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PATHOGENS AND PARASITIC NEMATODES ASSOCIATED WITH POPULATIONS OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) LARVAE IN MEXICO

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Abstract

Larvae of fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) and soil samples were collected in six Mexican states. Larvae were collected from whorl-stage corn, grain sorghum, forage sorghum, and Sudan grass fields in 64 locations during the summer of 2000, to determine the occurrence of entomopathogens and parasitic nematodes. A total of 5591 FAW larvae from 64 locations were examined for indigenous FAW biological control agents. Overall total larval mortality was 3.935%. The larval mortality percent due to entomopathogens and parasitic nematodes was 3.524%, other causes reached 0.411% of total mortality. Three species of entomopathogenic fungi representing two classes, Hyphomycetes (*Nomuraea rileyi*, and *Hirsutella* sp.) and Zygomycetes (*Entomophthora* sp.) were recovered from FAW larvae, and two species of Hyphomycetes (*Metarhizium anisopliae* and *Beauveria bassiana*) were isolated from soil samples. An unidentified microsporidian was recovered from four locations in the State of Jalisco, three from Michoacán, three from Nayarit, and one from Veracruz and Colima, respectively. Mermithid nematodes were recovered from 24 FAW larvae at three locations in Nayarit and three larvae were recovered from two locations in Veracruz. Six larvae showing symptoms of viral disease were collected from Sinaloa (2), Jalisco (2), Michoacán (1), and Nayarit (1). Entomopathogenic nematodes from the genus *Heterorhabditis* sp. and *Steinernema* sp. were isolated from soil samples from Colima in one and two locations, respectively. *Steinernema* sp., and *Heterorhabditis* sp. were isolated from soil in one location in Michoacán. *Steinernema* sp. was recovered from two locations of Jalisco. In this survey, *N. rileyi*, mermithid nematodes, and microsporidia were the most frequent pathogens and parasites.

Key Words: *Spodoptera frugiperda*, biological control, occurrence, survey, maize, mermithid nematodes, entomopathogenic microorganisms

Resumen

Larvas de gusano cogollero, *Spodoptera frugiperda* (J. E. Smith) (FAW), y muestras de suelo se colectaron de 64 localidades en seis estados mexicanos. Las larvas fueron recogidas de campos cultivados con maíz, sorgo para grano, sorgo forrajero y pasto Sudán, en estado de cogollo o verticilio, con la finalidad de determinar la presencia de entomopatógenos y nematodos parásitos, durante el verano de 2000. 5591 larvas se colectaron para buscar agentes de control biológico indígenos de esta plaga. En general la mortalidad total de larvas fue de 3.935%, la mortalidad larvaria provocada por patágenos y parásitos fue de 3.524%, otras causas provocaron 0.411%. Tres especies de hongos entomopatógenos pertenecientes a dos clases, los Hyphomycetes (*Nomuraea rileyi* e *Hirsutella* sp.) y los Zygomycetes (*Entomophthora* sp.) fueron recuperados de larvas de gusano cogollero, y dos especies de Hyphomycetes (*Metarhizium anisopliae* y *Beauveria bassiana*) fueron aislados de muestras de suelos. Un microsporidio no identificado fue recuperado en cuatro localidades de Jalisco, tres de Michoacán, tres de Nayarit, y una en Veracruz y Colima, respectivamente. Los nematodos mermítidos parasitaron a veinticuatro larvas en tres localidades de Nayarit, asi mismo, a tres larvas en dos localidades de Veracruz. Las larvas con síntomas de virosis se presentaron en dos sitios de Sinaloa, dos en Jalisco, una en Michoacán y una en Nayarit. Los nematodos entomopatógenos de los géneros *Steinernema* sp. y *Heterorhabditis* sp. fueron aislados de muestras de suelo, presentándose en Colima, en una y dos localidades, respectivamente; en Michoacán ocurrieron en una localidad, respectivamente, pero *Steinernema* sp. solo se presentó en dos localidades de Jalisco. En este inventario, *N. rileyi*, los nematodos mermítidos y el microsporidio fueron los patógenos y parásitos más frecuentes.

