Occurrence of Natural Enemies of Spodoptera frugiperda (Lepidoptera: Noctuidae) in Chihuahua, Mexico

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Occurrence of natural enemies of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Chihuahua, Mexico

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Abstract

All instars of fall armyworm (FAW), *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae), were collected in maize (corn) fields in 5 localities of the state of Chihuahua, Mexico, in 2014, with the main objectives of identifying its natural enemies and estimating the level of parasitism. Larvae were maintained under controlled conditions, fed with artificial diet, and observed daily until the emergence of parasitoids, until the appearance of mycosis, nematodes, or typical symptoms of baculoviral infection, or until they reached adulthood. Out of 5,870 larvae collected, 1,068 were attacked by natural enemies (parasitoids and entomopathogens), representing a total incidence of 18.2%. The incidence of parasitism by parasitoids was 8.1%, and parasitoids emerged from 5.8% of the larvae. The parasitoids found were: *Chelonus insularis* Cresson and *Meteorus arizonensis* Muesebeck (Hymenoptera: Braconidae); *Campoletis sonorensis* (Cameron), *Campoletis flavicincta* (Ashmead), *Pristomerus* sp. (Hymenoptera: Ichneumonidae); *Euplectrus platyhypenae* Howard (Hymenoptera: Eulophidae); and *Lespesia* sp. and *Archytas marmoratus* (Townsend) (Diptera: Tachinidae). *Meteorus arizonensis* and *C. flavicincta* were the most commonly encountered parasitoids, affecting 3% and 1.3% of the total collected larvae, respectively. Also, 2 species of entomopathogenic fungi were found: *Metarhizium rileyi* (Farl.) Kepler, S.A. Rehner & Humber comb. nov. (= *Nomuraea rileyi* [Farl.] Samson; Hypocreales: Clavicipitaceae) and *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae), with incidences of 8.6% and 0.65%, respectively. Forty-nine nucleopolyhedrovirus (Baculoviridae) isolates were obtained, corresponding to an incidence of 0.8%. Also, 0.07% of larvae were infected by entomopathogenic nematodes. In addition to the parasitoids and pathogens obtained, 34 specimens of the predator *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) were found during the surveys.

Key Words: biological control; fall armyworm; parasitoids; fungi; baculovirus; nematode

Resumen

Se recolectaron larvas de todos los instares de gusano cogollero *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae), en parcelas de maíz, en 5 localidades del estado de Chihuahua, México en el 2014, con el objetivo de identificar a sus enemigos naturales y estimar el nivel de parasitismo. Las larvas fueron mantenidas bajo condiciones controladas, alimentadas con dieta artificial, y observadas diariamente hasta la emergencia de parasitoides, la aparición de micosis, nematodos, síntomas típicos de infección viral o hasta que llegaron a la etapa adulta. De 5,870 larvas recolectadas, 1,068 fueron atacadas por enemigos naturales (parasitoides y entomopatógenos), representando una incidencia del 18.2%. La incidencia de parasitismo causada por parasitoides fue de 8.1%, y emergió el 5.8% de las larvas. Se encontraron los himenópteros parasitoides: *Chelonus insularis* Cresson, *Meteorus arizonensis* Muesebeck (Braconidae), *Campoletis sonorensis* (Cameron), *Campoletis flavicincta* (Ashmead), *Pristomerus* sp. (Ichneumonidae), *Euplectrus platyhypenae* Howard (Eulophidae), *Lespesia* sp. y *Archytas marmoratus* (Townsend) (Diptera: Tachinidae). *Meteorus arizonensis* y *C. flavicincta* fueron los parasitoides más comúnmente encontrados, afectando el 3% y 1.3%, del total de las larvas recolectadas, respectivamente. También, se encontraron 2 especies de hongos entomopatógenos: *Metarhizium rileyi* (Farl.) Kepler, S.A. Rehner & Humber comb. nov. (= *Nomuraea rileyi* [Farl.] Samson; Hypocreales: Clavicipitaceae) y *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) con incidencias de 8.6% y 0.65%, respectivamente. Se obtuvieron 49 aislados de nucleopolyhedrovirus (Baculoviridae) correspondiente a una incidencia del 0.8%. Un 0.07% de las larvas fueron infectadas por nematodos entomopatógenos. Adicionalmente, a los parasitoides y patógenos, se encontraron 34 especímenes del depredador *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) durante la recolecta.

