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Authors: Ruiz-Nájera, Ramiro E., Ruiz-Estudillo, Ramiro A., Sánchez-Yáñez, Juan M., Molina-Ochoa, Jaime, Skoda, Steven R., et al.

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OCCURRENCE OF ENTOMOPATHOGENIC FUNGI AND PARASITIC NEMATODES ON *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE) LARVAE COLLECTED IN CENTRAL CHIAPAS, MÉXICO

RAMIRO E. RUIZ-NÁJERA¹, RAMIRO A. RUIZ-ESTUDILLO², JUAN M. SÁNCHEZ-YÁÑEZ³, JAIME MOLINA-OCHOA^{2,6,*}, STEVEN R. SKODA⁵, ROBERTO COUTIÑO-RUIZ¹, RENÉ PINTO-RUIZ¹, FRANCISCO GUEVARA-HERNÁNDEZ¹ AND JOHN E. FOSTER⁶

¹Laboratorio de Entomología, Facultad de Ciencias Agronómicas, Universidad Autónoma de Chiapas, Campus V, apartado postal 78, Villaflores, Chiapas 30470, México

²Facultad de Ciencias Agronómicas, Universidad Autónoma de Chiapas, Campus V, apartado postal 78, Villaflores, Chiapas, México

³Laboratorio de Microbiología Ambiental, Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio B-3, Cd. Universitaria Morelia Michoacán 58140, México

⁴Secretaría de Investigación, Universidad de Colima, Av. Universidad No. 333, Colonia Las Víboras, Colima, Colima 28040, México

⁵USDA-ARS-KBUSLIRL Screwworm Research Unit, Kerrville, TX 78028, USA

⁶Department of Entomology, Insect Genetics Laboratory, University of Nebraska-Lincoln, 3B Entomology Hall, Lincoln, NE 68583-0816, USA

*Corresponding author; E-mail: jmolina@ucol.mx, jmolina18@hotmail.com

ABSTRACT

Fall armyworm larvae (FAW), *Spodoptera frugiperda* (J. E. Smith) were collected from whorl-stage corn, *Zea mays* (L.), between the V₂ and V₄ stages, in 22 localities of Central, Chiapas, México, called "La Frailesca" during late Jun 2009 to determine the occurrence of native entomopathogens and parasitic nematodes, and to determine the most parasitized instars. A total of 1,247 larvae were examined in search of native biological control agents. Overall total larval mortality was 16.36%. The percent larval mortality due to entomopathogens and parasitic nematodes was 12.99%. *Hexameris* sp., probably *albicans* Siebold (Mermitha: Mermithidae) were isolated from 105 FAW larvae (8.42%) with highest incidence from the 4th, 5th, 6th and 3rd instars, respectively. A hyphomycete, *Nomuraea rileyi* Farlow (Samson), was recovered from 38 larvae (about 3.05% parasitism) with highest incidence in the 5th, 3rd, 4th, and 6th instars, respectively. Lowest percentage of FAW larval parasitism was caused by unidentified microsporidian or microsporidium (1.52%), infecting 19 larvae in the 6th, 5th and 3rd instars, respectively. First and 2nd instars did not show parasitism. This information is useful in designing future biological control programs.

Key Words: Fall armyworm, natural enemies, mermithid nematodes, entomopathogenic microorganisms, corn

RESUMEN

Se recolectaron larvas de gusano cogollero (FAW), *Spodoptera frugiperda* (J. E. Smith) en maizales, *Zea mays* (L.) en etapa vegetativa de verticilio, entre las etapas V₂ y V₄, en 22 localidades de la región central de Chiapas, México, conocida como "La Frailesca" a finales de Junio de 2009, con la finalidad de determinar la presencia de entomopatógenos y nematodos parásitos, y para determinar los estadios larvarios más parasitados. Un total de 1247 gusanos cogolleros fueron examinados para agentes de control biológico nativos. La mortalidad total fue de 16.36%. El porcentaje de mortalidad larval debido a entomopatógenos y nematodos parásitos fue de 12.99%, nematodos mermítidos probablemente de la especie *Hexameris albicans* Siebold fueron aislados de 105 larvas (8.42%) provenientes de los estadios del 3° al 6°. Entre tanto, un hongo Hyphomycete, *Nomuraea rileyi* Farlow (Samson), se recuperó de 38 FAW larvas entre los estadios del 3° al 6° causando alrededor

de 3.05% de parasitismo. El porcentaje más bajo de parasitismo larvario fue causado por un microsporidio no identificado infectando a 19 larvas entre los estadios 6°, 5° y 3° (1.52%). En este inventario, los nematodos mermitidos, *N. rileyi* y un microsporidio no identificado fueron los parásitos y patógenos más frecuentes. Las larvas de primer y segundo estadios no mostraron parasitismo. Esta información es útil para el diseño futuro de programas de control biológico.