Translation provided by author

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), causes considerable economic losses in maize, sorghum, peanuts, cotton, soybeans and occasionally other crops, in most of the countries of the Western Hemisphere (Sparks 1986). Control of this pest is usually achieved through the application of synthetic insecticides (Hruska & Gould 1997), but their high cost, environmental contamination, development of resistance to chemicals, and pest resurgence (Colborn 1995; Crowe & Booty 1995) have encouraged the search for alternatives more compatible with the environment. Microbial control is an environmentally sound and a valuable alternative to the use of chemicals for controlling this pest.

Interactions between insect host, environment, insect host age (Fuxa et al. 1988; Molina-Ochoa et al. 1996), pathogens and plant to be protected (Bergman & Tingey 1979; Hamm & Wiseman 1986; Barbercheck 1993; Wiseman & Hamm 1993; Molina-Ochoa et al. 1997, 1999) determine the strategies for using pathogens in microbial control (Hamm 1984). FAW larvae are susceptible to entomopathogenic bacteria, fungi, nematodes, protozoa, and viruses (Gardner & Fuxa 1980; Agudelo-Silva 1986; Hamm et al. 1986; Patel & Habib 1988; Richter & Fuxa 1990; Lezama-Gutierrez et al. 1996; Molina-Ochoa et al. 1996; Molina-Ochoa et al. 1999). The insect host age, habitat and soil type, pesticide use, agricultural practices, and location, influence the natural distribution of biological control organisms (Croft & Brown 1975; Fuxa 1982; Agudelo-Silva 1986; Hamm et al. 1986; Sosa-Gomez & Moscardi 1994; Vanninen 1996; Chandler et al. 1997; Mietkiewski et al. 1997; Molina-Ochoa et al. 2001).

As a result of economic and environmental concerns, surveys for natural enemies of the FAW occurring in Mexico have been conducted to develop a better understanding of the pathogen complex, parasitic nematodes and parasitoids (Lezama-Gutierrez 2001, Molina-Ochoa 2001, Molina-Ochoa et al. unpublished). These two surveys conducted in four Mexican states reported the occurrence of the fungi *Beauveria bassiana*, *Nomuraea rileyi*, and *Hirsutella* sp., an unidentified microsporidian, mermithid nematodes, and an ascovirus affecting FAW larvae. The occurrence of the bacterium *Bacillus thuringensis* and steinernematid and heterorhabditid nematodes are reported from soil samples.

This paper reports on the presence of entomopathogens and parasitic nematodes in FAW larval populations and recovered from soil samples of corn, grain sorghum, forage sorghum, and Sudan grass fields from six Mexican states, during the summer of 2000.

Materials and Methods

Isolation of Entomopathogens from FAW Larvae

During August and September of 2000, FAW larvae were collected from whorl-stage corn, grain and forage sorghum, and Sudan grass fields in 64 locations in the Mexican states of Sinaloa, Nayarit, Jalisco, Colima, Michoacán, and Veracruz. Concurrently, four soil samples were obtained from each location in all of the states. Location 43 comprised a combination of collections from adjacent corn and grain sorghum field in whorl-stage. Sample size ranged from 33 to 119 FAW larvae per field, but most often sample size was about 90. The number collected was corrected by subtracting the number that died from injury or unknown causes during the first days after collection. Collection data and percent infection by entomopathogens and parasitic nematodes is presented in Table 1. Larval mortality due to insect parasitoids is reported elsewhere (Molina-Ochoa et al., in press).

Larvae were placed individually in 30 cc plastic cups with regular pinto bean diet (Burton & Perkins 1989) and maintained in the laboratory to record the larvae infected by entomopathogens and parasitic nematodes. Mermithid nematodes that emerged from larvae were collected and placed in crystal vials containing 2 ml of 70% ethanol. Dead FAW larvae showing signs of fungal infection were placed in plastic Petri dishes (60 × 10 mm) lined with a piece of 5.5 cm-diameter filter paper (Whatman No. 1) moistened with sterile distilled water until the fungus sporulated on the insect surface. A medium composed of 200 ml of V8 vegetable juice, 5 g glucose, 2 g yeast extract, 3 g $CaCO_3$, 15 g agar, and 800 ml distilled water (Fargues & Rodriguez-Rueda 1980) for isolating the fungus *Nomuraea rileyi* was used. A Sabouraud-dextrosa-agar medium enriched with 1% (w/v) yeast extract (SDAY), and 500 ppm chloramphenicol (Lezama et al. 1996) for growing other fungus species was used. Entomophthorales were not isolated.