Palabras Clave: control biológico; gusano cogollero; parasitoides; hongos; baculovirus; nematodos

The fall armyworm (FAW), *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae), is the main pest of maize (corn) and some other crops in Latin America (Hernández-Mendoza et al. 2008), causing yield reductions and economic losses (Sparks 1986; Casmuz et al. 2010). Chemical control is the practice most often used to control this insect pest; however, this method has been in-

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efficient due to incorrect and indiscriminate use, thus causing acute and chronic poisoning to farm workers, and inducing development of resistance, elimination of native natural enemies, and pollution of soil (Tinoco & Halperin 1998; Gómez-Valderrama et al. 2010). An alternative to the use of insecticides is to control this pest using native natural enemies (Rios-Velasco et al. 2011), which has great advantages, such as not having harmful effects on human health and environment. Also, natural enemies often are specific, some have high search capabilities, and the great majority can be handled easily and released in the field.

The parasitoids of FAW in the families Ichneumonidae, Braconidae, Eulophidae (order Hymenoptera) and Tachinidae (order Diptera) have been inventoried in the Mexican states Coahuila, Michoacán, Jalisco, Sinaloa, Nayarit, Veracruz, Colima, Yucatán, among others (Molina-Ochoa et al. 2004; Delfín-González et al. 2007; Rios-Velasco et al. 2011; Estrada-Virgen et al. 2013). Also, entomopathogenic bacteria, viruses, nematodes, and fungi have been reported (Molina-Ochoa et al. 2003; Hajek et al. 2007; Rios-Velasco et al. 2011). However, the fauna of natural enemies of FAW larvae in Chihuahua State has not been reported. Therefore, the main objectives of this study were to identify natural enemies of *S. frugiperda* and to estimate their level of parasitism in 5 localities of Chihuahua, Mexico.

Materials and Methods

All instars of *S. frugiperda* were collected from infested maize (corn) fields from Chihuahua, Mexico, in Aug and Sep 2014 (Table 1). Habitats surrounding maize fields were other crops as apple (*Malus pumila* Mill.; Rosales: Rosaceae) and soybean (*Phaseolus vulgaris* L.; Fabales: Fabaceae); a mix of noncrop vegetation predominated by sagebrush (*Artemisia* sp. L.; Asterales: Asteraceae) and grasses (Poales: Poaceae); and a forest-species complex composed primarily of hardwood/coniferous woodland species such as juniper *Juniperus* spp. (Pinales: Cupressaceae) and oak *Quercus* spp. (Fagales: Fagaceae). All corn fields had been without chemical pesticide application. Larvae were placed in 1 oz (29.6 mL) plastic cups (Grupo Convermex, S.A. de C.V. Puebla, Puebla, Mexico) with artificial diet (Southland Products, Inc., Village, Arkansas, USA). Collected larvae were transported to the Centro de Investigación en Alimentación y Desarrollo, A.C., (CIAD, Campus Cuauhtémoc, Chihuahua, Mexico) and held at 26 ± 2 °C, a 12:12 h photoperiod, and > 70% RH. Plastic containers were examined daily until the emergence of parasitoids, until the presence of mycosis, nematodes, or viral infections, or until FAW larvae reached adulthood.

The parasitoids obtained were identified using a stereoscope (Leica G26, Barrington, New Jersey, USA) and compound microscope (Carl Zeiss, Jena, Germany), and the keys published by Townes & Townes (1966), Cave (1993), Wharton et al. (1997), and Triplehorn & Johnson (2005). Confirmations of identifications were made by Dra. Juana Maria Coronado Blanco (Universidad Autónoma de Tamaulipas, Tamaulipas, México). Parasitoids were kept in 70% ethanol for preservation. Percentage parasitism was calculated based on the total number of FAW larvae that were positive for parasitoids or entomopathogens divided by the total number of larvae collected × 100 (Pair et al. 1986; Rios-Velasco et al. 2011).

The predator *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) was observed consuming FAW larvae in corn plots. Specimens were collected and placed in 1 oz (29.6 mL) plastic cups with 70% ethanol and identified using taxonomic and pictorial keys (University of Florida 2010; Rider 2012).

