiana* could be enhanced through addition of certain soil amendments (Lingg and Donaldson 1981, Rosin et al. 1996) or through improved formulation (Storey et al. 1989). Additionally, persistence may vary among different fungal species or strains. Shapiro-Ilan et al. (2002) reported that several new isolates of *B. bassiana* and *M. anisopliae* possess greater virulence to *C. caryae* than the GHA strain used in this study; future research will determine if these isolates also possess a greater ability to persist in soil.

Even if the persistence of *B. bassiana* control is improved, it is still not clear if a large enough proportion of *C. caryae* would become infected and die before damage thresholds are surpassed. The average time from *C. caryae* emergence to oviposition is 6.5 d (Criswell et al. 1975). Even in the most effective treatment (*B. bassiana* without irrigation), less than 50% mycosis occurred within 7 d post-emergence. Thus, it is questionable whether the perimeter application approach could act as a curative treatment for the current nut crop, or only as a preventative treatment for subsequent crops. On the other hand, fungus-infected insects can reduce feeding and other behaviors prior to death (Tanada and Kaya 1993); if such cessation of behavior could be demonstrated for *B. bassiana* versus *C. caryae* then the case for curative treatments would certainly increase.

Finally, it is not known if a 2 m band of fungus applied around each tree is a sufficient treatment area. We do not know what proportion of *C. caryae* crawl to the tree relative to those that fly, nor do we know if those that crawl across the treated area become infected as efficiently as those that emerge directly under the treated area. Clearly, the proportion of the *C. caryae* population flying to the tree would not be susceptible to *B. bassiana* infection using the perimeter application approach (unless they emerge from soil within the treated perimeter). Raney and Eikenbary (1968) reported less than 10% of emerging *C. caryae* crawl to the trunk. The study, however, was conducted on collected (and handled) *C. caryae* (Raney and Eikenbary 1968); research in progress (T.E.C., unpubl.) indicates the behavior of naturally-emerging adult *C. caryae* will diverge from the findings of Raney and Eikenbary (1968). The optimum area of application has yet to be determined.

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