Isolation of Entomopathogenic Fungi and Nematodes from Soil

In each location of the six surveyed states, a 2 kg combined soil sample was collected. A soil subsample, about 500 g from four different points a few meters apart, was obtained by digging to a depth of 10-15 cm with a small shovel. Soil samples were deposited into double plastic bags, tagged, stored in a plastic cooler, and taken to the laboratory where they were kept at 25°C until processing. The storage time ranged from a few days to three weeks. Soil was thoroughly mixed and passed through a 0.4 mm mesh sieve, breaking soil lumps and separating any litter.

For isolating entomopathogenic nematodes and fungi, greater wax moth (GWM) larvae, *Galleria mellonella* L., were used as bait (Bedding & Akhurst 1975; Tarasco et al. 1997). From the 2 kg combined soil sample from each location, two samples were placed in 1000 ml capacity plastic pots and five GWM last instar larvae were released into each pot. Pots were incubated at 25°C in the dark for a 10-day period (Woodring & Kaya

TABLE 1. GEOGRAPHIC LOCATION, DATE, ALTITIUDE, CROP (*), SAMPLE SIZE (N), AND TOTAL PERCENT FAW LARVAE INFECTED BY ENTOMOPATHOGENS AND PARASITIC NEMATODES IN SIX MEXICAN STATES (**) DURING 2000.

Code	Date	Location	Coordinates	Altitude (m)	Crop	n	Infected larvae (%)
C1	08/04	El poblado, Coquimatlán	19°13.698’N 103°47.722’W	422	c	90	0.000
C2	08/04	Pueblo Juárez, Coquimatlán	19°10.752’N 103°54.634’W	279	c	90	2.222
C3	08/04	Amachico, Coquimatlán	19°10.667’N 103°56.351’W	328	c	90	0.000
C4	08/06	Los mezcales, Comala	19°20.811’N 103°47.639’N	608	c	90	1.111
C5	08/06	El remate, Comala	19°24.825’N 103°47.639’W	817	c	90	0.000
C6	08/06	Carrizalillo, Quesería	19°25.389’N 103°41.000’W	1550	c	90	0.000
C7	08/06	Quesería	19°23.362’N 103°34.882’W	1304	c	90	0.000
C8	08/06	Villa de Alvarez	19°17.201’N 103°47.030’W	515	c	90	0.000
C9	08/06	Juluapan, Villa de Alvarez	19°18.890’N 103°49.611’W	539	c	90	1.111
C10	08/07	Tepames, Colima	19°08.231’N 103°37.996’W	519	c	90	2.222
C11	08/07	Estapilla, Colima	19°59.549’N 103°31.140’W	304 1557	c	90	1.111
J1	08/08	Ciudad Guzmán	19°40.11’N 103°28.830’W		c	90	2.222
J2	08/15	Los pinitos, Tonila	19°25.343’N 103°32.447’W	1326	c	90	11.111
J3	08/15	Pialla, Tuxpan	19°27.293’N 103°28.514’W	1079	c	90	44.444
J4	08/15	Atenquique, Tuxpan	19°31.778’N 103°27.851’W	1338	c	90	11.111
J5	08/17	Canoas, Zapotiltic	19°34.073’N 103°27.324’W	1391	c	90	12.111
J6	08/17	Apastepe	19°38.060’N 103°30.950’W	1709	c	90	3.333
J7	08/17	Teocuitatlán	20°07.035’N 103°32.704’W	1369	c	90	1.111
J8	08/17	Zacoalco de Torres	20°11.988’N 103°33.806’W	1425	c	90	1.111
J9	08/17	Acatlán de Juárez	20°25.362’N 103°33.406’W	1575	c	96	4.166
J10	08/17	Tlajomulco de Zúñiga	20°29.396’N 103°28.298’W	1607	c	92	4.347
J11	08/18	Zapopan	20°43.129’N 103°29.041’W	1670	c	90	10.000
J12	08/18	Magdalena	20°53.008’N 103°02.509’W	1496	c	93	2.150
J13	08/23	Crucero de Magdalena	20°56.300’N 104°02.509’W	1386	c	92	9.782
M1	08/09	Totolán	19°58.890’N 102°40.183’W	1590	c	90	0.000
M2	08/09	Santa Inés Tocumbo	19°44.502’N 102°34.967’W	1630	c	90	0.000

*Corn (c), Forage Sorghum (fs), Grain Sorghum (gs), and Sudan Grass (sg).
**Colima (C), Jalisco (J), Michoacan (M), Nayarit (N), Sinaloa (S), and Veracruz (V).

