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Presence of corn earworm and fall armyworm (Lepidoptera: Noctuidae) populations in sweet corn and their susceptibility to insecticides in Puerto Rico

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Corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), and fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), are important pests of sweet corn, *Zea mays* L. (Poaceae), in the tropics and elsewhere (Belay et al. 2012; Bohnenblust et al. 2013). Larvae of both species feed on different plant parts (e.g., leaves, tassels, and ears) during the entire growing season, causing yield losses of over 20% (Marenco et al. 1992; Bohnenblust et al. 2013; Aguirre et al. 2016). The use of insecticides is the most effective strategy to control larvae of corn earworm and fall armyworm. However, resistance or tolerance to organophosphates (Hamadain & Chambers 2001; Carvalho et al. 2013; Zhu et al. 2015), pyrethroids (Jacobson et al. 2009; Carvalho et al. 2013), and Cry proteins of *Bacillus thuringiensis* Berliner (Bacillaceae) (Blanco et al. 2010; Huang et al. 2014; Monnerat et al. 2015; Zhu et al. 2015; Santos-Amaya et al. 2016; Reisig & Kurtz 2018) were reported for both species.

The knowledge of the abundance of these or others Lepidopteran species in different plant stages and the levels of susceptibility to various insecticides are important tasks to evaluate in an integrated pest management program. Most of the bioassays to identify resistance or susceptibility to insecticides in Puerto Rico have been conducted in fall armyworm populations. For instance, acephate, spinetoram, thiodicarb active ingredients, and combinations of chlorantraniliprole and spinetoram with the entomopathogenic nematode *Steinernema carpocapsae* (Weiser) (Nematoda: Steinernematidae) caused larval mortality of fall armyworm of over 60% in populations from Santa Isabel, and Lajas (Belay et al. 2012; Viteri et al. 2018). However, there are no reports if the larvae of corn earworm have similar levels of susceptibility to biological and synthetic insecticides compared to fall armyworm in Puerto Rico. Our objectives were to (1) identify which species were most prevalent in vegetative and reproductive stages in sweet corn, (2) evaluate the efficacy of 9 biological and synthetic insecticides to control larvae of corn earworm and fall armyworm, and (3) determine the lethal concentrations (LC₅₀) of chlorpyrifos and the concentrations of the entomopathogenic nematode *S. carpocapsae* + rapeseed oil to cause corn earworm larval mortality ≥ 50%.

Larvae in later instars (fourth–sixth) were collected from leaves, tassels, and ears of sweet corn cultivar ‘Suresweet 2011’ planted in Isabela, Juana Díaz, and Lajas Research Substations at the University of Puerto Rico in 2017 through 2018. A total of 1,260 larvae with morphological characteristics of fall armyworm (e.g., brown or gray larva with the presence of Y inverted on head capsule and 4 spots on

the 8 abdominal segment [Hardke et al. 2015]) were separated from those larvae without these characteristics at the 3 stations. A sample of 1,192 larvae of corn earworm displayed different colors, but with microspines on the cuticle, whereas the 30 larvae of the sugarcane borer were creamy white with black tubercles on each body segment. One larva of each species was placed in a 20 mL plastic cup (Lion, Santo Domingo, Dominican Republic) containing soy-wheat germ-based artificial diet (Frontier Scientific Services Inc., Newark, Delaware, USA) and reared until it reached the adult stage. In the case of *S. frugiperda*, the brown forewing ground color with contrasting markings, the small conspicuous white spot at the junction of M3 and CuAl veins, and a white patch at the apex of the forewing were used as major morphological characteristics for their identification (Pogue 2002). Furthermore, the extraction of the aedeagus from 658 adult males of *Helicoverpa* species was conducted to confirm the presence of 3 ventral lobes and 15 cornuti used for the identification of *H. zea* (Brambila 2014). Also, the presence of a tegumen as long as wide in the aedeagus of the sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae), was used for the identification of adult males (Martorell 1976).

