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

DAVID I. SHAPIRO-ILAN, 1 WAYNE A. GARDNER, 2 JAMES R. FUXA, 3 BRUCE W. WOOD, 1 KHUONG B. NGUYEN, 4 BYRON J. ADAMS, 4 RICHARD A. HUMBER, 5 AND MICHAEL J. HALL 6

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-
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 steinerne- matids and heterorhabditids, respectively) (Kaya and Gaugler 1993). Infective juveniles (IJs), enter hosts through 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. anispolia) 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 entomopatho- genic 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 (Scap- teriscus 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
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 cm² (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 cm² (100,000 per cup) but, because of low mortality, 15,900 conidia per cm² (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
was significant (α = 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.**

### Table 2. Soil parameters for pecan orchards surveyed for entomopathogens

<table>
<thead>
<tr>
<th>Location</th>
<th>Orchard</th>
<th>Sand/Silt/Clay</th>
<th>pH</th>
<th>OM</th>
<th>N</th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
<th>Mn</th>
<th>P</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Helena, AR</td>
<td>AR1</td>
<td>38:56:6</td>
<td>6.1</td>
<td>4.3</td>
<td>0.13</td>
<td>1517</td>
<td>318.4</td>
<td>257.4</td>
<td>61.8</td>
<td>105.1</td>
<td>13.0</td>
</tr>
<tr>
<td>West Helena, AR</td>
<td>AR2</td>
<td>38:56:6</td>
<td>5.2</td>
<td>4.6</td>
<td>0.14</td>
<td>2004</td>
<td>394.5</td>
<td>335.3</td>
<td>161.1</td>
<td>115.4</td>
<td>14.9</td>
</tr>
<tr>
<td>Holly Springs, AR</td>
<td>AR3</td>
<td>38:56:6</td>
<td>5.7</td>
<td>4.7</td>
<td>0.13</td>
<td>2372</td>
<td>442.4</td>
<td>286.5</td>
<td>130.3</td>
<td>131.4</td>
<td>24.2</td>
</tr>
<tr>
<td>Jackson, GA</td>
<td>GA1</td>
<td>96:4</td>
<td>6.0</td>
<td>5.0</td>
<td>0.21</td>
<td>2648</td>
<td>348.1</td>
<td>440.4</td>
<td>50.1</td>
<td>154.1</td>
<td>25.6</td>
</tr>
<tr>
<td>Bullard, GA</td>
<td>GA2</td>
<td>na</td>
<td>5.0</td>
<td>3.6</td>
<td>na</td>
<td>1498</td>
<td>179.8</td>
<td>251.7</td>
<td>66.1</td>
<td>81.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Pineview, GA</td>
<td>GA3</td>
<td>na</td>
<td>5.7</td>
<td>3.0</td>
<td>na</td>
<td>2248</td>
<td>209.2</td>
<td>276.5</td>
<td>42.2</td>
<td>120.8</td>
<td>24.7</td>
</tr>
<tr>
<td>Weston, GA</td>
<td>GA4</td>
<td>90:4:6</td>
<td>6.0</td>
<td>1.9</td>
<td>0.06</td>
<td>1641</td>
<td>134.5</td>
<td>100.0</td>
<td>21.0</td>
<td>295.0</td>
<td>94.6</td>
</tr>
<tr>
<td>Barnesville, GA</td>
<td>GA5</td>
<td>95:12:2</td>
<td>6.3</td>
<td>3.6</td>
<td>0.09</td>
<td>2743</td>
<td>215.7</td>
<td>345.3</td>
<td>32.0</td>
<td>95.9</td>
<td>14.4</td>
</tr>
<tr>
<td>Waycross, GA</td>
<td>GA6</td>
<td>96:1:2</td>
<td>6.7</td>
<td>2.9</td>
<td>0.06</td>
<td>1408</td>
<td>140.5</td>
<td>252.4</td>
<td>47.1</td>
<td>184.1</td>
<td>29.6</td>
</tr>
<tr>
<td>Cochran, GA</td>
<td>GA7</td>
<td>96:2:2</td>
<td>5.3</td>
<td>3.0</td>
<td>0.11</td>
<td>2292</td>
<td>232.2</td>
<td>188.8</td>
<td>45.8</td>
<td>102.8</td>
<td>25.2</td>
</tr>
<tr>
<td>Byron, GA</td>
<td>GA8</td>
<td>80:14:6</td>
<td>6.1</td>
<td>3.6</td>
<td>0.08</td>
<td>2020</td>
<td>332.6</td>
<td>170.1</td>
<td>46.5</td>
<td>222.5</td>
<td>30.7</td>
</tr>
<tr>
<td>Benton, LA</td>
<td>LA1</td>
<td>24:72:4</td>
<td>6.9</td>
<td>5.2</td>
<td>0.17</td>
<td>4062</td>
<td>692.3</td>
<td>1151</td>
<td>37.0</td>
<td>360.0</td>
<td>6.2</td>
</tr>
<tr>
<td>Shreveport, LA</td>
<td>LA2</td>
<td>36:54:10</td>
<td>8.1</td>
<td>4.0</td>
<td>0.09</td>
<td>2646</td>
<td>304.2</td>
<td>840.0</td>
<td>57.9</td>
<td>170.4</td>
<td>41.2</td>
</tr>
<tr>
<td>Dixie, LA</td>
<td>LA3</td>
<td>24:76:10</td>
<td>5.2</td>
<td>3.8</td>
<td>0.1</td>
<td>8314</td>
<td>253.3</td>
<td>1187</td>
<td>10.4</td>
<td>163.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Robson, LA</td>
<td>LA4</td>
<td>34:62:4</td>
<td>5.9</td>
<td>3.7</td>
<td>0.08</td>
<td>2757</td>
<td>302.6</td>
<td>951.3</td>
<td>47.1</td>
<td>184.1</td>
<td>29.6</td>
</tr>
<tr>
<td>Cloutierville, LA</td>
<td>LA5</td>
<td>62:32:6</td>
<td>7.5</td>
<td>1.4</td>
<td>0.04</td>
<td>1147</td>
<td>209.9</td>
<td>254.2</td>
<td>43.3</td>
<td>120.3</td>
<td>25.3</td>
</tr>
<tr>
<td>Benton LA</td>
<td>LA6</td>
<td>22:78:0</td>
<td>8.0</td>
<td>4.0</td>
<td>0.1</td>
<td>9163</td>
<td>315.7</td>
<td>934.1</td>
<td>27.6</td>
<td>195.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Rena Lara, MS</td>
<td>MS1</td>
<td>54:8:38</td>
<td>4.7</td>
<td>6.4</td>
<td>0.12</td>
<td>3325</td>
<td>500.7</td>
<td>764.6</td>
<td>69.3</td>
<td>46.4</td>
<td>5.8</td>
</tr>
<tr>
<td>Rena Lara, MS</td>
<td>MS2</td>
<td>52:25:22</td>
<td>5.0</td>
<td>5.6</td>
<td>0.15</td>
<td>2628</td>
<td>473.2</td>
<td>469.2</td>
<td>74.6</td>
<td>87.6</td>
<td>9.6</td>
</tr>
<tr>
<td>Rena Lara, MS</td>
<td>MS3</td>
<td>32:38:30</td>
<td>5.0</td>
<td>6.5</td>
<td>0.15</td>
<td>3868</td>
<td>676.1</td>
<td>862.4</td>
<td>44.3</td>
<td>81.8</td>
<td>7.3</td>
</tr>
<tr>
<td>Clarksdale, MS</td>
<td>MS4 (2)</td>
<td>46:28:26</td>
<td>4.3</td>
<td>4.9</td>
<td>0.13</td>
<td>2194</td>
<td>305.3</td>
<td>438.3</td>
<td>37.2</td>
<td>33.5</td>
<td>4.2</td>
</tr>
</tbody>
</table>

