Survey of Entomopathogenic Nematodes and Fungi Endemic to Pecan Orchards of the Southeastern United States and Their Virulence to the Pecan Weevil (Coleoptera: Curculionidae)

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ABSTRACT The pecan weevil, *Curculio caryae* (Horn), is a major pest of pecans in the Southeastern United States. Entomopathogenic nematodes and fungi are potential alternatives to chemical insecticides for *C. caryae* control. Our objective was to survey pecan orchards in the southeastern United States for entomopathogenic nematodes and fungi and determine the virulence of the new isolates to *C. caryae* larvae. Soil was collected from 105 sites in 21 orchards in Arkansas, Georgia, Louisiana, and Mississippi. Entomopathogens were isolated by exposing soil to *C. caryae* and greater wax moth larvae, *Galleria mellonella*, (L.). We isolated entomopathogenic fungi and nematodes from 16 and 6 of the 21 orchards surveyed, respectively. The entomopathogenic fungi included *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin, and nematodes included *Heterorhabditis bacteriophora* Poinar, *Steinernema carpocapsae* (Weiser), *Steinernema glaseri* (Steiner), and *Steinernema rarum* (Doucet). This is the first report of *Steinernema rarum* in the United States. Soil characteristics in orchards were analyzed for pH, organic matter, and nutrients; we detected a negative relationship between fungal occurrence and manganese levels in soil and a positive relationship between *M. anisopliae* occurrence and calcium or magnesium levels. In laboratory assays, virulence of 15 nematode and 22 fungal isolates to *C. caryae* larvae was tested in small plastic cups containing soil. Results indicated poor susceptibility of the *C. caryae* larvae to entomopathogenic nematodes. Several fungal isolates that caused significantly higher mortality in *C. caryae* larvae than other strains (including a commercial strain of *B. bassiana*) should be investigated further as potential control agents of *C. caryae*.

KEY WORDS *Beauveria bassiana*, *Curculio caryae*, *Heterorhabditis*, *Metarhizium anisopliae*, pecan, *Steinernema*

THE PECAN WEEVIL, *Curculio caryae* (Horn) is a major pest of pecans throughout the southeastern United States (Mizell 1985). The insects have a 2 or 3 yr life-cycle (Harris 1985). Adults emerge from soil in late July–August, and then feed on and oviposit in the nuts (Harris 1985). Larvae develop within the nut and fourth instars drop to the soil where they burrow to a depth of 8–25 cm. The following year ≈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 yr in the soil as larvae emerging as adults in the third year (Harris 1985).

Control recommendations for the pecan weevil currently consist solely of applications of chemical insecticides (e.g., carbaryl) to the canopy to suppress adults (Ellis et al. 2000). Late season applications of carbaryl, however, can result in resurgence of damaging aphid populations, because carbaryl suppresses certain aphid predators (e.g., coccinellids) but does not suppress the pecan aphid complex (Dutcher and Payne 1985). As a result of the problems associated with aphid resurgence, as well as other environmental and regulatory concerns, research on developing alternative control strategies is warranted. Microbial control (use of entomopathogenic virus, bacteria, protozoa, fungi, or nematodes) is one of the potential alternatives to chemical insecticides.

Among entomopathogens of *C. caryae* studied thus far, nematodes (genera *Heterorhabditis* and *Steinernema*) and certain hyphomycete fungi i.e., *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium aniso-*

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pliae (Metschnikoff) Sorokin, have shown the most promise as microbial control agents (Gottwald and Tedders 1983, Sikorowski 1985, Fuxa et al. 1998, Shapiro-Ilan 2001a). Fungi in the class Hyphomycetes generally invade the insect host through the cuticle, replicate within the host's hemocoel, and form external conidiophores to disperse their spores (Tanada and Kaya 1993). Entomopathogenic nematodes kill insects with the aid of a mutualistic bacterium (*Xenorhabdus* spp. and *Photorhabdus* spp. for steinernematids and heterorhabditids, respectively) (Kaya and Gaugler 1993). Infective juveniles (IJs), enter hosts through natural openings (mouth, anus, and spiracles), or in some cases, through the cuticle, and complete 1–3 generations within the insect cadaver after which IJs are released to search out new hosts (Kaya and Gaugler 1993). Both the nematodes (*Heterorhabditis* and *Steinernema* spp.) and fungi (*B. bassiana* and *M. anispoliae*) are pathogenic to a wide variety of insects including a number of curculionid or other coleopteran pests (Booth et al. 2000, McCoy et al. 2000, Shapiro-Ilan et al. 2002). Because of susceptibility to environmental extremes (e.g., low relative humidity, ultraviolet light, temperature) both groups are most successful when applied to soil or other "protected" environments (Fuxa and Tanada 1987). Microbial control of *C. caryae* with these agents is most likely to be successful when nematodes or fungi are applied to soil as a barrier treatment when larvae are dropping from nuts, or when adults are emerging (Sikorowski 1985, Shapiro-Ilan unpublished).

Although some promising results have been reported in suppressing *C. caryae* with entomopathogenic nematodes and fungi (e.g., >60% suppression) (Tedders et al. 1973, Gottwald and Tedders 1983), other laboratory and field experiments indicate a lack of consistency (i.e., <35% suppression) (Nyczepir et al. 1992, Harrison et al. 1993, Smith et al. 1993). Virulence can depend substantially on the strain or species of the particular entomopathogen being used (Tanada and Kaya 1993, Shapiro-Ilan et al. 2002). Thus, discovery of new strains or species of entomopathogenic nematodes or fungi may lead to enhanced potential for microbial control. For example, discovery of new entomopathogenic nematode species within the past decade has substantially bolstered the commercial success of microbial control programs versus important pests including mole crickets (*Scapteriscus* spp.) in turf and the diaprepes root weevil, *Diaprepes abbreviatus* (L.) in citrus (Shapiro-Ilan et al. 2002). Likewise, susceptibility of *C. caryae* varies among species or strains of entomopathogenic fungi or nematodes (Harrison et al. 1993; Shapiro-Ilan 2001a, 2001b). For example, certain entomopathogenic nematode species and strains were highly virulent to *C. caryae* adults (Shapiro-Ilan 2001a) but only poorly or moderately virulent to the larval stage with no difference among strains (Shapiro-Ilan 2001b). Conceivably, undiscovered entomopathogen strains or species with superior virulence exist in pecan orchards within the range of *C. caryae.* Our objective was to survey pecan orchards of the southeastern United States for entomopathogenic nematodes and fungi and determine the relative virulence of the new isolates to *C. caryae* larvae. Future studies will be directed to screening the isolates for virulence to adult *C. caryae* and to surveying for nonendemic entomopathogens.