Palabras Clave: Gusano cogollero del maíz, enemigos naturales, nematodos mermitidos, microorganismos entomopatógenos, maíz

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is well known in the Western Hemisphere as a voracious insect pest of multiple agricultural crops (Clark, 2007; Murúa et al. 2009). It is an important economic pest of corn, *Zea mays* (L.) (Poales: Poaceae), other grain crops and forage sorghums, peanuts, cotton, soybeans, alfalfa, and occasionally other crops (Sparks 1986; Lu & Adang 1996).

Corn is one of the most valuable field crops in the United States of America (USA). The FAW is one of the most destructive insects attacking corn; FAW feeds on tender leaves and stalks causing severe damage in every stage of plant development, resulting in limited production or complete destruction of whorl-stage plants (Wiseman et al. 1967, 1996). In Latin America maize losses in grain production are about 90% (Andrews 1988; Hruska & Gould 1997). Chemical control of FAW is a common practice; however, the adverse effects of the use of insecticides lead to a search for alternatives (Aktar et al. 2009). Microbial control is an environmentally sound and valuable alternative for controlling the FAW. FAW larvae are susceptible to entomopathogenic microorganisms such as bacteria, fungi, nematodes, viruses, and protozoa (Gardner & Fuxa 1980; Hamm et al. 1986; Patel & Habib 1988; Lezama-Gutiérrez et al. 1996; Molina-Ochoa et al. 1999; Molina-Ochoa et al. 2003; Ríos-Velasco et al. 2010).

The natural enemy complex, particularly pathogens, is poorly known in México, and there is an increased interest in developing biological control methods for FAW. Most concentrated efforts for biological control appear to be directed towards the "rear and release" augmentation, followed by importation and thirdly by conservation; Lewis et al. (1997) suggested a reversed order of priorities. There is a need to understand, promote and maximize the effectiveness of indigenous populations of natural enemies, and based on the knowledge and results of these, we should supplement any gaps by importation. The information on the naturally occurring natural enemy complex of the FAW larvae in "La Frailesca" located in central-western Chiapas, México is scarce, so we surveyed whorl-stage corn to determine the occurrence, and parasitism rates of

entomopathogenic microorganisms and parasitic nematodes of FAW larvae, as well as to determine which of the larval instars were the most parasitized.

MATERIALS AND METHODS

This survey was conducted during late Jun 2009 in the region known as "La Frailesca" located in the central-western of the state of Chiapas (municipalities of Villaflores, Villacorzo, La Concordia and Ángel Albino Corzo) located between the coordinates 15°45' and 16°30' N latitude, and between 92°30' and 93°45' W longitude; elevation ranging between 700 and 800 m asl (Anuario Estadístico de la Producción Agrícola 2008). The region has an annual mean temperature of 22 °C, and annual rainfall between 700 to 1200 mm (Table 1).

During late Jun 2009 collections of FAW larvae were made from whorl-stage corn in the phenological stages V₂ to V₄ (Ritchie et al. 1992). For each locality, at least 274 larvae were sampled. Each sample consisted of different FAW instars. FAW larvae were individually placed into 250 mL plastic cups, covered on top with a paper lid, held in the laboratory (25 ± 3 °C, 80% RH, and 12:12 h L:D) to record infected larvae. Larvae were fed with fresh corn leaves, each about 20 cm²/cup, replenished every 36 h until pupation. Corn leaves were disinfected by immersion for 2 min in a 0.5% sodium hypochlorite solution, then washed with sterile, distilled water (Sánchez & Bellotti 1997).

Mermithid nematodes that emerged from larvae were placed in 2 mL crystal vials with 70% ethanol, and were identified according Nickle (1972). Dead larvae showing signs of fungus infection were placed in plastic Petri dishes (60 × 10 mm) lined with filter paper moistened with sterile distilled water until the fungus sporulated on the insect (Fargues & Rodríguez-Rueda 1980). *Nomuraea rileyi* Farlow (Samson) (Moniliales: Moniliaceae) was isolated from dead larvae on medium composed of 200 mL of "V8" vegetable juice, 3 g CaCO₃, 5 g glucose, 2 g yeast extract, 15 g agar, and 800 mL distilled water (Fargues & Rodríguez-Rueda 1980). Fungal identification was according to Brady (1979) and Barnett & Hunter (1998). Larvae showing signs of infection by microsporidia were examined microscopically.

