Pathogens Associated with Southwestern Corn Borers and Southern Corn Stalk Borers (Lepidoptera: Crambidae)

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ABSTRACT A study was undertaken to isolate entomopathogens of southwestern corn borer, *Diatraea grandiosella* Dyer, and southern corn stalk borer, *Diatraea crambidoides* (Grote). Field-collected diapausing larvae of southwestern corn borer (three sites in Mississippi) and southern corn stalk borer (one site in North Carolina), and a laboratory strain of *D. grandiosella* in the diapause state were maintained in a simulated winter followed by a simulated spring environment. Few larvae (≤6%) collected from any of the field sites died in the winter environment, and most insect mortality (11–25%) occurred after transfer of the larvae to the simulated spring environment. Mortality during the simulated spring period differed among the collection sites, and the highest mortality was recorded for southwestern corn borers from Washington County (25%), followed by Marshall (16%) and Oktibbeha (11%) Counties. A high level of mortality was also observed in southern corn stalk borers during the simulated spring period (27%). No viruses were observed, but a number of bacteria, microsporidia, and fungi were isolated from both southwestern corn borer and southern corn stalk borer larvae and pupae. In most instances, numerous bacterial taxa were isolated from cadavers, but on some occasions a single taxon predominated. The most prevalent bacterial taxon from larval and pupal cadavers was *Enterococcus faecalis* (Andrewes & Horder) Schleifer & Kilpper-Balz, but *Bacillus* spp., *Pseudomonas aeruginosa* (Schroeter) Migula, and *Serratia marcescens* Bizio were frequently isolated as well. Few fungi (1–7%) were recovered from southwestern corn borer and southern corn stalk borer larvae and pupae. The most common entomopathogenic taxon isolated was *Beauveria bassiana* (Balsamo) Vuillemin from southern corn stalk borer larvae. Microsporidia were not isolated from southern corn stalk borers. However, *Nosema* spp. were isolated from southwestern corn borer cadavers from Washington (15%), Marshall (1%), and Oktibbeha (3%) Counties in Mississippi. In addition, we observed parasitism of southern corn stalk borer larvae by *Macrocentrus cingulum* Reinhard (Hymenoptera: Braconidae). No parasitism of southwestern corn borers was observed. Isolates of *Bacillus*, *Beauveria*, *Entercoccus*, *Nosema*, *Pseudomonas* and *Serratia* were all pathogenic to southwestern corn borer larvae under controlled environmental conditions, and with the exception of *B. bassiana*, these are novel pathogens of *Diatraea* corn borers.

KEY WORDS *Diatraea grandiosella*, *Diatraea crambidoides*, disease, entomopathogens

THE SOUTHWESTERN CORN borer, *Diatraea grandiosella* Dyer, is a nonindigenous pest of corn, *Zea mays* L., in the United States, and is currently established in the southern corn belt region (as far north as Kansas, Missouri, and Illinois). *D. grandiosella* larvae and pupae are virtually indistinguishable from those of the southern corn stalk borer, *Diatraea crambidoides* (Grote), and although the distribution of the two species overlap, southern corn stalk borers are primarily limited to the southeast coastal region (Maryland to northern Florida) of the United States (Steffey et al. 1999). Under the appropriate conditions, southwestern corn borers and southern corn stalk borers can cause substantial yield losses in corn (Ainslie and Phillips 1954, Davis and Williams 1994), and management of these pests primarily relies on the implementation of cultural control measures (e.g., early planting and fall cultivation) and on the use of well-timed insecticide applications. In southwestern corn borers, prediapause larvae migrate to the base of the stalk of the corn plant below the soil surface to overwinter. Many of these larvae girdle the stalk just above the soil level while preparing a chamber for overwintering (Chippendale 1979). Southern corn stalk borer larvae also overwinter in tap roots (Ainslie and Phillips 1954) but they do not girdle the stalks as do southwestern corn borers. Substantial mortality to both species of corn borer larvae can occur during the overwintering period, and population decreases as high as 90% have been recorded (Leiby 1920, Davis et al. 1973, Chippendale and Reddy 1974, Langille 1975). Entomopathogens are thought to contribute to the mortality of southwestern corn borers, and both fungi and bacteria have been implicated in the death of overwintering of larvae (Davis et al. 1933, Langille 1975). A number of microorganisms, including *Bacillus* sp., *Beauveria bassiana* (Balsamo) Vuillemin, *Hirsutella* sp., *Isaria* sp. (this genus contained a diverse assemblage of species that have primarily been transferred to other genera including, *Beauveria*, *Metarhizium*, *Nomuraea*, and *Paecilomyces*), *Metarhizium aniso-*

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pliae (Metschnikoff) Sorokin, have been isolated from southwestern corn borer or southern corn stalk borer larvae (Leiby 1920, Langille 1975, Knutson and Gilstrap 1990a), but limited information is available on naturally occurring entomopathogens of these pests. The three objectives of the current study were to (1) measure mortality of field-collected southwestern corn borer and southern corn stalk borer larvae in a simulated winter environment; (2) subsequently measure mortality of larvae and pupae in a simulated spring environment; and (3) isolate and characterize entomopathogens responsible for the death of larvae and pupae. An understanding of the pathogens responsible for overwintering and spring mortality of southwestern corn borer and southern corn stalk borer larvae and pupae may eventually lead to the utilization of entomopathogens in integrated pest management strategies for *Diatraea* spp. in corn.