Materials and Methods

Isolation of Entomopathogens. Twenty-one pecan orchards (varieties included Stuart, Schley, and Desirable) were surveyed in Arkansas, Georgia, Louisiana, and Mississippi (Table 1). In each orchard five sample sites were chosen ≈50 m from the next. Within each site, two subsamples consisting of approximately 2 liters of soil were removed by shovel (to a depth of 30 cm) from each of two adjacent trees (four subsamples total per site). One-half of the subsamples were taken 25 cm from the trunk of the tree, and the other half were taken 2 m from the trunk. The subsamples from each site were combined into a single plastic bag and mixed thoroughly. Approximately one-half of the soil from each site (4 liters) was removed, placed in a refrigerated cooler, and taken to the laboratory for processing. The samples were collected during October and November 2000.

Pathogens were isolated by the insect-baiting method (Bedding and Akhurst 1975, Zimmermann 1986). In the laboratory, soil samples from each site were split into two plastic pots. Ten last instar greater wax moth larvae, *Galleria mellonella* (L.) (obtained from Sunfish Bait Co., Webster, WI) were added to one pot and five fourth instar *C. caryae* to the other. We chose to isolate entomopathogens using both hosts because some species or strains can be host-specific; e.g., *Steinernema scapterisci* Nguyen and Smart shows considerable specificity to *Scapteriscus* spp. (Nguyen and Smart 1990). Dead insects were removed from pots and replaced with healthy ones every 5 days for 15 d. Soil was kept moist (approximately at field capacity) during this period. Entomopathogenic nematodes and fungi were isolated from insect cadavers exhibiting signs of infection (Tanada and Kaya 1993) on Sabouraud dextrose agar (for fungi) (Goettel and Inglis 1997) or on White traps (for nematodes) (Kaya and Stock 1997). The pathogenicity of fungi and nematodes was confirmed through Koch's postulates (Lacey and Brooks 1997). Entomopathogenic fungi were identified according to procedures described by Humber (1997), and nematodes were identified using morphological characteristics and molecular techniques (Nguyen and Smart 1996, Nguyen et al. 2001). After pathogen isolation, soil from the five sites in each orchard was mixed thoroughly and analyzed for pH, organic matter, and nutrients.

Virulence Assays. Nematodes for all experiments were reared simultaneously on last instar *G. mellonella* at 25°C according to procedures described in Kaya and Stock (1997). Fungi were cultured on Sabouraud dextrose agar (Goettel and Inglis 1997). Before experimentation, fungi and nematodes were stored at 4 and 13°C for less than 2 and 6 wk, respectively. Subculturing of fungi and nematodes did not exceed three

Table 1. Entomopathogenic fungus and nematode isolates from soil in southeastern U.S. pecan orchards

Orchard	Location	Fungal isolates	Nematode isolates
AR1	West Helena, AR	BbAR1	ScAR1, ScAR2
AR2	West Helena, AR	–	–
AR3	Holly Springs, AR	–	–
GA1	Jackson, GA	BbGA3, BbGA4	–
GA2	Bullard, GA	BbGA5	–
GA3	Pineview, GA	BbGA6	–
GA4	Weston, GA	BbGA7	–
GA5	Barnesville, GA	BbGA1	HbGA1, HbGA2, HbGA3, SgGA1, SgGA2
GA6	Waycross, GA	BbGA8	–
GA7	Cochran, GA	–	–
GA8	Byron, GA	BbGA2	–
LA1	Benton, LA	BbLA1	–
LA2	Shreveport, LA	BbLA2, BbLA3, MaLA1, MaLA2	–
LA3	Dixie, LA	MaLA3, MaLA4	SgLA1
LA4	Robson, LA	MaLA5, MaLA6	–
LA5	Cloutierville, LA	MaLA7,Ma LA8	SgLA2, SgLA3
LA6	Benton LA	MaLA9	SrLA1, SrLA2
MS1	Rena Lara, MS	–	–
MS2	Rena Lara, MS	–	–
MS3	Rena Lara, MS	BbMS1	SrMS1, SrMS2, SrMS3
MS4	Clarksdale, MS	BbMS2, BbMS3	–

AR, Arkansas; Bb, *Beauveria bassiana;* GA, Georgia; Hb, *Heterorhabditis bacteriophora;* LA, Lousiana; Ma, *Metarhizium anisopliae;* MS, Mississippi; Sc, *Steinernema carpocapsae;* Sg, *S. glaseri;* Sr, *S. rarum.*

passages in the host or on agar before use in experiments.

Virulence experiments were conducted in plastic cups (Bioserv Inc., Frenchtown, NJ) based on procedures described by Harrison et al. (1993) and Shapiro-Ilan (2001a, 2001b). All cups (3–4 cm i.d., 3.5-cm deep) contained oven-dried soil from the USDA-ARS pecan orchard (Byron, GA) and one larva each. Cups in the nematode experiments held 27 g autoclaved soil (that was kept at least 2 wk before use), and cups in fungus experiments held 10 g of soil. The soil was a loamy sand with the percentage sand:silt:clay = 84: 10:6, pH = 6.1, and organic matter = 2.8% by weight. Pecan weevil larvae (fourth instar), collected from infested nuts on the USDA-ARS Research Station (Byron, GA), were stored in sterile (autoclaved) soil at 25°C for 2 wk, at which time diseased larvae were removed. Remaining larvae were then stored at 4–10°C (Shapiro-Ilan 2001b) until use.