TABLE 1. (CONTINUED) GEOGRAPHIC LOCATION, DATE, ALTITIUDE, CROP (*), SAMPLE SIZE (N), AND TOTAL PERCENT FAW LARVAE INFECTED BY ENTOMOPATHOGENS AND PARASITIC NEMATODES IN SIX MEXICAN STATES (**) DURING 2000.

Code	Date	Location	Coordinates	Altitude (m)	Crop	n	Infected larvae (%)
M3	08/09	Peribán	19°33.106'N 102°26.586'W	1475	c	90	0.000
M4	08/10	Cointzio	19°41.609'N 101°16.398'W	1932	c	90	2.222
M5	08/10	Cerro "La Esperanza"	19°41.233'N 101°18.890'W	1998	c	90	2.222
M6	08/11	Tejabán	19°13.342'N 101°53.714'W	587	c	90	0.000
M7	08/11	Carretera a Nueva Italia	19°03.290'N 102°02.458'W	442	c	90	2.222
M8	08/11	Presa de Zicuirán	18°56.191'N 101°54.650'W	292	c	63	12.698
M9	08/11	El ceñidor, Nueva Italia	18°59.651'N 102°11.577'W	350	c	57	1.754
M10	08/12	La Guadalupe Parácuaro	19°07.472'N 102°12.519'W	540	fs	90	2.222
M11	08/12	Las yeguas Parácuaro	18°57.308'N 102°16.733'W	359	fs	90	2.222
M12	08/12	El cirián, Nueva Italia	18°53.661'N 102°07.483'W	255	c	90	0.000
N1	08/18	Santa María del Oro	21°20.121'N 104°40.174'W	1160	c	90	2.222
N2	08/18	El rincón, Tepic	21°32.472'N 104°56.123'W	849	c	96	2.083
N3	08/18	El pichón, Tepic	21°33.479'N 104°56.937'W	774	c	95	4.210
N4	08/19	Xalisco	21°19.601'N 104°55.060'W	1042	c	107	7.476
N5	08/19	El refilión, Xalisco	21°19.407'N 104°55.323'W	964	c	90	2.222
N6	08/19	Compostela	21°17.858'N 104°54.044'W	920	c	93	21.505
N7	08/19	La presa, Compostela	21°13.714'N 104°52.162'W	928	c	90	2.222
N8	08/20	Las lumbres, Acaponeta	22°20.795'N 105°18.141'W	48	C&gs	60	13.333
N9	08/23	Seboruco	21°20.850'N 104°40.749'W	1134	c	90	2.222
N10	08/23	Ahuacatlán	21°06.331'N 104°27.427'W	1120	c	90	1.111
S1	08/21	Bacurimi, Culiacán	24°51.688'N 107°29.478'W	70	gs	97	0.000
S2	08/21	La campana, Culiacán	24°58.415'N 107°33.517'W	143	gs	100	2.000
S3	08/21	Pericos, Mocorito	25°03.574'N 107°39.547'W	80	gs	95	1.052
S4	08/21	Rancho viejo Mocorito	25°06.033'N 107°43.165'W	89	gs	98	1.020
S5	08/22	Aguapepito Mocorito	25°03.861'N 107°39.547'W	68	sg	95	1.052
S6	08/22	Comanito Mocorito	25°09.006'N 107°39.645'W	91	gs	95	0.000

*Corn (c), Forage Sorghum (fs), Grain Sorghum (gs), and Sudan Grass (sg).
**Colima (C), Jalisco (J), Michoacan (M), Nayarit (N), Sinaloa (S), and Veracruz (V).

TABLE 1. (CONTINUED) GEOGRAPHIC LOCATION, DATE, ALTITIUDE, CROP (*), SAMPLE SIZE (N), AND TOTAL PERCENT FAW LARVAE INFECTED BY ENTOMOPATHOGENS AND PARASITIC NEMATODES IN SIX MEXICAN STATES (**) DURING 2000.