* 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.
BBAR1 caused greater *C. caryae* mortality relative to the control, and BBGA2 caused greater mortality than all other isolates \((F = 11.89; \text{df} = 7,154; P = 0.0001)\) (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 \((F = 13.82; \text{df} = 9,294; P = 0.0001)\) (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 \((F = 51.98; \text{df} = 7,280; P = 0.0001)\) (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 \((F = 83.88; \text{df} = 6,156; P = 0.0001)\) (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. 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 \(\sim 39\) infective juveniles per cm\(^2\).

**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 \(\sim 7,950\) 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 cm²) was comparable to recommended field rates of 25–75 IJs per cm² (Georgis and Hague 1991, Georgis et al. 1995). Although it is difficult to predict field performance...
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) *Sphenocephorus* 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, Cotrell, 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.

![Figure 5](image1.png)

**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 (α = 0.05). See Table 1 and text for source of isolates. Rate of fungus application was ~15,900 conidia per cm².

![Figure 6](image2.png)

**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 (α = 0.05). See Table 1 and text for source of isolates. Rate of fungus application was ~15,900 conidia per cm².
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 cm²) is 10-fold less than recommended field rates (e.g., 2–8 × 10⁷ per cm²). 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.

Acknowledgments

We thank L. Buchanan, W. Evans, K. Halat, S. James, H. Peeler, and A. Richter, for technical assistance; Certis USA, Emerland BioAgriculture, and Randy Gaugler for providing pathogens; and J. Davis for statistical consultation. We are grateful to S. Burt, J. Curry, R. Dearing, R. Elston, D. Glidewell, G. Harrison, T. Jenkins, S. Manley, G. Maeurer, R. & J. Merrit, S. Richardson, D. & C. Sonnier, V. Stewart, D. Strickland, and B. Trunell for use of their orchards. This work was supported in part by the Georgia Agriculture Commodity Commission for Pecans.

References Cited


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.


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: 75–82.


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 Neoplectana dutkyi. J. Econ. Entomol. 66: 723–725.


Received for publication 3 January 2002; accepted 30 August 2002.

February 2003 SHAPIRO-ILAN ET AL.: ENTOMOPATHOGEN SURVEY IN PECAN ORCHARDS 195