It was not feasible to include all nematode or fungal isolates in a single experiment. Therefore, the isolates were tested in a series of assays, which included at least two common isolates in each test for comparison. The nematode experiments were split into two assays (hereafter referred to as Assay 1 and 2) and the fungi into four assays (hereafter referred to as Assays 3–6). All isolates were tested with the exception of BbGA5 and MaLA1 (see Table 1 for identification and source of isolates) because these cultures were inadvertently lost before experimentation. Each assay included three replicates of 10 cups per treatment (isolate) and was conducted twice (two trials). Nematode experiments included *Heterorhabditis bacteriophora* Poinar (Hb strain) and or *Steinernema riobrave* Cabanillas, Poinar, and Raulston as standards for qualitative comparison with Shapiro-Ilan (2001b); the nematode standards were subcultured from isolates originally provided by R. Gaugler (Rutgers University, NJ) and Certis Corp. (Columbia, MD), respectively. Also, to facilitate comparisons, one of the new isolates, SrMS1 was also included in both nematode assays. All fungus experiments included Mycotrol (*B. bassiana* GHA strain), which is currently registered for pecan weevil control by Emerald BioAgriculture Corp., as a standard and the new isolate BbMS1. All experiments included an untreated control (only water added) and were arranged in completely randomized designs.

Nematodes and fungi were pipetted onto the soil surface of each cup in 0.5 and 1.4 ml of water and 0.05% Tween 80, respectively, so the final moisture was standardized at field capacity (14%). Nematodes were applied at a rate of ≈39 IJs per cm^2 (500 IJs per cup), and larval mortality was recorded after 13 or 14 d of incubation at 25°C. In the first fungus assay (Assay 3, trial 1), the application rate was ≈7,950 conidia per cm^2 (100,000 per cup) but, because of low mortality, 15,900 conidia per cm^2 (200,000 per cup) were applied thereafter. After inoculation, fungus experiments were incubated at 25°C, and mortality as a result of fungi, i.e., signs of mycosis (Tanada and Kaya 1993), was recorded every 1–3 d beginning 5 d postinoculation. Preliminary experimentation indicated it was not necessary to make observations before 5 d. The rates of application were based on previous laboratory experiments that showed differences among strains at similar rates, e.g., Harrison et al. (1993), Shapiro and McCoy (2000a), and Shapiro-Ilan (2001a, 2001b).

Data Analysis. Correlation analysis (SAS Institute 1985) was used to determine relationships between soil parameters (listed in Table 2) and pathogen occurrence (number of positive sites). Virulence assays were analyzed using analysis of variance; if the F value

Table 2. Soil parameters for pecan orchards surveyed for entomopathogens[a]

Location	Orchard	Sand:Silt:Clay	pH	OM	N	Ca	K	Mg	Mn	P	Zn
West Helena, AR	AR1	38:56:6	6.1	4.3	0.13	1517	318.4	287.4	61.8	108.1	13.0
West Helena, AR	AR2	38:56:6	5.2	4.6	0.14	2004	394.5	338.3	161.1	115.4	14.9
Holly Springs, AR	AR3	38:56:6	5.7	4.7	0.13	2372	442.4	268.5	130.3	131.4	24.2
Jackson, GA	GA1	9:6:4	6.0	8.0	0.21	2848	348.1	440.4	50.1	154.1	25.6
Bullard, GA	GA2	na[b]	5.0	3.6	na	1498	179.8	251.7	66.1	81.8	8.4
Pineview, GA	GA3	na	5.7	3.0	na	2248	209.2	276.5	42.2	120.8	42.7
Weston, GA	GA4	90:4:6	6.0	1.5	0.06	1641	134.5	100.0	21.0	295.0	94.6
Barnesville, GA	GA5	86:12:2	6.5	3.8	0.09	2743	215.7	345.3	32.0	95.9	14.4
Waycross, GA	GA6	96:2:2	5.7	2.9	0.06	1408	140.5	252.4	15.0	194.9	33.5
Cochran, GA	GA7	96:2:2	5.3	3.0	0.11	2292	223.2	188.8	45.8	102.8	25.2
Byron, GA	GA8	80:16:4	6.1	3.6	0.08	2020	332.6	170.1	46.5	222.5	30.7
Benton, LA	LA1	24:72:4	6.0	5.2	0.17	4602	692.3	1151	37.0	360.0	6.2
Shreveport, LA	LA2	36:54:10	8.1	4.0	0.09	2646	304.2	840.0	57.9	170.4	41.2
Dixie, LA	LA3	24:76:10	5.2	3.8	0.1	8314	253.3	1187	42.5	163.6	2.6
Robson, LA	LA4	34:62:4	5.9	3.7	0.08	2787	302.6	951.3	47.1	184.1	29.6
Cloutierville, LA	LA5	62:32:6	7.5	1.4	0.04	1147	209.9	254.2	43.3	120.3	25.3
Benton LA	LA6	22:78:0	8.0	4.0	0.1	9163	315.7	934.1	27.6	195.0	11.0
Rena Lara, MS	MS1	54:8:38	4.7	6.4	0.12	3525	500.7	764.6	69.3	46.4	5.8
Rena Lara, MS	MS2	52:25:22	5.0	5.6	0.15	2628	473.2	469.2	74.6	87.6	9.6
Rena Lara, MS	MS3	32:38:30	5.0	6.5	0.15	3868	676.1	862.4	44.3	81.8	7.3
Clarksdale, MS	MS4 (2)	46:28:26	4.3	4.9	0.13	2194	305.3	438.3	37.2	33.5	4.2

[a] OM (organic matter) and N are percentages; Ca, K, Mg, Mn, P, and Zn are approximations of Kg of nutrient/ha.
[b] na, not analyzed.

was significant ($\alpha = 0.05$) then means were differentiated by LSMEANS (SAS Institute 1985). In the fungus assays, mycosis was recorded through time, therefore analysis of variance was applied for each day separately as well as over the entire experimental period (repeated measure analysis, Proc Mixed) (SAS Institute 1985, McCoy et al. 2000, Shapiro et al. 2000). In the Proc Mixed procedure, default covariance structure (variance components) was used; no additional options were specified, and there were no missing values. In each assay, trials were combined for analysis except for Assay 3 because it had different application rates of conidia in each of the trials.