Materials and Methods

Field Collection of Larvae. Diapausing southwestern corn borer larvae (fifth to sixth instar) were collected from the base of the stem of late-seeded (mid- to late May) corn at various sites in Mississippi during October of 1998; corn was purposely seeded late to increase the probability of infestation by overwintering corn borers. The three collection sites were Marshall, Oktibbeha, and Washington Counties. The Marshall collection site was located in north central Mississippi near the community of Holly Springs (34.8218° N and 89.5627° W), the Oktibbeha collection site was located in the hill country of the northeastern part of the state near the town of Starkville (33.4750° N and 88.7800° W), and the Washington collection site was located in the Mississippi River Delta region of west Mississippi near Leland (33.4494° N and 90.9176° W). At the Oktibbeha collection site, southwestern corn borer larvae reared in the laboratory (Davis 1989) were released during July 1998 as a source of insects for plant-resistance studies. Therefore, insects collected from this site may have co-originated from both wild and laboratory-reared southwestern corn borers. Larvae were transported to the Corn Host Plant Resistance Research Unit, USDA–ARS (Mississippi State University). Southern corn stalk borer larvae were collected from corn stalks on 28 September 1998 in Washington County near the town of Roper, NC (35.8300° N and 77.3780° W), and were sent to Mississippi State, MS.

Maintenance of Corn Borers. Field-collected larvae were placed individually in 30-ml polystyrene cups capped with a polysaran-coated paperboard lid. A group of healthy diapausing larvae from a laboratory colony of southwestern corn borers was also included as a comparative treatment; these larvae did not exhibit any signs or symptoms of disease at the commencement of the experiment. All larvae were maintained at 21°C and a photoperiod of 12:12 (L:D) h for 7–10 d, and cadavers were collected daily during this period. Larvae surviving the prewinter period were individually transferred to clean cups containing 10 ml of water agar (2%) containing neomycin sulfate (0.047%), sorbic acid (0.047%), and methyl paraben (0.142%). They were placed at 10°C under a photoperiod of 12:12 (L:D) h to simulate winter conditions, and cadavers were removed at 7- to 10-d intervals. Commencing 73 d after placement of southwestern corn borers at 10°C (4 January 1999), one-third of the larvae were transferred to a simulated spring environment (27°C with a photoperiod of 16:8 [L:D] h). At 7- to 10-d intervals, the remainder of the larvae were transferred to the spring environment in one-third allotments. Southern corn stalk borer larvae were transferred to the simulated spring environment in two allotments at a 10-d interval beginning 89 d after placement at 10°C (4 January 1999). Larvae placed at 27°C were examined on a regular basis for mortality, completion of pupation, and pupal eclosion. The total numbers of southwestern corn borer larvae included in the experiment were 635, 400, 526, and 629 for Marshall County, Oktibbeha County, Washington County, and the Laboratory colony, respectively. Four hundred and ninety-eight southern corn stalk borer larvae were used. At the end of the experimental period (181–188 d), noneclosed pupae were classified as moribund and were treated as dead pupae.

Pathology and Microbiology. Using a stereomicroscope, signs of disease were recorded for all cadavers. For cadavers exhibiting external fungal growth, hyphae were aseptically transferred to Sabouraud's dextrose agar containing 100 μg ml^{-1} each of tetracycline and chloramphenicol and cultures were maintained at 25°C for ≈3–5 d. After isolations of fungi, all cadavers were individually homogenized in sterile water using a micropestle. A drop of the homogenate was placed on a microscope slide, air-dried, stained with Buffalo Black, and examined microscopically to visualize viral occlusion bodies, bacterial, fungal and microsporidian cells. In addition, the homogenates (≈20–30 μl) were individually spread on trypticase soy agar, cultures were incubated at 30°C for 24–48 h, and unique colonies (i.e., based on colony appearance) were subcultured on trypticase soy agar and purity confirmed by streaking. In instances where no microorganisms were observed microscopically and none were isolated on trypticase soy agar, representative homogenates were processed for transmission electron microscopy (i.e., for nonoccluded viruses and rickettsiae) using negative staining (2% phosphotungstic acid) and examined at ≈27,000× magnification at 60 kV. Representative isolates of microsporidian spores from the various locations were fixed in Karnovsky's (0.5× concentration), dehydrated in ethanol, embedded in Spurr's resin, ultrathin-sectioned, and examined with the transmission electron microscope at 60 kV. Spores were fixed also (2% glutaraldehyde), dehydrated in ethanol, dried in liquid CO_2, and examined with a Leo S360 scanning electron microscope at an accelerating voltage of 15 kV.

Filamentous fungi were identified on the basis of conidiogenesis. The Gram stain, catalase and oxidase reactions of all bacteria were determined after 24-h growth on TSA, and representative isolates were further characterized using the Microscan identification

system (Baxter Travenol, Deerfield, IL). Microsporidia were characterized to genus based on spore shape, cell wall sculpture, spore ultrastructure, and polar filament morphology (Poinar and Thomas 1984). Purified bacterial and fungal isolates were stored on trypticase soy agar or Sabouraud's dextrose agar in slant culture at 5°C until identified. Microsporidian spores were stored at −20°C.

Koch's Postulates. To confirm pathogenicity, Koch's postulates were applied such that (1) the microorganism was isolated from dead or moribund southwestern corn borers and southern corn stalk borers, and the signs of disease were recorded; (2) the isolated microorganism was purified and characterized; (3) the isolated microorganism was then inoculated on/in healthy southwestern corn borer insects, and signs and symptoms of disease were determined to be the same as in step 1; and (4) the microorganism was re-isolated in axenic culture and its characteristics determined to be the same as in step 2.