FAW larvae with mycosis were incubated in humid chambers, and the possible entomopathogenic fungi were isolated and purified in an artificial medium of potato dextrose agar (PDA) for *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) and V8-Agar for *Metarhizium rileyi* (Farl.) Kepler, S.A. Rehner & Humber comb. nov. (= *Nomuraea rileyi* [Farl.] Samson; Hypocreales: Clavicipitaceae) (Kepler et al. 2014). The purified entomopathogenic fungi were identified according to their microscopic and macroscopic characteristics using taxonomic keys in Barnett & Hunter (1998), Dugan (2006), and Watanabe (2010).

Larvae infected with nematodes were observed with a stereoscope (Leica G26, Barrington, New Jersey, USA) and compound microscope (Carl Zeiss, Jena, Germany) for identification of the nematodes with the taxonomic keys in Nickle (1972) and Nguyen & Smart (1996). However, identification to species level was not possible.

Larvae with symptoms of viral infection, such as white or dark color and fragility of the integument when dead, were obtained (Nicholls-Estrada 2008). To verify that virus was the cause of death, a sample was mounted on a slide and stained with 0.4% Giemsa stain (Sigma Aldrich, St. Louis, Missouri, USA) and/or Naphthol Blue Black (Sigma Aldrich, St. Louis, Missouri, USA) to observe the occlusion bodies (OBs) that showed polyhedric and granular characteristics of the nucleopolyhedrovirus (NPV) and granulovirus (GV) genera, respectively, for their identification. Also, a pathogenicity test was performed in which 100 µL of the OBs suspension was mixed with 100 µL of a solution containing 4% sucrose and blue food dye at 1%. In accordance with the droplet feeding method described by Hughes & Wood (1981), a suspension of 2 µL droplets was administered orally to a group of 2nd instars that previously had been subjected to a 24 h starvation period. After FAW larvae ingested the viral suspension, which was evidenced by a blue color inside the body, these larvae were transferred to plastic cups with artificial diet, held under controlled conditions (26 ± 2 °C, 12:12 h

Table 1. Geographical location of maize fields in the state of Chihuahua, Mexico, where *Spodoptera frugiperda* larvae were collected in 2014.

County	Plot	Geographical location	Altitude (m asl)
Cuauhtemoc	Los Adobes	28°40’59”N, 106°48’50”W	2,079
	Los Adobes II	28°41’10”N, 106°47’48”W	2,089
Cusihuiriachi	Reynaldo Ordóñez	28°11’56”N, 107°0’6”W	2,117
	Ernesto Márquez	28°12’48”N, 106°58’50”W	2,128
	Juan Ordóñez	28°12’44”N, 106°59’45”W	2,125
Namiquipa	Namiquipa	29°08’14”N, 107°23’38”W	1,875
	El Molino	29°12’19”N, 107°25’22”W	1,861
Bachíniva	Bachíniva	28°51’45”N, 107°18’47”W	1,949
Guerrero	Guerrero	28°33’52”N, 107°28’40”W	2,001

m asl: meters above sea level

photoperiod, and > 70% RH), and checked daily for symptoms of viral infection, such as loss of appetite and mobility, color change, or fragility and rupture of the integument (Caballero et al. 2001; Vasquez et al. 2002). Infected FAW larvae were placed in 15 mL polypropylene tubes with sterile distilled water and stored at −70 °C.