Results

Entomopathogenic nematodes and fungi isolated in our survey are listed in Table 1. Entomopathogenic nematodes were isolated from 6 of the 21 orchards (28.5%) and 11 out of 105 of the sites (10.4%). Entomopathogenic fungi were isolated from 16 of 21 of the orchards (76.2%) and 21 of 105 of the sites (20.0%). The entomopathogenic nematode species isolated were *H. bacteriophora*, *Steinernema carpocapsae* (Weiser), *Steinernema glaseri* (Steiner), and *Steinernema rarum* (Doucet). The entomopathogenic fungi isolated were *B. bassiana* and *M. anisopliae* variety *anisopliae. Beauveria bassiana* was found in 12 orchards (57.1%) and 15 sites (14.3%), whereas *M. anisopliae* was isolated in five orchards (23.8%) and six sites (5.7%). Some sites yielded more than one isolate.

Soil characteristics from each orchard are listed in Table 2. Correlation analysis indicated several significant relationships. We detected a negative relationship between fungal (*B. bassiana* and *M. anisopliae* combined) occurrence and manganese levels in soil ($r = -0.49$; $P = 0.03$). Levels of calcium and magnesium were each positively correlated with *M. anisopliae* occurrence ($r = 0.61$; $P = 0.003$ and $r = 0.59$; $P = 0.005$ for calcium and magnesium, respectively). We did not detect any other significant correlations among fungal or nematode isolates and soil characteristics ($P > 0.05$).

In virulence assays, all entomopathogenic nematode isolates and standards caused <30% mortality in *C. caryae* larvae. In assay 1, several nematode isolates caused greater mortality than the control ($F = 2.61$; df = 12,50; $P = 0.009$), and with the exception of the *S. glaseri* isolates (which caused the least mortality), there were no significant differences among new isolates and standards (Fig. 1). Assay 2 compared the virulence of six isolates (SrMS1, SgLA1, SgLA2, SgLA3, SrLA1, and SrLA2) with the Hb(hb) strain and a control; no significant treatment effects were detected ($F = 1.84$; df = 7,30; $P = 0.12$), and average (SE) mortality among nematode treatments ranged from 11.9 ± 5.7 to 28.6 ± 3.7.

Significant differences in virulence were detected among the fungal isolates. For the sake of brevity, only the average mortality over the entire experimental period and the cumulative mortality for the last day, are presented for each assay (Figs. 2–6). A number of isolates caused greater *C. caryae* mortality than the control and the standard (Mycotrol) (Figs. 2–6). In Assay 3, trial 1, no differences in *C. caryae* larval mortality were detected among treatments on day 14 of the experiment ($P > 0.05$) (Fig. 2). When averaged over the entire experimental period, however, two isolates (BbMS1 and BbGA2) caused significantly greater *C. caryae* mortality compared with the control ($F = 12.77$; df = 7,182; $P = 0.0001$) (Fig. 2). Additionally, the overall average mortality caused by each of the new fungal isolates, except for BbAR1 and BbMS2, were greater than the standard (Fig. 2). In the second trial of Assay 3, when averaged over the entire experimental period, all the fungal isolates except

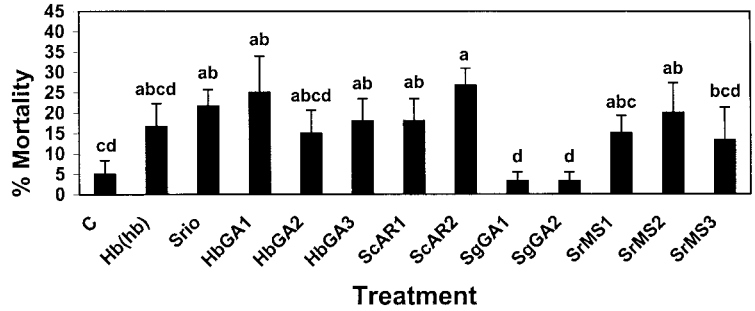

Fig. 1. Mortality of *Curculio caryae* larvae after 14 d of exposure to entomopathogenic nematodes isolated from soil in southeastern U.S. pecan orchards (Assay 1). Different letters above bars indicate statistical significance ($\alpha = 0.05$). See Table 1 and text for source of isolates. C, control (water); Hb, *Heterorhabditis bacteriophora*; Srio, *Steinernema riobrave*; Sc, *S. carpocapsae*; Sg, *S. glaseri*; Sr, *S. rarum;* Hb (hb), *H. bacteriophora* (Hb strain). Rate of nematode application was ≈39 infective juveniles per cm^2.

BbAR1 caused greater *C. caryae* mortality relative to the control, and BbGA2 caused greater mortality than all other isolates ($F = 11.89$; df = 7,154; $P = 0.0001$) (Fig. 3).

In Assay 4, 14 d after exposure several isolates caused greater mortality than the control, and MaLA4, MaLA7, and BbLA1 caused the highest mortality (Fig. 4). When averaged over the entire experimental period, all the new fungal isolates caused greater mortality than the control and standard, which were not different from each other, and MaLA4 caused the highest mortality ($F = 13.82$; df = 9,294; $P = 0.0001$) (Fig. 4).

In Assay 5, BbMS1 and BbGA6 caused greater mortality than the control and Mycotrol at 15 d post inoculation ($P < 0.05$) (Fig. 5). Similarly, when analyzed over the whole experimental period, BbMS1 caused greater mortality than all other isolates followed by BbGA6 ($F = 51.98$; df = 7,280; $P = 0.0001$) (Fig. 5).