To prepare bacterial inocula, cultures were grown on trypticase soy agar for 18–24 h at 30°C, the cultures were flooded with sterile water and a suspension of bacterial cells was created by agitation. For the microsporidia, spore suspensions were washed three times with sterile water by centrifugation and the spores were resuspended in a solution containing 0.04% neomycin sulfate. Fungal inoculum was prepared by aseptically removing conidia from the actively growing colony margins, and suspending the conidia in water using micropestles. Bacteria were tested immediately after preparation of inoclum whereas, fungal and microsporidian inocula were maintained at 5°C overnight.

Larvae (7–9 d old) from a laboratory colony of southwestern corn borers were used in all pathogenicity tests. The colony was maintained on a laboratory diet (Davis 1989) containing sorbic acid (0.047%), methyl paraben (0.142%), and neomycin sulfate (0.047%); the colony undergoes an infusion of wild genes each year and the ensuing offspring are monitored to ensure they are healthly (e.g., developmental rate, size, adult emergence, fecundity) before they are introduced into the main colony (Davis 1989). For each microbial isolate to be tested, a minimum of 30 southwestern corn borer larvae were inoculated. For bacteria and microsporidia, diet squares ($\approx$1 cm^2 for bacteria and $\approx$0.5 cm^2 for microsporidia) were aseptically removed with a scalpel and were placed in vials containing a cell suspension of the test isolate, and gently swirled to ensure adequate coverage of diet surfaces. An individual diet piece was then placed in a clean polystyrene rearing cup containing a moistened paper towel per isolate to which a larva was added. Cups were covered with a paper lid and larvae were placed at 24°C under a photoperiod of 14:10 (L:D) h. After ingestion of the inoculum, larvae were transferred to a new cup containing fresh diet and placed at 24°C. For fungi, larvae were individually dipped in 4 ml of each conidial suspension and subsequently transferred to a polystyrene cup containing diet. For each bioassay, a control treatment was included in which larvae were inoculated with water. Twenty-six isolates were tested and included: *Aspergillus* sp. (58 and 59 from Washington Country, NC); *Bacillus* sp. (156 and 392 from Washington County, MS); *B. bassiana* (1, 3, 4, 5, 6, 41, and 42 from Washington County, NC); *Enterococcus faecalis* (Andrewes & Horder) Schleifer & Kilpper-Balz (482 from Marshall County; 500 from Oktibbeha County; and 515 from Washington County, MS); *Nosema* spp. (167 from Marshall County; 295 and 504 from Oktibbeha County; and 181, 513 and 522 from Washington County, MS); *Pseudomonas aeruginosa* (Schroeter) Migula (258 from Marshall County; 183 from Oktibbeha County; and 162 from Washington County, MS); and *Serratia marcescens* Bizio (126 from Marshall County; 230 from Oktibbeha County; and 98 from Washington County, MS).

Mortality of larvae and pupae was monitored daily, and signs of disease were recorded. Dead larvae or pupae originally inoculated with bacteria or microsporidia were homogenized in sterile water and the homogenates examined microscopically as previously described. For dead individuals initially inoculated with bacteria, the homogenate was also spread on trypticase soy agar, cultures incubated at 30°C, and representative colonies were streaked for purity, their Gram stain reaction determined, and they were further characterized using the Microscan identification method. In surviving individuals, the meconia from newly emerged adults was homogenized, and the homogenate streaked on trypticase soy agar or examined microscopically. After death of larvae and pupae inoculated with fungi, cadavers were left on the diet until external hyphae could be transferred to Sabouraud's dextrose agar.

Results

Field Conditions. All corn plants had completed development (i.e., reached physiological maturity) and were senescent at the time of insect collection. At the Washington County site, the 1998 growing season was hot and dry, and corn plants were conspicuously dry on the day of collection. The density of southwestern corn borers (second and third generations) was very high, and evidence of reduced food quality and competition among southwestern corn borers for food and space was observed (F.M.D. unpublished data). The corn at the Marshall County site was more normal than at Washington County, and while considerable numbers of southwestern corn borers were observed, the density of larvae was less than at Washington County. The growing conditions in Oktibbeha County were also hot and dry during 1998, and populations of southwestern corn borers were similar to that observed at Marshall County.

Mortality of Larvae. For southwestern corn borers, overall mortality was highest for larvae from Washington County (33%) followed by Marshall County (22%), Oktibbeha County (15%), and the laboratory colony (7%) (Table 1). Total mortality for southern corn stalk borers from North Carolina was 31%. During

Table 1. Total mortality and stage of development at which southwestern corn and southern corn stalk borers died or became moribund

Groups	% mortality (no. of insects)				
	Southwestern corn borers[a]				Southern corn stalk borers[b]
	MC	OC	WC	LAB	NC
Larvae	14.1 (90)	10.0 (40)	18.1 (95)	1.0 (6)	19.7 (98)
Pupation[c]	2.7 (17)	2.8 (11)	4.2 (22)	1.1 (7)	3.0 (15)
Pupae	4.9 (31)	2.5 (10)	10.3 (54)	4.9 (31)	8.0 (40)
Total mortality	21.7 (138)	15.3 (61)	32.5 (171)	7.0 (44)	30.7 (153)
Total individuals	(635)	(400)	(526)	(629)	(498)

Pupae that did not eclose were classified as moribund.

[a] Field-collected southwestern corn borer (*D. grandiosella*) larvae collected from Marshall County (MC), Oktibbeha County (OC), and Washington County (WC) in Mississippi. Larvae from a laboratory-reared colony of southwestern corn borer larvae (LAB) were also included.