Results and Discussion

In total, 5,870 FAW larvae were collected from 9 locations with 1 or 2 sampling dates per location. From these samples, 473 larvae were found to be parasitized, but there was no emergence from 133 larvae, in which only the immature stages of the parasitoids were observed. Adult parasitoids emerged from 340 FAW larvae. These parasitoids represent species of Hymenoptera (Ichneumonidae, Braconidae, and Eulophidae) and Diptera (Tachinidae) (Table 2). Molina-Ochoa et al. (2004) found FAW larvae parasitized by specimens belonging to the families Braconidae (*Aleoides*, *Chelonus*, *Cotesia*, *Glyptapanteles*, *Homolobus*, and *Meteorus* genera), Ichneumonidae (*Campoletis*, *Eiphosoma*, *Ophion*, and *Pristomerus* genera), and Eulophidae (*Aprostocetus*, *Euplectrus*, and *Horismenus*) in Michoacán, Jalisco, Sinaloa, Nayarit, Veracruz, and Colima, Mexico, in various crops. In the present study, we found only 5 of the 13 genera reported by these authors. Parasitoid species found in this study were *Meteorus arizonensis* Muesebeck, *Campoletis sonorensis* (Cameron), *Campoletis flavicincta* (Ashmead), *Pristomerus* sp., *Chelonus insularis* (Cresson), *Euplectrus platyhypenae* Howard, *Lespesia* sp. *Archytas marmoratus* (Townsend), and other unknown tachinids of which twelve morphospecies were identified (Table 3). These results were similar to those reported by Delfín-González et al. (2007), who found *Lespesia archippivora* (Riley), *A. marmoratus*, and *E. platyhypenae* in the state of Yucatán, Mexico. Murúa et al. (2009) reported *Campoletis grioti* (Blanchard*), Ch. insularis*, *A. marmoratus*, and *E. platyhypenae*, as in this study. Additionally, they found *Archytas incertus* (Giglio-Tos), *Ophion* sp., and *Incamyia chilensis* (Aldrich). Further Jourdie et al. (2008) found *Cotesia marginiventris* (Cresson), *Chelonus cautus* Cresson, *Meteorus laphygmae* Viereck, *Pristomerus spinator* (F.), and *Eiphosoma vitticolle* Cresson. The data obtained suggest the existence of a great diversity of parasitoids species and parasitism levels, depending on geographic region, though all sites share many of the same species. The regional differences in parasitoid species and levels of parasitism may be caused largely by environmental differences, besides weather, adjacent crops, and alternate hosts. Also, extent of sampling, population density of the host, the level of adaptation of natural enemies, and population growth in both host and parasitoids, among other factors, will affect determination of natural enemies.

The overall parasitism level by natural enemies observed in this study, including both parasitoids and entomopathogens, was 18.2% (Table 3). In contrast, Estrada-Virgen et al. (2013) reported a parasitism level of 29.7% in the state of Nayarit, which was substantially greater than in this study. The most commonly encountered species of parasitoids were *M. arizonensis* and *C. flavicincta*, parasitizing 3.0% and 1.3% FAW larvae, respectively. These results are different from what was reported by Molina-Ochoa et al. (2003), who indicated that *Ch. insularis* was the most common and widely distributed parasitoid species occurring in Central America and North America. The tachinid flies recovered in this study, *Lespesia* sp. and *A. marmoratus,* showed parasitism rates of only 0.1% and 0.02%, respectively. However, a parasitism incidence of 0.56% by other unknown species of tachinids was found (Table 3).

Thirty-four specimens of the predator *P. maculiventris* were found consuming FAW larvae in corn fields. This species has been recognized

Table 2. Incidence of natural enemies of *Spodoptera frugiperda* larvae in Chihuahua, Mexico, in 2014.

				Parasitoids			Entomopathogens				
							Fungi[b]				
County	Plot	Date (day and month)	Total no. larvae	Parasitized larvae	Parasitoids emerged[a]	Parasitism (%)	*M. rileyi*	*B. bassiana*	Virus[c] NPV	Nematodes	Unknown causes
Cuauhtémoc	Los Adobes	29-Aug	375	20	16	0.34	22	0	0	1	66
		05-Sep	920	68	51	1.20	56	33	17	2	64
	Los Adobes II	19-Sep	888	84	45	1.40	16	0	0	0	65
		26-Sep	284	14	12	0.24	31	0	0	0	3
Cusihuiriachi	Reynaldo Ordóñez	28-Aug	136	10	3	0.17	1	2	0	0	3
	Ernesto Márquez	31-Aug	105	11	8	0.19	3	0	0	0	0
	Juan Ordóñez	03-Sep	780	93	79	1.60	18	3	8	0	88
		30-Sep	723	15	14	0.26	51	0	20	0	81
Bachíniva	Bachíniva	10-Sep	697	89	62	1.50	167	0	0	0	5
Namiquipa	Namiquipa	10-Sep	99	20	6	0.34	15	0	4	0	1
	El Molino	10-Sep	266	35	32	0.60	33	0	0	0	50
Guerrero	Guerrero	23-Sep	597	14	12	0.24	91	0	0	1	57
Total			5,870	473	340	8.10	504	38	49	4	483

[a]In the rest of the larvae, the parasitoids did not complete their development, and only the immature stage was observed.
[b]Fungal species were *Metarhizium rileyi*, *Beauveria bassiana*.
[c]NPV: Nucleopolyhedrovirus.