In Assay 6, BbMS1 and BbLA3 caused greater mortality than other fungal isolates from pecan orchards 14 d after exposure (Fig. 6) ($P < 0.05$). When analyzed over the whole experimental period, BbMS1 caused greater mortality among isolates followed by BbLA3 ($F = 83.88$; df = 6,156; $P = 0.0001$) (Fig. 6).

To summarize the fungus virulence assays, isolates BbGA2 and MaLA4 may be considered the most virulent because they are the only isolates that caused greater mortality than all other isolates in at least one assay, and in all assays no other isolate caused greater mortality than they did (Figs. 2–6). Other isolates that showed relatively high virulence include BbMS1, BbGA6, and BbLA3 (Figs. 2–6). The standard, Mycotrol, caused greater mortality than the control in two of the four fungal assays.

Discussion

The occurrence (percentage positive samples) of entomopathogenic fungi and nematodes in our soil surveys is within the range of what has been found elsewhere, e.g., in surveys conducted in Czechoslovakia (Mracek et al. 1999), Georgia, USA (Harrison and Gardner 1991), Hawaii (Hara et al. 1991), Israel (Glazer 1991), the United Kingdom (Chandler et al. 1997), and western Canada (Mracek and Webster 1993). In a previous survey in Georgia, Harrison and Gardner (1991) detected *B. bassiana* in each of the 19 pecan orchards surveyed; similarly, we isolated *B. bassiana* in each of the Georgia orchards surveyed, con-

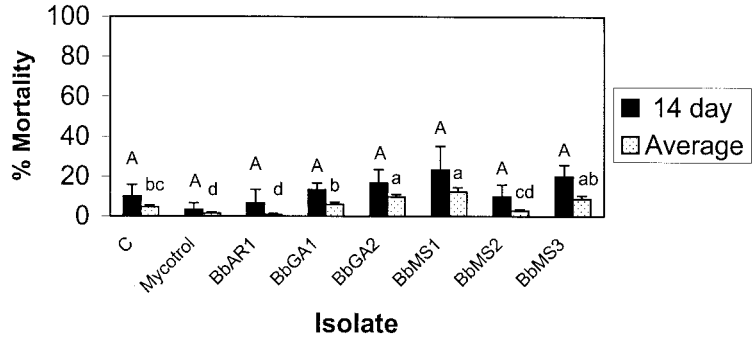

Fig. 2. Mortality of *Curculio caryae* after exposure to *Beauveria bassiana* isolates from Southeastern pecan orchards (Assay 3, trial 1). Black bars represent cumulative mortality after 14-d exposure (the last date of the assay); white bars with pattern represent daily mortality averaged over the experimental period. Different upper case letters (for black bars) and lower case letters (for white bars with pattern) indicate statistical significance within each series ($\alpha = 0.05$). See Table 1 and text for source of isolates. Rate of fungus application was ≈7,950 conidia per cm^2.

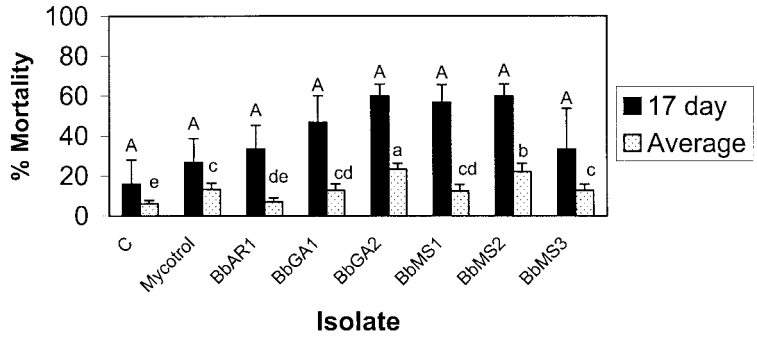

Fig. 3. Mortality of *Curculio caryae* after exposure to *Beauveria bassiana* isolates from Southeastern pecan orchards (Assay 3, trial 2). Black bars represent cumulative mortality after 17-d exposure (the last date of the assay); white bars with pattern represent daily mortality averaged over the experimental period. Different upper case letters (for black bars) and lower case letters (for white bars with pattern) indicate statistical significance within each series ($\alpha = 0.05$). See Table 1 and text for source of isolates. Rate of fungus application was ≈15,900 conidia per cm^2.

firming the widespread occurrence reported by Harrison and Gardner (1991) in this state.

Our findings concerning the relationship among soil nutrients and entomopathogenic fungi may have implications on pecan management practices to conserve the natural populations of these pathogens in the orchard. Interestingly, the nutrients we found to be positively related to *M. anisopliae* occurrence (calcium and magnesium) are required by pecan trees in relatively large amounts, whereas manganese, which is negatively correlated to fungal occurrence, is required by pecan trees in relatively small amounts (O'Barr et al. 1989). Thus, if a causal relationship between the nutrients and fungal prevalence can be demonstrated through further research, management practices to enhance the fungi will likely be compatible with those recommended for pecan husbandry. Other soil parameters such as nitrogen content and organic matter are known to affect entomopathogenic fungi (Lingg and Donaldson 1981, Studdert et al. 1990, Rosin et al. 1997), yet we did not detect any other significant relationships. We also did not detect significant relationships among soil parameters and nematode occurrence despite various studies that have demonstrated otherwise (Kung et al. 1990a, 1990b; Barbercheck and Kaya 1991; Shapiro et al. 2000). It is likely that we would detect more relationships between soil parameters and pathogen occurrence with increased sampling.