[b] Southern corn stalk borer (*D. crambidoides*) larvae collected from Washington County, North Carolina (NC).

[c] Larvae that died during pupation (e.g., incomplete pupal cuticle formation).

the prewinter period, mortality was similar (2–4%) for southwestern corn borers and southern corn stalk borers from North Carolina (Fig. 1). Relatively few larvae of either species died during the simulated winter conditions. Mortality during this period ranged from 4 to 6% for southwestern corn borers from Marshall and Washington Counties, and 1 to 2% for southwestern corn borers from Oktibbeha County and southern corn stalk borers from North Carolina. No mortality occurred in the southwestern corn borers from the laboratory colony during the simulated winter. Regardless of the location, most mortality in southwestern corn borers and southern corn stalk borers occurred during the simulated spring period. The highest mortality during this period was recorded for southwestern corn borers from Washington County (25%), followed by Marshall County (16%), Oktibbeha County (11%), and the laboratory colony of southwestern corn borers (7%). A high level of mortality was also observed in southern corn stalk borers during the simulated spring period (27%). Many of the field-collected southwestern corn borers died as larvae (10–18%), but substantial losses also occurred during pupation (3–4%) and as pupae (3–10%). Both larval and pupal mortality were appreciably higher for insects collected at the Washington County site relative to the other two field sites (Table 1). Relative to the field-collected insects, very few southwestern corn borers from the laboratory colony died as larvae or during pupation (≈1%). However, pupal mortality was similar to that observed in pupae from the Marshall and Oktibbeha County sites. Considerable numbers of southern corn stalk borers died as larvae (20%), during pupation (3%), and as pupae (8%).

Isolation of Microorganisms. Bacteria were isolated from 63 to 78% of moribund or dead southwestern corn borers from the three field environments, and from 54% of moribund or dead southern corn stalk borers (Table 2). In contrast, only 18% of the cadavers from the laboratory colony was extensively colonized by bacteria at the time of processing. In most instances numerous bacterial taxa were isolated from cadavers, but a single taxon predominated in a few instances. The most frequently isolated bacterial taxon was *E. faecalis* (Table 2), but *Bacillus* spp., *P. aeruginosa*, and *S. marcescens* were also isolated from cadavers for all five treatments.

Few fungi were isolated from southwestern corn borer and southern corn stalk borer larvae and pupae. Fungi were isolated from 1 to 8% of the cadavers, and they were primarily isolated from individuals that died within the first 7–10 d after placement of larvae in polystyrene cups in the prewinter period. The most common entomopathogenic fungus isolated was *B. bassiana* from southern corn stalk borer larvae.

Nosema spp. were not isolated from the southwestern corn borer laboratory colony nor from southern corn stalk borers. A low frequency of infection was observed in southwestern corn borers from Marshall County (1%) and Oktibbeha County (3%) whereas, microsporidia were recovered from 15% of the larval and pupal cadavers from Washington County. Spores of the *Nosema* strains isolated were not enclosed in a cyst wall, they were smooth-walled, broadly ellipsoidal to ellipsoidal and relatively uniform in size (2.9–3.5 by 1.6–2.5 μm). We were unable to induce polar filament extrusion using a combination of mechanical pressure, incubation in hydrogen peroxide or potassium hydroxide. Examination of spore thin sections indicated the presence of polaroplasts and a well-developed polar filament consisting of a minimum of 11 coils.

We did not observe viral occlusion bodies in any of the collected cadavers. Furthermore, virions were not detected in negative stain preparations of the homogenates from the 77 cadavers examined with the TEM (23 insects from Washington County in North Carolina, 12 insects from the laboratory colony, and 21, 7, and 14 and insects from Marshall, Oktibbeha, and Washington Counties, respectively).

No insect parasitoids were observed in any of the 1,561 field-collected southwestern corn borers. In contrast, nine southern corn stalk borer larvae (2%) were parasitized. In all instances, the parasitoid was identified as the hymenopteran, *Macrocentrus cingulum* Reinhard; this parasitoid was previously known as *Macrocentrus grandii* Goidanich, and it is distinguished from other *Macrocentrus* spp. by its minute trochantellar teeth (van Achterberg and Haeselbarth 1983).