Table 3. Natural enemy taxa of *Spodoptera frugiperda* larvae found in maize fields from Chihuahua, Mexico in 2014.

	Localities						
Natural enemies	Cuauhtémoc	Cusihuiriachi	Bachíniva	Namiquipa	Guerrero	Total	Parasitism (%)
Parasitoids not emerged[a]	62	25	27	17	2	133	2.30
Hymenoptera							
Braconidae							
Chelonus insularis	8	1	5	1	0	15	0.25
Meteorus arizonensis	54	67	34	16	5	176	3.00
Ichneumonidae							
Campoletis sonorensis	2	2	0	0	1	5	0.10
Campoletis flavicincta	39	7	18	12	0	76	1.30
Pristomerus sp.	6	5	0	1	0	12	0.20
Eulophidae							
Euplectrus platyhypenae	6	1	4	4	0	15	0.25
Other hymenopteran species	1	1	0	0	0	2	0.03
Diptera							
Tachinidae							
Lespesia sp.	0	5	0	0	0	5	0.10
Archytas marmoratus	0	1	0	0	0	1	0.02
Unknown tachinids	8	14	1	4	6	33	0.56
Entomopathogens							
Metarhizium rileyi	125	73	167	48	91	504	8.60
Beauveria bassiana	33	5	0	0	0	38	0.65
Nucleopolyhedrovirus	17	28	0	4	0	49	0.80
Nematodes	3	0	0	0	1	4	0.07
Total	364	235	256	107	106	1,068	18.20

[a]Parasitoids did not complete development; only the immature stage was observed.

as a polyphagous predator associated with several orders of insects including Lepidoptera (De Clercq & Degheele 1992; Zanuncio et al. 2008).

The incidence of entomopathogenous fungi, viruses, and nematodes in this study was 10.1%, which was higher than the 3.8% found by Wyckhuys & O'Neil (2006) in Honduran subsistence maize. Five hundred forty-two (9.2%) FAW larvae showed fungal mycosis, of which 504 (8.6%) were infected by *M. rileyi* and 38 (0.65%) by *B. bassiana.* The high incidence of fungal infections in Bachiniva County (Table 2) may possibly be due to the significant level of precipitation (166.8 mm) and cool weather (18.5 °C and 18.8 °C for Aug and Sep 2014, respectively) that occurred in the days just prior to the sampling date (Unifrut 2014). Growth and germination of fungi are influenced strongly by environmental conditions, mainly by temperature and RH (Ignoffo & García 1985; Vimala-Devi et al. 1996; Rios-Velasco et al. 2010).

Forty-nine isolates of nucleopolyhedrovirus (Baculoviridae) were obtained from FAW larvae. Each infected larva was considered to be an isolate, and 0.8% of the larvae were infected with this entomopathogen. Similar results were obtained in Colombia by Gómez-Valderrama et al. (2010), who collected 2,140 larvae of which only 3 larvae were infected with nucleopolyhedrovirus, which corresponded to an occurrence rate of 0.14%. This result is also similar to that reported by Valicente & Barreto (1999), who collected 14,000 larvae, which produced 21 nucleopolyhedrovirus isolates, corresponding to an occurrence rate of 0.15%.

Among the collected larvae, 0.07% showed infection by nematodes. Molina-Ochoa et al. (2003) asserted that entomopathogenic nematodes have been shown to be agents with high potential for the control of the FAW. Four hundred eighty-three FAW larvae (8.2%) died from unknown causes, and the remaining larvae (4,319) reached adulthood (Table 2).

A great diversity of natural enemies of the FAW occurs in the state of Chihuahua, which is similar to other locations in Mexico and Central America, and may have potential to regulate population of this pest. Properly managed, this diversity may represent a viable alternative for implementation in integrated FAW management programs.

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