The virulence of the new nematode isolates was comparable with the standards (*S. riobrave* and *H. bacteriophora* Hb strain), which had previously been shown to possess only low to moderate virulence toward *C. caryae* larvae (Shapiro-Ilan 2001b). The relatively low susceptibility of *C. caryae* larvae to entomopathogenic nematodes was demonstrated in an experiment in which larvae of *C. caryae* and the diaprepes root weevil, *Diaprepes abbreviatus* (L), were tested in parallel; the nematodes caused substantially greater mortality in the latter insect (Shapiro-Ilan 2001b), one that is commercially controlled by nematodes, (Shapiro-Ilan et al. 2002). None of the nematode isolates from our survey caused >25% mortality in *C. caryae* larvae. The nematode application rate used in our laboratory assays (39 IJs per cm^2) was comparable to recommended field rates of 25–75 IJs per cm^2 (Georgis and Hague 1991, Georgis et al. 1995). Although it is difficult to predict field performance

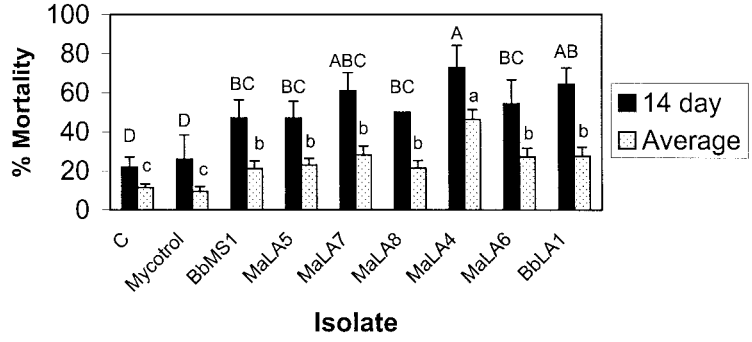

Fig. 4. Mortality of *Curculio caryae* after exposure to *Beauveria bassiana* or *Metarhizium anispoliae* isolates from Southeastern pecan orchards (Assay 4). Black bars represent cumulative mortality after 14 d exposure (the last date of the assay); white bars with pattern represent daily mortality averaged over the experimental period. Different upper case letters (for black bars) and lower case letters (for white bars with pattern) indicate statistical significance within each series ($\alpha = 0.05$). See Table 1 and text for source of isolates. Rate of fungus application was ≈15,900 conidia per cm^2.

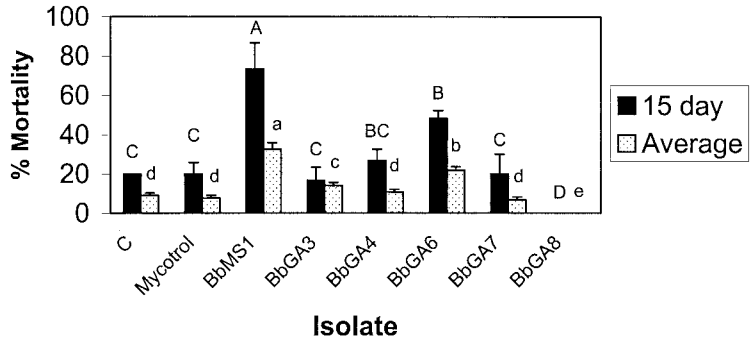

Fig. 5. Mortality of *Curculio caryae* after exposure to *Beauveria bassiana* or *Metarhizium anispoliae* isolates from Southeastern pecan orchards (Assay 5). Black bars represent cumulative mortality after 15-d exposure (the last date of the assay); white bars with pattern represent daily mortality averaged over the experimental period. Different upper case letters (for black bars) and lower case letters (for white bars with pattern) indicate statistical significance within each series ($\alpha = 0.05$). See Table 1 and text for source of isolates. Rate of fungus application was ≈15,900 conidia per cm^2.

from laboratory results, it is most likely that the nematodes will cause less insect mortality in the field than under controlled greenhouse or laboratory conditions (Kaya and Gaugler 1993, Shapiro and McCoy 2000b). Therefore, the nematodes we isolated in this survey appear to have little promise for control of *C. caryae* larvae. Perhaps these nematodes are more virulent to adult *C. caryae*, as is the case with some other entomopathogenic nematodes (Shapiro-Ilan 2001a).

Before our survey, *S. rarum* had not been reported to occur outside of its original region of isolation, the Province of Cordoba, Argentina (Doucet 1986, Koppenhöfer and Kaya 1999). Host range studies on the Argentinean *S. rarum* strain demonstrated a high affinity to larvae of the curculionid, (billbug) *Sphenophorus* sp. (Koppenhöfer and Kaya (1999). Thus, one may hypothesize that the Argentinean strain would have exhibited higher virulence to *C. caryae* than the strains we isolated. However, numerous strains and species of entomopathogenic nematodes that are highly virulent toward other curculionids have all exhibited relatively low virulence to *C. caryae* larvae (see Klein 1990 and Shapiro-Ilan et al. 2002 compared with Shapiro-Ilan 2001b), and, hence, it is probable that the Argentinean strain is no exception. Nonetheless, future studies are needed to compare host range and other ecological characteristics of the Argentinean strain (Koppenhöfer and Kaya 1999) with the new United States strains.

The fungal standard, Mycotrol, had relatively low virulence against *C. caryae* larvae, perhaps because of a dosage effect. For example, the virulence of Mycotrol to adult *C. caryae* was demonstrated in two field trials in which >70% of weevils were killed by Mycotrol within 1 wk of application (Shapiro-Ilan, Cottrell, and Gardner, unpublished data). The application rates in the field study (which were comparable to rates used in other field studies, Booth et al. 2000) were >10-fold those used in the current study. If we had used a higher application rate in the current study, mortality rates likely would have been greater; thus, it is not necessary to conclude that Mycotrol is not pathogenic to *C. caryae* larvae (at least two assays prove otherwise) but rather that the new isolates are more virulent.