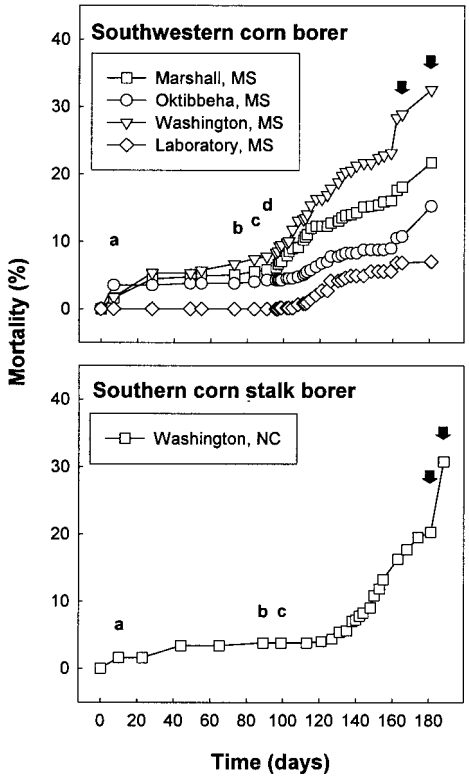

Fig. 1. Cumulative mortality curves for southwestern corn borer (*D. grandiosella*) and southern corn stalk borer (*D. crambidoides*) larvae and pupae. Southwestern corn borers were collected from three locations in Mississippi (Marshall, Oktibbeha, and Washington Counties) and placed at 21°C for 7–10 d, at which point they were transferred to 10°C (simulated winter) (a). Commencing 73 d after placement at 10°C, equal numbers of southwestern corn borer larvae were moved at 7- to 10-d intervals (three times) to 27°C (simulated spring) (b–d). In addition to the field-collected insects, larvae from a laboratory-reared colony of southwestern corn borer larvae were included for comparison. Southern corn stalk borer larvae were collected from Washington County, NC. Larvae were maintained at 21°C for 10 d and then transferred to the simulated winter environment (a). Beginning 89 d after placement at 10°C, larvae were transferred to the simulated spring environment in two allotments at a 10-d interval (b–c). Southwestern corn borer and southern corn stalk borer pupae that did not eclose (during the period between arrows) were classified as moribund and were also processed for entomopathogens and parasites.

Pathogenicity. Eight hundred and seventy-seven larvae were used to confirm the pathogenicity of microorganisms toward southwestern corn borers. Total control mortality was very low (1%). Of the eight isolates of *B. bassiana* tested, total mortality ranged from 10 to 21%. Most of the individuals died as larvae (≈90%), and all but two larvae and one pupa exhibited the characteristic evidence of mycosis by *B. bassiana.* Neither of the two isolates of *Aspergillus* tested were pathogenic.

Mortality by the two *Bacillus* isolates tested ranged from 13 to 50%, and the bacterium was recovered in large numbers from 28 to 100% of the larval and pupal cadavers, and from 15 to 20% of surviving adults. Three isolates of *E. faecalis* were tested, and total mortality ranged from 18 to 22%. Approximately equal numbers of individuals inoculated with *E. faecalis* died as larvae (4–11%) and pupae (10–14%). The bacterium was recovered from three of seven larval cadavers but was not isolated from any of the 10 pupal cadavers. In addition, *E. faecalis* was recovered from 19–30% of the surviving adults. None of the larvae treated with either *Bacillus* spp. or *E. faecalis* exhibited consistent signs of infection. Of the individuals inoculated with *P. aeruginosa*, 41–68% died as larvae, 0–19% died as pupae, and the bacterium was recovered from 73–100% of the cadavers. Similarly to the original cadavers, insects inoculated with *P. aeruginosa* were smaller in size, dark in appearance, and lost tissue integrity upon death. Some larvae ($n = 8$) inoculated with *P. aeruginosa* did not pupate, and the bacterium was isolated from 50% of these larvae. A low prevalence (7–17%) of larvae inoculated with *S. marcescens* died. The bacterium was recovered from all of the larval cadavers and all but one of the pupal cadavers, and 22–59% of the surviving adults was positive for *S. marcescens.* None of the cadavers inoculated with *S. marcescens* exhibited consistent signs of infection. In no instance, were the test bacteria isolated from control larvae.

All six isolates of *Nosema* were pathogenic to southwestern corn borer larvae. After inoculation, total mortality ranged from 0 to 55% in larvae and from 7 to 29% in pupae. Large numbers of *Nosema* spores were observed in the homogenates of 28% the larval and 88% of the pupal cadavers. A number of larvae did not pupate during the experimental period, and spores were observed in the homogenates of 90% of these larvae. In addition, 70 of the 129 surviving adults were positive for *Nosema* spores, but no spores were observed in control adults. No similarity in external signs of disease were observed in larvae or pupae infected with *Nosema.*

Discussion

Substantial overwintering mortality occurs in southwestern corn borer and southern corn stalk borer larvae in field environments, and several researchers have speculated that pathogens incite disease in diapausing larvae. *M. anisopliae* was associated with dead overwintering southern corn stalk borer larvae in North Carolina (Leiby 1920), and 3–15% of diapausing southwestern corn borer larvae were infected by *B. bassiana* in Texas from November to March (Knutson and Gilstrap 1990a). Rather than monitor mortality in the field, we collected larvae in the fall and placed them in a simulated winter environment. Using this strategy, we observed minimal overwintering mortality (≤6%) in larvae of both corn borer species. Cold temperatures are important determinants of larval survival (Chippendale and Reddy 1974, Knutson and Gilstrap 1990b) and southwestern corn borer larvae do not survive if mean temperatures fall below −7°C for ≈30 d (Chippendale and Reddy 1974). Cold stress has been shown to increase the sensitivity of some insects to infection. For example, *Nosema pyrausta* (Paillot)

Table 2. Microbial taxa and insect parasites recovered from dead or moribund southwestern corn and southern corn stalk borer larvae and pupae