TABLE 1. LOCATION, DATE, COORDINATES AND ALTITUDE OF THE COLLECTION SITES OF FAW LARVAE IN LA FRAILESCA CHIAPAS, MEXICO.

Location	Date	Coordinates	Altitude (m)
Villaflores			
Úrsulo Galván	06/25/09	N 16° 16' 47" W 093°24'53"	0700
Juan María Garza	06/25/09	N 16°23'38" W 093°17'33"	0619
Rivera Horizonte	06/25/09	N 16°15'29" W 093°13'59"	0544
Dr. Domingo Chanona	06/25/09	N 16°20'46" W 093°25'00"	0669
Francisco Villa	06/25/09	N 16°12'45" W 093°20'11"	0581
Joaquín Miguel Gutiérrez	06/25/09	N 16°22'12" W 093°21'56"	0624
Villa Hidalgo	06/25/09	N 16°18'14" W 093°09'15"	0540
Villacorzo			
San Pedro Buena Vista	06/26/09	N 16°08'01" W 093°09'50"	0581
Revolución Mexicana	06/26/09	N 16°10'12" W 093°04'26"	0539
Parral	06/26/09	N 16°21'58" W 093°00'23"	0649
Valle Morelos	06/26/09	N 16°08'10" W 092°59'56"	0561
Monterrey	06/26/09	N 16°03'37" W 093°22'08"	0665
La Concordia			
La Tigrilla	06/26/09	N 15°59'13" W 092°51'04"	0580
Diamante de Echeverría	06/26/09	N 16°06'48" W 092°52'48"	0568
Independencia	06/27/09	N 16°05'23" W 092°50'25"	0546
Plan de Agua Prieta	06/27/09	N 16°07'52" W 092°47'29"	0540
Niños Héroes	06/27/09	N 16°06'35" W 092°37'23"	0602
A. Albino Corzo			
Querétaro	06/28/09	N 15°50'11" W 092°45'28"	0664
Nueva Palestina	06/29/09	N 15°48'19" W 092°44'59"	0725
Salvador Urbina	06/29/09	N 15°46'51" W 092°49'01"	0813
Francisco I. Madero	06/29/09	N 15°49'39" W 092°41'08"	0654
Libertad el Pajal	06/29/09	N 15°46'46" W 092°39'32"	0758

RESULTS AND DISCUSSION

Out of 1,247 larvae collected from corn in 22 locations of "La Frailesca" Chiapas, 204 larvae were found to be infected by mermithid nematodes, entomopathogenic fungi and microsporidia. Total mortality by these agents was 12.99%. Larval mortality by unknown causes was about 3.37% (42).

Mermithid nematode species *Hexameris* probably *albicans* Siebold (Nematoda: Mermithidae) caused the highest mortality rate of 8.42% (105 larvae), followed by *N. rileyi* causing 3.05% mortality (38 larvae), and an unidentified microsporidia causing 1.52% (19 dead larvae).

Thus mermithid nematodes were the most important natural enemies of FAW larvae. In the four locations, the 4th, 5th, and 3rd FAW instars were most parasitized by the mermithid *Hexameris* sp. with 34, 25, and 22 larvae or 2.73%, 2.00%, and 1.76% parasitized, respectively (Table 2).

Hexameris sp. has a wide distribution range, having been reported infesting FAW larvae in Honduras, Brazil, Nicaragua, and Argentina

(Van Huis 1981; Valicente 1989; Wheeler et al. 1989; Vera et al. 1995). In México, *Hexameris* sp. was reported in the states of Colima (Lezama-Gutiérrez et al. 2001), Nayarit and Veracruz (Molina-Ochoa et al., 2003) causing percentage mortality ranging from 0.0-14.9%, 0.0-3.33% and 0.0-15.05%, respectively. *Hexameris* sp., has been also reported attacking other insect pests such as *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae), and *Hypsipyla grandella* (Zeller) (Lepidoptera: Pyralidae) in Colombia (Vergara 2004).