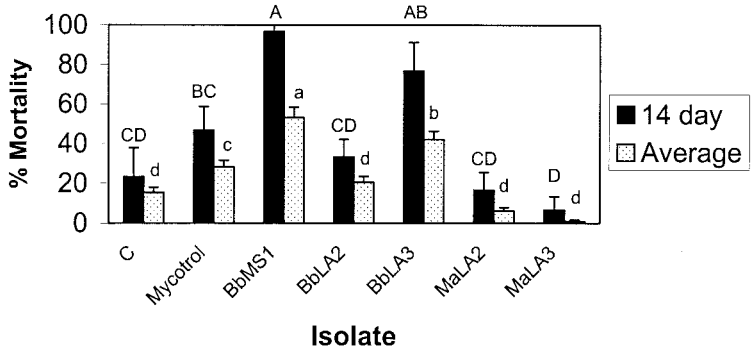

Fig. 6. Mortality of *Curculio caryae* after exposure to *Beauveria bassiana* or *Metarhizium anispoliae* isolates from Southeastern pecan orchards (Assay 6). Black bars represent cumulative mortality after 14-d exposure (the last date of the assay); white bars with pattern represent daily mortality averaged over the experimental period. Different upper case letters (for black bars) and lower case letters (for white bars with pattern) indicate statistical significance within each series ($\alpha = 0.05$). See Table 1 and text for source of isolates. Rate of fungus application was ≈15,900 conidia per cm^2.

Several fungal isolates were highly virulent and appear to be promising candidates for microbial control of *C. caryae* larvae. Previous studies have indicated greater virulence in *B. bassiana* isolates compared with *M. anisopliae* (Tedders et al. 1973, Gottwald and Tedders 1983). Our findings, however, indicate that some *M. anisopliae* isolates can be equal or greater in virulence. The relatively low *C. caryae* mortality caused by all isolates in Assay 3, trial 1 was clearly caused by the lower application rate in the trial; despite the low mortality, the assay was useful in distinguishing virulence among the isolates. The application rate used in most of our assays (15,900 per cm^2) is >10-fold less than recommended field rates (e.g., $2–8 \times 10^5$ per cm^2). Again, it is difficult to predict field performance based on laboratory results; nevertheless, it is promising that a number of isolates caused >60% *C. caryae* mortality with such a low rate. Two isolates in particular, BbGA2 and MaLA4, were consistently among the most virulent. Future studies will determine the ability of these promising fungal isolates and others to suppress *C. caryae* under field conditions.

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References Cited

Barbercheck, M. E., and H. K. Kaya. 1991. Effect of host condition and soil texture on host finding by the entomogenous nematodes *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) and *Steinernema carpocapsae* (Rhabditida: Steinernematidae). Environ. Entomol. 20: 582–589.

Bedding, R. A., and R. J. Akhurst. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica 21: 109–116.

Booth, S. R., F. A. Drummond, and E. Groden. 2000. Small fruits, pp. 597–615. *In* L. A. Lacey, and H. K. Kaya [eds.], Field manual of techniques in invertebrate pathology. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Chandler, D., D. Hay, and A. P. Reid. 1997. Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils. Appl. Soil. Ecol. 5: 133–141.

Doucet, M.M.A. de. 1986. A new species of *Neoaplectana* Steiner, 1929 (Nematoda: Steinernematidae) from Cordoba, Argentina. Rev. Nematol. 9: 317–323.

Dutcher, J. D., and J. A. Payne. 1985. The impact of pecan weevil control strategies on non-target arthropods, pp. 39–50. *In* W. W. Neel [ed.], Pecan weevil: research perspective. Quail Ridge Press, Brandon, MS.

Ellis, H C, P. Bertrand., and T. F. Crocker. 2000. Georgia pecan pest management guide. University of Georgia Cooperative Extension Service Bulletin No. 841.

Fuxa, J. R., and Y. Tanada (eds.). 1987. Epizootiology of insect diseases. Wiley, New York

Fuxa, J. R., R. Ayyappath., and R. A. Goyer. 1998. Pathogens and microbial control of North American forest insect pests. Forest Heath Technology Enterprise Team, USDA Forest Service, Morgantown, WV.

Georgis, R., and N.G.M. Hague. 1991. Nematodes as biological insecticides. Pesticide Outlook. 2: 29–32.

Georgis R., D. B. Dunlop, and P. S. Grewal. 1995. Formulation of entomopathogenic nematodes, pp. 197–205. *In* F. R. Hall and J. W. Barry [eds.], Biorational Pest Control Agents: Formulation and Delivery, American Chemical Society, Washington, DC.

Glazer, I. 1991. A survey of entomopathogenic nematodes (Rhabditida) in the Negev desert. Phytoparasitica. 19: 291–300.

Goettel, M. S., and D. Inglis. 1997. Fungi: Hyphomycetes, pp. 213–249. *In* L. A. Lacey [ed.], Manual of techniques in insect pathology, Academic Press, San Diego.

Gottwald, T. R., and W. L. Tedders. 1983. Suppression of pecan weevil (Coleoptera: Curculionidae) populations with entomopathogenic fungi. Environ. Entomol. 12: 471–474.

Hara, A., R. Gaugler, H. K. Kaya, and L. M. LeBeck. 1991. Natural populations of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) from the Hawaiian islands. Environ. Entomol. 20: 211–216.

Harris, M. K. 1985. Pecan phenology and pecan weevil biology and management, pp. 51–58. *In* W. W. Neel [ed.], Pecan weevil: research perspective. Quail Ridge Press, Brandon, MS.

Harrison, R. D., and W. A. Gardner. 1991. Occurrence of entomogenous fungus *Beauveria bassiana* in pecan orchard soils in Georgia. J. Entomol. Sci. 26: 360–366.

Harrison, R. D., W. A. Gardner, and D. J. Kinard. 1993. Relative susceptibility of pecan weevil fourth instars and adults to selected isolates of *Beauveria bassiana.* Biol. Control. 3: 34–38.

Humber, R. A. 1997. Fungi: identification, pp. 153–185. *In* L. A. Lacey [ed.], Manual of techniques in insect pathology. Academic Press, San Diego.

Kaya, H. K., and R. Gaugler. 1993. Entomopathogenic nematodes. Annu. Rev. Entomol. 38: 181–206.

Kaya, H. K., and S. P. Stock. 1997. Techniques in insect nematology, pp. 281–324. *In* L. A. Lacey [ed.], Manual of techniques in insect pathology. Academic Press, San Diego.

Klein, M. G. 1990. Efficacy against soil-inhabiting insect pests, pp. 195–214. *In* R. Gaugler, and H. K. Kaya [eds.], Entomopathogenic nematodes in biological control. CRC, Boca Raton, FL.