Code	Date	Location	Coordinates	Altitude (m)	Crop	n	Infected larvae (%)
S7	08/22	La poma Badiraguato	25°15.749'N 107°40.739'W	157	c c	100	0.000
S8	08/22	La majada Badiraguato	25°14.076'N 107°39.781'W	145	c	92	0.000
V1	09/02	Seis de Enero, Xalapa	19°34.115'N 96°50.207'W	950	c	91	0.000
V2	09/02	Altolucero, Almolonga	19°35.063'N 96°47.384'W		c	33	6.060
V3	09/02	Actopan	19°34.623'N 96°48.589'W		c	64	1.562
V4	09/02	Los González, Actopan	19°31.894'N 96°41.294'W	432	c	113	0.884
V5	09/02	Bocana, Actopan	19°24.416'N 96°36.731'W	311	c	119	0.000
V6	09/03	El volador, Coatepec	19°21.594'N 96°51.037'W	709	c	90	3.333
V7	09/03	Palmillas	19°12.293'N 96°46.221'W	702	c	59	0.000
V8	09/03	Tierra Colorada	19°13.255'N 96°21.916'W	46	c	45	4.444
V9	09/04	Cerro gordo	19°25.252'N 96°39.566'W	443	c	45	0.000
V10	09/04	Lacumbre	19°23.320'N 96°38.807'W	366	c	66	0.000

*Corn (c), Forage Sorghum (fs), Grain Sorghum (gs), and Sudan Grass (sg).
**Colima (C), Jalisco (J), Michoacan (M), Nayarit (N), Sinaloa (S), and Veracruz (V).

1988, Bidochka et al. 1998). Larval cadavers were removed and surface-sterilized with 1% Sodium hypochlorite for a 3 minute-period, then washed three times with sterile distilled water and placed on damp filter paper in a 60 mm diameter sealed Petri dish, and incubated at 25°C for 12 days (Chandler et al. 1997). Entomopathogenic fungi from the larvae were isolated using SDAY, with 500 ppm of chloramphenicol (Lezama-Gutierrez et al. 1996). Fungi were identified by microscopic inspection of morphological characteristics *in situ* or after isolation in SDAY according to the criteria by Brady (1979) and Samson et al. (1988).

The entomopathogenic nematodes were separated to genera by identifying coloration of *Galleria* cadavers according to Woodring & Kaya (1988).

Entomopathogenic viruses and bacteria from FAW larvae and soil, respectively, have not been yet isolated or identified.

Geographical Coordinates and Collection Data

A Garmin GPS III Plus™ was used for obtaining the coordinates and altitude data. Location, date, place, coordinates, altitude, crop, sample size, and percentage of infected larvae are shown in Table 1.

RESULTS

In this survey, out of 5591 FAW larvae collected from 64 locations in six Mexican states, the entomopathogens and parasitic nematodes killed 197 larvae. Overall larval mortality percentage due to these organisms was 3.524%. Mortality percentage per location ranged from 0.000 to 44.444% (Table 1). Considering the total mortality due to entomopathogens and parasitic nematodes, 137 (69.54%) larvae were killed by entomopathogenic fungi, 26 (13.19%) larvae were killed by microsporidia, six (3.04%) larvae were killed by viruses, and 28 (14.21%) larvae were killed by mermithid nematodes. Two classes of entomopathogenic fungi were collected. The class Zygomycetes was represented by *Entomophthora* sp. which infected a larva from Colima and a larva from Veracruz. The class Hyphomycetes was represented by *Nomuraea rileyi* and *Hirsutella* sp. which infected 134 and one larvae, respectively. *N. rileyi* was responsible for 68.020% of total mortality due to entomopathogens and parasitic nematodes, and was the most abundant and widely distributed, occurring in all the states. *Hirsutella* sp. occurred in Sinaloa, only. Mermithids were collected from the states of Nayarit (23), and Veracruz (5), only, and accounted for approximately 14.21% of total mortality of FAW larvae.