Pathogen/parasite	% larvae (no. of individuals)				
	Southwestern corn borer[a]				Southern corn stalk borer[b]
	MC	OC	WC	LAB	NC
Fungi	0.7 (1)	8.2 (5)	0.6 (1)	2.3 (1)	7.2 (11)
Aspergillus sp.	0.7 (1)	0	0	0	1.3 (2)
Beauveria bassiana	0	0	0	0	5.2 (8)
Fusarium spp.	0	4.9 (3)	0	0	0.7 (1)
Penicillium sp.	0	1.6 (1)	0	0	0
Rhizopus sp.	0	1.6 (1)	0	0	0
Unidentified	0	0	0.6 (1)	2.3 (1)	0
Bacteria	63.0 (87)	60.7 (37)	77.8 (133)	18.2 (8)	54.2 (83)
Bacillus spp.	5.8 (8)	1.6 (1)	7.0 (12)	4.5 (2)	9.2 (14)
Clostridium sp.	0	1.6 (1)	0	0	0
Enterobacter sp.	0	0	0.6 (1)	0	0
Enterococcus faecalis	21.0 (29)	14.8 (9)	38.6 (66)	2.3 (1)	26.1 (40)
Pseudomonas aeruginosa	4.3 (6)	6.5 (4)	8.2 (14)	0	2.0 (3)
Serratia marcescens	5.8 (8)	3.3 (2)	7.0 (12)	2.3 (1)	12.4 (19)
Unidentified Gram +	2.9 (4)	0	2.3 (4)	0	0
Unidentified Gram −	23.2 (32)	32.8 (20)	14.0 (24)	9.1 (4)	4.6 (7)
Protozoa					
Nosema spp.	0.7 (1)	3.3 (2)	15.2 (26)	0.0	0.0
Parasite					
Macrocentrus cingulum	0.0	0.0	0.0	0.0	5.9 (9)
Total individuals	(138)	(61)	(171)	(44)	(153)

[a] Field-collected southwestern corn borer (*D. grandiosella*) larvae collected from Marshall County (MC), Oktibbeha County (OC), and Washington County (WC) in Mississippi. Larvae from a laboratory-reared colony of southwestern corn borer larvae (LAB) were also included.
[b] Southern corn stalk borer (*D. crambidoides*) larvae collected from Washington County, North Carolina (NC).

incited more mortality in European corn borer, *Ostrinia nubilalis* (Hübner), larvae that were exposed to low temperatures (Siegel et al. 1986). Our simulated winter environment was relatively mild (constant 10°C), and it is probable that low and fluctuating temperatures will predispose overwintering southwestern corn borer and southern corn stalk borer larvae to infection. The interaction between temperature and the susceptibility of *Diatraea* spp. to entomopathogens is unknown.

In early spring, larvae of both species break diapause and become active for a period before pupation (i.e., line tunnels with silk and prepare an exit hole for the moth) (Davis et al. 1933; Ainslie and Phillips 1954). Limited research has focused on mortality of larvae and pupae after diapause, and we observed that the highest levels of mortality occurred during the simulated spring period for both corn borer species. Furthermore, appreciable differences existed in mortality of individuals collected from the different field sites during this period. The highest mortality was for southwestern corn borers from Washington County, followed by Marshall and Oktibbeha Counties. Reasons for the differential mortality among the collection sites are unknown. The rearing facility in which larvae were maintained after field collection uses filtered air (Davis 1989), and we took care to prevent cross-contamination between sites and from the laboratory. Furthermore, very low mortality was observed in the laboratory colony of southwestern corn borers, supporting the supposition that the pathogens infecting field-collected southwestern corn borers and southern corn stalk borers originated in the field and not the laboratory. Precise reasons for the higher overall mortality of southwestern corn borers from Washington County (33%) than the other two field sites (15–22%) are unknown, but a number of factors may have contributed. Corn plants at this site were conspicuously dry, the density of larvae was very high, and there was evidence of reduced food quality (i.e., the hot dry conditions resulted in premature drying of plants) and competition among larvae for food and space. It has long been recognized that "stressed" insects are more susceptible to entomopathogens and less likely to survive infection (e.g., Steinhaus 1958, 1959). Although environment conditions and larval density may predispose insects to infection, the relative importance of the direct (e.g., transmission) and indirect (e.g., increased susceptibility caused by reduced vigor) factors on the severity of diseases warrants additional study.

A number of entomopathogenic fungi, including *B. bassiana*, have been isolated from southwestern corn borer or southern corn stalk borer larvae during the summer and fall (Langille 1975, Knutson and Gilstrap 1990a). We did not observe *B. bassiana* infection in any of the field-collected southwestern corn borer larvae, but 2% of the southern corn stalk borer larvae were colonized by the fungus. Six of the eight larvae colonized by *B. bassiana* died before placement in the simulated winter conditions, and the remaining two larvae died relatively early into the simulated winter period. This is consistent with previous observations of *B. bassiana* inciting mortality in southwestern corn borer and southern corn stalk borer larvae in the fall and early winter. Although we did not isolate *B. bassiana* from southwestern corn borers in the current study, we have observed mycosis of diapausing larvae in previous years (F.M.D., unpublished data). Reasons for the low levels of *B. bassiana* infection are unknown,

but Knutson and Gilstrap (1990a) observed considerable mycosis of southwestern corn borers by *B. bassiana* in two growing seasons but not in a third. Elucidation of the factors controlling the periodicity of *B. bassiana* epizootics are needed.

The most prevalent bacterium recovered from southwestern corn borer cadavers was *E. faecalis.* Although *Enterococcus* spp. are cosmopolitan and are commonly found in the alimentary canals of insects, *E. faecalis* is a pathogen of a number of insects including bees, silkworm, and tobacco budworm (Yamashita 1992, Miller and Miller 1996). We observed relatively low levels of mortality (≤22%) in southwestern corn borer larvae inoculated with *E. faecalis* consistent with the facultative nature of this bacterium. *P. aeruginosa* and *S. marcescens* were also isolated from cadavers of all five populations, although none of the *S. marcescens* strains produced the red pigment, prodigiosin, that is often associated with entomopathogenic strains (Sikorowski and Lawrence 1999). Both bacteria are commonly encountered facultative pathogens of a variety of insects from both field and artificial rearing settings (e.g., Sri-Arunotal et al. 1975, Banerjee and Dangar 1995). *Bacillus* spp. were recovered from a number of cadavers during the simulated winter and spring periods. Although we did not identify any of the isolates to species, none produced parasporal bodies typical of *B. thuringiensis. Bacillus* spp. were implicated as the cause of mortality (10%) in southwestern corn borer in Missouri (Langille 1975). However, *Bacillus* spp. are cosmopolitan, all are saprotrophic to varying degrees, many taxa are nonpathogenic or facultative pathogens, and they are commonly isolated from living and dead insects. With the exception of *P. aeruginosa*, none of the bacteria tested exhibited consistent signs of infection, a requisite for Koch's Postulates. Nonetheless, the considerably higher mortality in inoculated larvae and the recovery of substantial numbers of inoculated microorganisms from the hemolymph of cadavers implicates these taxa as pathogens of southwestern corn borers.