Koppenhöfer, A. M., and H. K. Kaya. 1999. Ecological characterization of *Steinernema rarum.* J. Invertebr. Pathol. 73: 120–128.

Kung, S., R. Gaugler, and H. K. Kaya. 1990a. Soil type and entomopathogenic nematode persistence. J. Invertebr. Pathol. 55: 401–406.

Kung, S., R. Gaugler, and H. K. Kaya. 1990b. Influence of soil pH and oxygen on persistence of *Steinernema* spp. J. Nematol. 22: 440–445.

Lacey, L. A., and W. M. Brooks. 1997. Initial handling and diagnosis of diseased insects, pp. 1–15. *In* L. A. Lacey [ed.], Manual of techniques in insect pathology. Academic Press, San Diego.

Lingg, A. J., and M. D. Donaldson. 1981. Biotic and abiotic factors affecting stability of Beauveria bassiana conidia in soil. J. Invertebr. Pathol. 38: 191–200.

McCoy, C. W., D. I. Shapiro, L. W. Duncan, and K. Nguyen. 2000. Entomopathogenic nematodes and other natural enemies as mortality factors for larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Biol. Control. 19: 182–190.

Mizell, R. F. 1985. Risk rating: A fruitful approach to management of the pecan weevil, pp. 69–78. *In* W. W. Neel [ed], Pecan weevil: research perspective. Quail Ridge Press, Brandon, MS.

Mracek, Z., and J. M. Webster. 1993. Survey of Heterorhabditidae and Steinernematidae (Rhabditida, Nematoda) in Western Canada. J. Nematol. 25: 710–717.

Mracek, Z., B. Stanislav, and P. Kindlmann. 1999. Survey of entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) in the Czech Republic. Folia Parasitologica. 46: 145–148.

Nguyen, K. B., and G. C. Smart, Jr. 1990. *Steinernema scapterisci* n. sp. (Rhabditida: Steinernematidae). J. Nematol. 22: 187–199.

Nguyen. K. B., and G. C. Smart, Jr. 1996. Identification of entomopathogenic nematodes in the Steinernematidae and Heterorhabditidae (Nemata: Rhabditida). J. Nematol. 28: 286–300.

Nguyen, K. B., J. Maruniak, and B. J. Adams. 2001. The diagnostic and phylogenetic utility of the rDNA internal transcribed spacer sequences of Steinernema. J. Nematol. 33: 73–82.

Nyczepir, A. P., J. A. Payne, and J. J. Hamm. 1992. *Heterorhabditis bacteriophora:* a new parasite of pecan weevil *Curculio caryae.* J. Invertebr. Pathol. 60: 104–106.

O'Barr, R. D., M. Smith, G. Taylor, and B. Goff. 1989. Pecan nutrition, pp. 61–71. *In* W. Goff, J. R. McVay, and W. S. Gasaway [eds.], Pecan production in the Southeast. Alabama Cooperative Extension Service Circular ANR-459, AL.

Rosin, F., D. I. Shapiro, and L. C. Lewis. 1997. Effects of fertilizers on survival of *Beauveria bassiana.* J. Invertebr. Pathol. 68: 194–195.

SAS Institute. 1985. SAS users guide, version 5th ed. SAS Institute, Cary, NC.

Shapiro, D. I., and C. W. McCoy. 2000a. Virulence of entomopathogenic nematodes to *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in the laboratory. J. Econ. Entomol. 93: 1090–1095.

Shapiro, D. I., and C. W. McCoy. 2000b. Effect of culture method and formulation on the virulence of *Steinernema riobrave* (Rhabditida: Steinernematidae) to *Diaprepes abbreviatus* (Curculionidae). J. Nematol. 32: 281–288.

Shapiro, D. I., C. W. McCoy, A. Fares, T. Obreza, and H. Dou. 2000. Effects of soil type on virulence and persistence of entomopathogenic nematodes in relation to control of *Diaprepes abbreviatus.* Environ. Entomol 29: 1083–1087.

Shapiro-Ilan, D. I. 2001a. Virulence of entomopathogenic nematodes to pecan weevil adults (Coleoptera: Curculionidae). J. Entomol. Sci. 36: 325–328.

Shapiro-Ilan, D. I. 2001b. Virulence of entomopathogenic nematodes to pecan weevil larvae *Curculio caryae* (Coleoptera: Curculionidae) in the laboratory. J. Econ. Entomol. 94: 7–13.

Shapiro-Ilan D. I., D. H. Gouge and A. M. Koppenhofer. 2002. Factors affecting commercial success: case studies in cotton, turf, and citrus, pp. 333–356. *In* R. Gaugler [ed.], Entomopathogenic Nematology, CABI, New York, NY.

Sikorowski, P. P. 1985. Pecan weevil pathology, pp. 87–101. *In* W. W. Neel (ed.), Pecan weevil: research perspective. Quail Ridge Press, Brandon, MS.

Smith, M. T., R. Georgis, A. P. Nyczepir, and R. W. Miller. 1993. Biological control of the pecan weevil, *Curculio caryae* (Coleoptera: Curculionidae), with entomopathogenic nematodes. J. Nematol. 25: 78–82.

Studdert, J. P., H. K. Kaya, and J. M. Dunaway. 1990. Effect of water potential, temperature, and clay-coating on *Beauveria bassiana* conidia in a loam and peat soil. J. Invertebr. Pathol. 55: 417–427.

Tanada Y., and H. K. Kaya. 1993. Insect pathology. Academic Press, San Diego, CA.

Tedders, W. L., D. J. Weaver, and E. J. Wehunt. 1973. Pecan weevil: suppression of larvae with the fungi *Metarhizium anisopliae* and *Beauveria bassiana* and the nematode *Neoaplectana dutkyi.* J. Econ. Entomol. 66: 723–725.

Zimmermann, G. 1986. The '*Galleria* bait method' for detection of entomopathogenic fungi in soil. J. Appl. Entomol. 102: 213–215.

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