Nomuraea rileyi was the only entomopathogenic fungus species isolated from dead FAW larvae, mostly 3rd to 6th instars. Twelve 3rd instars (0.96%), ten 4th and ten 5th instars (0.80%, respectively), and six 6th instars (0.48%) were infected by this fungus in the 4 locations (Table 2); larvae were fully covered with whitish hyphae, dusty green mass of *N. rileyi* spores, conidia as well as phialides (Bosa et al. 2004). The highest infection percentage caused by *N. rileyi* was exhibited by 3rd instars at 0.96%. We did not observe 1st and 2nd instars parasitized by *N. rileyi*; however, Bosa et al. (2004) emphasized that

TABLE 2. FALL ARMYWORM (FAW) LARVAL MORTALITY CAUSED BY PARASITIC NEMATODES, ENTOMOPATHOGENIC MICROORGANISMS AND UNKNOWN CAUSES IN "LA FRAILSCA", CHIAPAS, MÉXICO.

Instar	Locations				No. Infected FAW larvae	% parasitism per instar	FAW larval mortality (%)
	1	2	3	4			
<i>Hexameris</i> probably <i>albicans</i>							
I	0	0	0	0	0	0	
II	0	0	0	0	0	0	
III	7	6	4	5	22	1.764	
IV	9	10	7	8	34	2.726	
V	7	8	6	4	25	2.004	
VI	4	5	3	3	15	1.202	
VII	2	4	0	3	9	0.721	
					105		8.420
<i>Nomuraea rileyi</i>							
I	0	0	0	0	0	0	
II	0	0	0	0	0	0	
III	3	4	2	3	12	0.962	
IV	2	3	3	2	10	0.801	
V	5	2	2	1	10	0.801	
VI	2	1	1	2	6	0.481	
VII	0	0	0	0	0	0	
					38		3.047
Microsporidia							
I	0	0	0	0	0	0	
II	0	0	0	0	0	0	
III	2	1	1	2	6	0.481	
IV	1	2	0	0	3	0.240	
V	1	1	2	1	5	0.400	
VI	1	2	1	1	5	0.400	
VII	0	0	0	0	0	0	
					19		1.523
Unknown causes							
I	0	0	0	0	0	0	
II	0	0	0	0	0	0	
III	3	4	2	2	11	0.882	
IV	2	3	1	2	8	0.641	
V	3	3	1	1	8	0.641	
VI	1	2	2	1	6	0.481	
VII	3	2	2	2	9	0.721	
					42		3.368
Overall FAW larval mortality							16.358

Location 1, Villaflores; Location 2, Villacorzo; Location 3, La Concordia; Location 4, Ángel Albino Corzo.

2nd instar FAW was the most susceptible, but we found that the 5th instars suffered the highest mortality with 0.40% at Villaflores (Table 2). However, Molina-Ochoa et al. (2003), and Lezama-Gutiérrez et al. (2001) reported 44.4% and 16.7% for a single location in the state of Jalisco. *Nomuraea rileyi* caused an epizootic in Coahuila, México, and the number of rainy days and the

rainfall amount were possibly the most important environmental factors favoring the growth of the fungus (Ríos-Velasco et al. 2010).

An unidentified microsporidia that infected FAW larvae was the third most frequent cause of FAW total larval mortality (1.52%) (Table 2). Mortality ranged from 0.00 to 0.48%. Similar mortality percentages were reported by Lezama-Gutiérrez

rez et al. (2001) in the Mexican states of Colima, Jalisco and Michoacán. In these studies FAW larvae showed no obvious symptoms prior to their death, but after death the cadavers were often dry and fragile, resembling the ash of a cigarette. The identification was not verified by electron microscopy. Of the total larval mortality 4.24% was attributed to unknown causes (Table 2).

Regardless of location, the highest mortality rates caused by entomopathogens and parasitic nematodes were observed on the 3rd, 4th, 5th and 6th FAW instars (Table 2). Similar results were reported by Alcocer & Méndez (1965), who asserted that FAW last instar larvae allow the development of entomopathogens and parasitic nematodes. Gross & Pair (1991), stated that a parasitoid *Ophion flavidus* Brulle (Hymenoptera: Ichneumonidae), parasitized 4th, 5th, and 6th FAW instars with equal success, but was minimally successful in completing development on late 6th instar larvae.

Ruiz-Nájera et al. (2007) stated that the parasitoid complex, as part of the native natural enemy complex in corn fields of Chiapas can cause a reduction in the FAW larval populations. We also found that the distribution of the entomopathogens indicates a potential for biological control.

Results of this survey suggest a need for more taxonomic studies of microsporidia and parasitic nematodes in Mexico, particularly for the mermithid species of *Hexameris* in central Chiapas. We speculate that the augmentation and release of *N. rileyi* in La Frailesca, Chiapas corn fields during the rainy season would reduce FAW infestations and their damage to the crop.

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