Microsporidia infect all insect orders but most host species occur in Diptera and Lepidoptera (Brooks 1988). The spores that we recovered were uniform in size and shape, and produced a polar filament that is coiled at least 11 times within the spore. Similarly to most bacterial taxa, consistent signs of infection were not observed in our pathogenicity tests. However, *Nosema* spp. often cause sublethal infections in insects (Brooks 1988), and the large number of spores that we recovered from the homogenates of cadavers inoculated with *Nosema* spp. indicates parasitism. To our knowledge there are few reports of microsporidia infecting *Diatraea.* Alves et al. (1985) observed an unidentified microsporidian in field and laboratory-collected sugarcane borers, *Diatraea saccharalis* (F.), in Brazil, and an unidentified *Nosema* was also recovered from a laboratory colony of sugarcane borers in Texas (L. Solter, personal communication). *N. pyrausta* commonly infects European corn borer, and both southwestern corn borers and European corn borers occur in Mississippi. Furthermore, both of these insect pests were observed to inhabit the same corn plant at Washington County, MS, and the highest frequency (15%) of cadavers with spores were from this site. Many *Nosema* spp., including *N. pyrausta*, possess relatively wide host ranges (Brooks 1988), but it is currently unknown whether *N. pyrausta* infects southwestern corn borers. However, the size and morphology of *N. pyrausta* spores (Andreadis 1980) are conspicuously different from the spores of the *Nosema* spp. that we isolated from southwestern corn borers. Microsporidia are capable of infecting insects via oral, cuticular, and ovarian pathways (Kramer 1976); and *N. pyrausta* is transmitted transovarially in European corn borers (Siegel et al. 1988), but transmission through contact with feces (Andreadis 1987) and ingestion of infected insects and infested substrates is also possible (Solter et al. 1991). Characterization of the microsporidia (e.g., phylogenetics) that we isolated from southwestern corn borers and elucidation of their pathology, including host range, are currently in progress.

Parasites may play a significant role in the decline of corn borer populations. However, we did not observe any parasitism of southwestern corn borer larvae, and this is consistent with previous observations of low levels of parasitism in field environments (F.M.D., unpublished data). Little is known about the influence of predators and parasitoids on southern corn stalk borers, and we observed low-frequency parasitism of larvae by the braconid, *M. cingulum.* In all instances, southern corn stalk borer larvae from which *M. cingulum* were observed died or became moribund at 27°C after the simulated winter period. *M. cingulum* is a nonindigenous parasitoid of European corn borer and *Ostrinia furnacalis* (Guenée) that was introduced into the United States from France and Korea in the late 1920s and early 1930s to control *O. nubilalis* (Baker et al. 1949, Edwards and Hopper 1999). *M. cingulum* has never been reported from *Diatraea* corn borers. However, another species, *M. prolificus,* has been reported from *Diatraea considerata* (Heinrich), *D. grandiosella*, or *D. saccharalis* (Wharton 1984, Overholt and Smith 1990, Rodriguez and Smith 1997).

We observed substantial mortality in field-collected southwestern corn borer and southern corn stalk borer postdiapause larvae and pupae in simulated conditions. However, limited mortality was observed in the simulated winter and most mortality occurred during the spring period. A number of microorganisms were identified as novel pathogens of southwestern corn borers and southern corn stalk borers and included isolates of *Bacillus, Beauveria, Entercoccus, Nosema, Pseudomonas,* and *Serratia.* In addition, *M. cingulum* was observed in southern corn stalk borer larvae and this is the first report of this braconid from *Diatraea.*

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References Cited

van Achterberg, C., and E. Haeselbarth. 1983. Revisionary notes on the European species of *Macrocentrus* Curtis *sensu stricto* (Hymenoptera: Braconidae). Entomofauna 4: 37–60.

Ainslie, G. G., and W. J. Phillips. 1954. The southern corn stalk borer. U.S. Dep. Agric. Leafl. 363.

Alves, S. B., P.S.M. Botelho, N. Macedo, and G. M. Riscado. 1985. The occurrence of some pathogens on sugar cane borer *Diatraea saccharalis* in field and laboratory conditions. Entomol. News 17: 4–5.

Andreadis, T. G. 1980. *Nosema pyrausta* infection in *Macrocentrus grandii*, a braconid parasite of the European corn borer, *Ostrinia nubilalis.* J. Invertebr. Pathol. 35: 229–233.

Andreadis, T. G. 1987. Horizontal transmission of *Nosema pyrausta* (Microsporidia: Nosematidae) in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Environ. Entomol. 16: 1124–1129.

Baker, W. A., W. G. Bradley, and C. A. Clark. 1949. Biological control of the European corn borer in the United States. U.S. Dep. Agric. Tech. Bull. 983.