The 26 FAW larvae infected by microsporidia were collected in five of six states sampled (Michoacán (10), Nayarit (9), Jalisco (5), Colima (1), Veracruz (1) and Sinaloa (0)). These entomopathogens accounted for 13.19% of the total FAW larval mortality. The symptoms of larvae infected with these entomopathogens were similar to the unidentified microsporidia reported by Lezama-Gutierrez et al. (2001). They often were dry and fragile when dead, resembling cigarette ashes. Few FAW larvae showed symptoms of ascovirosis infection (Hamm et al. 1986). Two were detected in Jalisco in one location, two in two separate locations in the same municipality in Sinaloa, one in Michoacán, and one in Nayarit. The viruses were not identified by electron microscopy. The percentage of fall armyworm larvae infected by pathogens and parasitic nematodes at each location is shown in Table 2.

Entomopathogens Isolated from Soil

Two species of entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*, were recovered from 10 of 64 soil samples. *M. anisopliae* was recovered from four of eleven locations in Colima, and in one location in each of Nayarit, Jalisco, and Michoacán. *Beauveria bassiana* was recovered from two locations in Veracruz and one in Michoacán.

Two genera of entomopathogenic nematodes, *Steinernema* sp., and *Heterorhabditis* sp. were collected from soil samples. Steinernematid nematodes were recovered from five of 64 locations (two locations in Colima, two in Jalisco, and in one in Michoacán). Heterorhabditid nematodes were recovered in two locations, one in Colima and one in Michoacán.

Discussion

Current research efforts are focused on selecting native and exotic entomopathogens, which are highly virulent to arthropod pests, for developing efficient and environmentally-sound bioinsecticides. The high susceptibility of fall armyworm larvae and other lepidopterous pests to strains of *N. rileyi*, *B. bassiana* and *M. anisopliae* has been demonstrated (Bustillo & Posada 1986; Habib & Patel 1990; Lecuona & Lanteri 1999). *N. rileyi* has been reported infecting FAW larvae naturally in Brasil (Valicente 1989), Venezuela (Agudelo-Silva 1986), Puerto Rico (Pantoja et al. 1985), Colombia (Vargas & Sánchez 1983), United States (Fuxa 1982), Mexico (Lezama-Gutiérrez et al. 2001), and other countries. In this survey, *N. rileyi* caused 68.020% of the total FAW larval mortality due to pathogens and parasitic nematodes, and was the most abundant and widely distributed entomopathogen, occurring in each of the six states surveyed. Similar results were reported by Lezama-Gutiérrez et al. (2001) from a survey conducted in Colima, Jalisco, and Michoacán.

Entomophthora aulicae was reported attacking FAW larvae on grain sorghum in Georgia (Hamm 1980; Schwehr & Gardner 1982), and Argentina (Vera et al. 1995). *Entomophthora* sp. and *Hirsutella* sp. were reported attacking larvae of this pest with parasitism rates that ranged from 0.6% to 1.1%, respectively (Lezama-Gutiérrez et al. 2001). In this study, parasitism rates for *E.* sp., and *H.* sp. were 3.030 and 1.520%, respectively. Total FAW mortality caused by all the pathogens was 3.524% (197 larvae killed).

An unidentified microsporidian was the third cause of FAW total larval mortality with 13.19%, and was similar to that reported by Lezama-Gutierrez et al. (2001). Most larvae infected with the microsporidian were collected from Michoacán, Nayarit, and Jalisco, with 10, 8, and 5 infected larvae, respectively. The arrangement of the spores of this microsporidian suggested that this entomopathogen was neither *Nosema* nor *Vairimorpha* as previously reported by Gardner & Fuxa (1980).

A few larvae showed symptoms and signs similar to those from ascoviruses, but the identity of these viruses was not verified by electron microscopy. Occurrence of entomopathogenic viruses has been reported in Latin America, in Puerto Rico, Argentina, Brasil, and Mexico (Valicente 1989; Pantoja & Fuxa 1992; Vera et al. 1995; Lezama-Gutierrez et al. 2001).

In this survey, the mermithid nematodes were important natural enemies of FAW larvae. They were the second most important mortality factor, causing 14.21% of total mortality. Nematodes from the genus *Hexamermis* have been reported attacking FAW larvae in Honduras, Brasil, Nicaragua, and Argentina (Van Huis 1981; Valicente 1989; Wheeler et al. 1989; Vera et al. 1995). Mermithids attacking FAW larvae in Mexico were reported by Alcocer-Gómez & Méndez-Villa (1965). They found parasitism ranging from 8 to 100% during a 3-year study. An association between the pest density and percent of parasitism was determined. Rainfall also was cited as an important factor in influencing percent parasitism. In a recent survey conducted in Mexico, mermithid nematodes caused larval mortality ranging from 0.0 to 14.9% in Colima (Lezama-Gutierrez et al. 2001). But in our survey, mermithids were not recovered in Colima. However, similar percentages of mortalities were recorded (0.000 to 15.054%) from other locations, with the highest rate of parasitism from Nayarit and Veracruz. A possible reason for the difference between the findings of Lezama-Gutierrez et al. (2001) and those we report is that most of the locations in Colima were

TABLE 2. PERCENTAGE OF FAW LARVAE INFECTED BY ENTOMOPATHOGENS AND MERMITHIDS AT EACH LOCATION.

Code*	*N. rileyi*	*Hirsut.*	*Entomoph.*	Mermithid	Microspo.	Viruses
C1	0	0	0	0	0	0
C2	2.222	0	0	0	0	0
C3	0	0	0	0	0	0
C4	0	0	1.111	0	0	0
C5	0	0	0	0	0	0
C6	0	0	0	0	0	0
C7	0	0	0	0	0	0
C8	0	0	0	0	0	0
C9	1.111	0	0	0	0	0
C10	1.111	0	0	0	1.111	0
C11	1.111	0	0	0	0	0
M1	0	0	0	0	0	0
M2	0	0	0	0	0	0
M3	0	0	0	0	0	0
M4	2.222	0	0	0	0	0
M5	2.222	0	0	0	0	0
M6	0	0	0	0	0	0
M7	2.222	0	0	0	0	0
M8	0	0	0	0	12.698	0
M9	0	0	0	0	1.754	0
M10	1.111	0	0	0	1.111	0
M11	1.111	0	0	0	0	1.111
M12	0	0	0	0	0	0
J1	2.222	0	0	0	0	0
J2	8.888	0	0	0	0	2.222
J3	44.444	0	0	0	0	0
J4	11.111	0	0	0	0	0
J5	12.222	0	0	0	0	0
J6	2.222	0	0	0	1.111	0
J7	1.111	0	0	0	0	0
J8	0	0	0	0	1.111	0
J9	4.166	0	0	0	0	0
J10	4.347	0	0	0	0	0
J11	7.777	0	0	0	2.222	0
J12	2.150	0	0	0	0	0
J13	8.696	0	0	0	1.086	0
N1	0	0	0	0	2.222	0
N2	1.041	0	0	0	0	1.042
N3	3.157	0	0	0	1.053	0
N4	2.803	0	0	0	4.673	0
N5	2.222	0	0	0	0	0
N6	6.451	0	0	15.054	0	0
N7	1.111	0	0	1.111	0	0
N8	0	0	0	13.333	0	0
N9	2.222	0	0	0	0	0
N10	1.111	0	0	0	0	0
S1	0	0	0	0	0	0
S2	2.000	0	0	0	0	0
S3	0	0	0	0	0	1.052
S4	0	0	0	0	0	1.020
S5	0	1.052	0	0	0	0
S6	0	0	0	0	0	0
S7	0	0	0	0	0	0
S8	0	0	0	0	0	0

*Locations are described in Table 1.

N. rileyi = *Nomuraea rileyi*, *Hirsut.* = *Hirsutella* sp., *Entomoph.* = *Entomophthora* sp., Mermithid = mermithid nematode, Microspo. = Microsporidia, Viruses = with virosis signs.

TABLE 2. (CONTINUED) PERCENTAGE OF FAW LARVAE INFECTED BY ENTOMOPATHOGENS AND MERMITHIDS AT EACH LOCATION.

Code*	*N. rileyi*	*Hirsut.*	*Entomoph.*	Mermithid	Microspo.	Viruses
V1	0	0	0	0	0	0
V2	3.030	0	3.030	0	0	0
V3	0	0	0	1.562	0	0
V4	0	0	0	0	0.884	0
V5	0	0	0	0	0	0
V6	0	0	0	3.333	0	0
V7	0	0	0	0	0	0
V8	2.222	0	0	2.222	0	0
V9	0	0	0	0	0	0
V10	0	0	0	0	0	0

*Locations are described in Table 1.
N. rileyi = *Nomuraea rileyi*, *Hirsut.* = *Hirsutella* sp., *Entomoph.* = *Entomophthora* sp., Mermithid = mermithid nematode, Microspo. = Microsporidia, Viruses = with virosis signs.

different than those sampled by Lezama-Gutierrez et al. (2001) during the summer of 1998.