Banerjee, A., and T. K. Dangar. 1995. *Pseudomonas aeruginosa*, a facultative pathogen of red palm weevil, *Rhynchophorus ferrugineus.* World J. Microbiol. Biotech. 11: 618–620.

Brooks, W. M. 1988. Entomogenous protozoa, pp. 1–149. *In* C. Ignoffo [ed.], CRC handbook of natural pesticides: microbial insecticides, part A: entomogenous protozoa and fungi. CRC, Boca Raton, FL.

Chippendale, G. M. 1979. The southwestern corn borer, *Diatraea grandiosella:* case history of an invading insect. Research Bulletin 1031. Missouri Agricultural Experiment Station, Columbia, MO.

Chippendale, G. M., and A. S. Reddy. 1974. Diapause of the southwestern corn borer, *Diatraea grandiosella:* low temperature mortality and geographical distribution. Environ. Entomol. 3: 233–238.

Davis, F. M. 1989. Rearing the southwestern corn borer and fall armyworms at Mississippi State, pp. 27–36. *In* J. A. Mihm [ed.], Toward Insect Resistant Maize for the Third World: Proceedings of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects. CIMMYT (International Maize and Wheat Improvement Center), Mexico, D.F.

Davis, F. M., C. A. Henderson, and T. G. Oswalt. 1973. Mortality of overwintering southwestern corn borers in Mississippi. Environ. Entomol. 2: 86–88.

Davis, F. M., and W. P. Williams. 1994. Evaluation of reproductive stage maize for resistance to the southwestern corn borer (Lepidoptera: Pyralidae) using visual rating scores of leaf sheath and husk damage. J. Econ. Entomol. 87: 1105–1112.

Davis, E. G., J. R. Horton, C. H. Gable, E. V. Walter, and R. A. Blanchard. 1933. The southwestern corn borer. U.S. Dep. Agric. Tech. Bull. 388.

Edwards, O. R., and K. R. Hopper. 1999. Using superparasitism by a stem borer parasitoid to infer a host refuge. Ecol. Entomol. 24: 7–12.

Knutson, A. E., and F. E. Gilstrap. 1990a. Seasonal occurrence of *Beauveria bassiana* in the southwestern corn borer (Lepidoptera: Pyralidae) in the Texas High Plains. J. Kans. Entomol. Soc. 63: 243–251.

Knutson, A. E., and F. E. Gilstrap. 1990b. Life tables and population dynamics of the southwestern corn borer (Lepidoptera: Pyralidae) in Texas corn. Environ. Entomol. 19: 684–696.

Kramer, J. P. 1976. The extra-corporeal ecology of microsporidia, pp. 127–135. *In* L. A. Bulla and T. C. Cheng [eds.], Comparative pathobiology. Plenum, New York.

Langille, R. H. 1975. Observations on the overwintering survival and spring development of the southwestern corn borer, *Diatraea grandiosella* Dyar. Ph.D. dissertation, University of Missouri, Columbia.

Leiby, R. W. 1920. The larger corn stalk-borer in North Carolina. Bull. NC Dep. Agric. 41(13).

Miller, S. G., and R. D. Miller. 1996. Infectious *Enterococcus* from *Heliothis virescens* x *H. subflexa* backcross hybrids (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 89: 420–427.

Overholt, W. A., and J. W. Smith. 1990. Colonization of six exotic parasites (Hymenoptera) against *Diatraea grandiosella* (Lepidoptera: Pyralidae) in corn. Environ. Entomol. 19: 1889–1902.

Poinar, G. O., and G. M. Thomas. 1984. Laboratory guide to insect pathogens and parasites. Plenum, New York.

Rodriguez, B.L.A., and J. W. Smith. 1997. Biological control of maize and sugarcane stemborers in Mexico: a review. Insect Sci. Appl. 17: 305–314.

Siegel, J. P., J. V. Maddox, and W. G. Ruesink. 1986. Lethal and sublethal effects of *Nosema pyrausta* on the European corn borer (*Ostrinia nubilalis*) in central Illinois. J. Invertebr. Pathol. 48: 167–173.

Siegel, J. P., J. V. Maddox, and W. G. Ruesink. 1988. Seasonal progress of *Nosema pyrausta* in the European corn borer, *Ostrinia nubilalis.* J. Invertebr. Pathol. 52: 130–136.

Sikorowski, P. P., and A. M. Lawrence. 2000. Effects of *Serratia marcescens* on rearing of *Heliothis virescens.* Am. Entomol. (in press).

Solter, L. F., J. V. Maddox, and D. W. Onstad. 1991. Transmission of *Nosema pyrausta* in adult European corn borers. J. Invertebr. Pathol. 57: 220–226.

Sri-Arunotal, S., P. P. Sikorowski, and W. W. Neel. 1975. Study of the pathogens of the pecan weevil larvae. Environ. Entomol. 4: 790–792.

Steffey, K. L., M. E. Rice, J. All, D. A. Andow, M. E. Gray, and J. W. Van Duyn. 1999. Handbook of corn insects. Entomological Society of America, Lanham, MD.

Steinhaus, E. A. 1958. Crowding as a possible stress factor in insect disease. Ecology 39: 503–514.

Steinhaus, E. A. 1959. *Serratia marcescens* Bizio as an insect pathogen. Hilgardia 28: 351–380.

Wharton, R. A. 1984. The status of certain Braconidae (Hymenoptera) cultured for biological control programmes, and description of a new species of *Macrocentrus.* Proc. Entomol. Soc. Wash. 86: 902–912.

Yamashita, T. 1992. Infection of honey bees by *Enterococcus faecalis.* Honeybee Sci. 13: 111–114.

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