The entomopathogenic fungi and nematodes were recovered in 26.5% of the soil samples (17 of 64 locations). *M. anisopliae* and *B. bassiana* were isolated in 15.6% of the samples. They occurred in Colima, Michoacán, Nayarit, and Veracruz, but were not found in Jalisco and Sinaloa. *M. anisopliae* was recovered from five locations in Colima, and one location each in Michoacán and Nayarit. *B. bassiana* was recovered in two locations of Veracruz and one location in Michoacán. In a study conducted in Szczecin, Poland using soils collected from forests during the spring and autumn, the entomopathogenic fungi *M. anisopliae* and *B. bassiana* infected wax moth larvae (Mietkiewski et al. 1998), and *M. anispoliae* was the dominant species. Recently, Lezama-Gutierrez et al. (2001) reported three species of entomopathogenic fungi recovered from soil samples using the *Galleria* technique; *M. anisopliae*, *B. bassiana*, and *Paecilomyces fumosoroseus*, with *M. anisopliae* being the most dominant species. In our soil samples, *M. anisopliae* was also the most dominant, occurring in 10.9% of the locations, while *B. bassiana* occurred only in 4.7% of the locations (Table 3).

Steinernematid and Heterorhabditid nematodes were found in seven of 64 locations (10.9%). Steinernematid nematodes were recovered from Colima in two locations, one in Michoacán, and two in Jalisco. Heterorhabditids occurred in Colima and Michoacán, in one location, respectively. Low rates

TABLE 3. ENTOMOPATHOGENIC FUNGI (HYPHOMYCETES) AND NEMATODES (RHABDITIDA: STEINERNEMATIDAE AND HETERORHABDITIDAE) RECOVERED FROM SOIL SAMPLES IN DIFFERENT MEXICAN LOCATIONS.

Code*	Location	Entomopathogen
C2	Pueblo Juárez, Coquimatlán	*Heterorhabditis* sp.
C5	El remate	*Metarhizium anisopliae*
C5	El remate	*Steinernema* sp.
C6	Carrizalillo	*Steinernema* sp.
C6	Carrizalillo	*Metarhizium anisopliae*
C7	Quesería	*Metarhizium anisopliae*
C10	Tepames	*Metarhizium anisopliae*
J1	Ciudad Guzmán	*Metarhizium anisopliae*
M2	Santa Inés, tocumbo	*Metarhizium anisopliae*
M4	Cointzio	*Beauveria bassiana*
M6	Tejabán	*Steinernema* sp.
M7	Carretera a Nueva Italia	*Heterorhabditis* sp.
J2	Los pinitos, Tonila	*Steinernema* sp.
J3	Pialla, Tuxpan	*Steinernema* sp.
N6	Compostela	*Metarhizium anisopliae*
V8	Tierra Colorada	*Beauveria bassiana*
V9	Cerro Gordo	*Beauveria bassiana*

*Locations are described in Table 1.

of entomopathogenic nematode recovery have been reported in different regions around the world, and range from 3.9% to 21.4% (Constant et al. 1998; Tangchitsomkid et al. 1998; Griffin et al. 2000; Rosa et al. 2000; Lezama-Gutierrez et al. 2001). Soil pH and type, altitude, habitat, soil temperature, croplands, orchards, pastures, and proximity to coastal lands were discussed as possible factors affecting the occurrence of these entomopathogens.

The diversity and distribution of entomopathogens and parasitic nematodes occurring in Mexico could play an important role in regulating the FAW larval populations. Additional research is needed on the identification, biology, and potential of the microsporidia frequently recovered in the surveys conducted during 1998 and 2000. There is also a need to identify the role of mermithid nematodes as potential biological control agents. Additional research has already been conducted at the Universidad de Colima, Mexico, to identify the steinernematid and heterorhabditid nematodes isolated, and to determine their potential for biological control of fall armyworm larvae and other lepidopterous pests.

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