For the bioassays, one fifth-instar larva of corn earworm or fall armyworm was placed separately in the aforementioned artificial diet cups. Fifteen larvae per repetition were treated topically with 200 µL insecticide solution of 3 biological agents, and 6 synthetic insecticides at high dosages (converted to lab dosages) (Table 1). The control was treated only with distilled water. Treated cups were held in a randomized complete block design with 4 replications (total $n = 60$ per location and species) in the lab at 18 to 20 °C, and a photoperiod of 12:12 h (L:D). Larval mortality was evaluated at 96 h after application. Also, in separate bioassays, insecticide dilutions of 1/2, 1/4, 1/8, and 1/16 of the low registered concentration of chlorpyrifos and *S. carpocapsae* + oil were applied to 60 larvae per dilution ($n = 240$ per treatment) to calculate the lethal concentrations (LC₅₀) of chlorpyrifos and *S. carpocapsae* + rapeseed oil after 120 h (where the low concentration was 2,400 ppm for chlorpyrifos and 1,250,000 nematodes per L + 2,500 ppm for *S. carpocapsae* + oil). Abbott’s formula (Fleming & Retnakaran 1985) was used to correct the data for control larval mortality in the bioassays and PROBIT analysis was conducted for chlorpyrifos. Also, LSD ($P \leq 0.05$) values were calculated to differentiate means among treatments.

Fall armyworm was observed in vegetative and reproductive stages in sweet corn in the 3 locations. However, the number of fall armyworm larvae was low in Isabela, and it was not possible to con-

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Table 1. Active ingredients, laboratory dosages, and percentages of mortality caused by 9 biological and synthetic chemical insecticides to fifth instar larvae of corn earworm (*Helicoverpa zea* (Boddie); Lepidoptera: Noctuidae) and fall armyworm (*Spodoptera frugiperda* (J. E. Smith); Lepidoptera: Noctuidae) at 96 h in 3 locations in Puerto Rico in 2017 and 2018.

Insecticides	Active ingredient and percentage	Commercial name	Manufacturer	Lab dosage		Corn earworm			Fall armyworm					
				g or mL per L	ppm	Isabela	Juana Díaz	Lajas	Juana Díaz	Lajas	% larval mortality			
												% larval mortality		
Biological agents														
<i>Chromobacterium subtsugae</i> 30%	Grandexo®	Marrone Bio Innovations		9.6 g	9,600	3.3	0.0	3.6	7.1	5.1				
	Heligen	AgBiTech		0.4 mL	400	19.0	0.0	0.0	—	—				
	Capsanem + Addit	Koppert		2.2 g + 2.5 mL	2,268 + 2,500	100.0	91.5	96.3	53.3	35.0				
Low-toxicity insecticides														
<i>Bacillus thuringiensis</i> 23.7%	Dipel®WG	Bayer		4.8 g	4,800	27.5	30.0	39.9	10.3	6.7				
	Coragen®	DuPont		0.6 mL	640	38.6	78.3	11.1	67.8	37.0				
	Radiant®SC	Dow AgroSciences		1.5 mL	1,480	63.8	77.6	37.0	78.6	54.3				
High-toxicity insecticides														
β-cyfluthrin 12.7%	Baythroid®XL	Bayer		0.4 mL	420	53.9	34.4	55.6	53.3	12.0				
	Warhawk®	Loveland		4.8 mL	4,800	97.9	100.0	100.0	98.1	100.0				
	Lannate®SP	DuPont		2.4 mL	2,400	31.0	8.9	13.3	88.5	96.4				
Methomyl 90%	—	—	—	—	—	48.3	46.7	39.6	57.1	43.3				
Mean	—	—	—	—	—	16.7	22.3	11.9	19.2	19.2				
LSD (<i>P</i> ≤ 0.05)														

duct the insecticide bioassays for this location. This might be caused by the absence of host plants (e.g., field corn, sorghum, and soybean) (Hardke et al. 2015) in this area compared to the southern part of Puerto Rico, where these crops are planted extensively, providing higher insect pressure during the entire yr. In fact, in Juana Díaz, the ratio of corn earworm and fall armyworm was, in general, 1:1 in ears except for Jun 2018, where an increase of the corn earworm population was observed (Table 2). This station is located close to farms where field corn and sorghum are the major crops planted for the entire yr. In Lajas and Isabela, corn earworm was the most abundant species observed in ears (Table 2). However, fall armyworm populations increased up to 46% from Oct to Nov 2018 in both locations. The sugarcane borer was observed only in Oct and Nov 2017, and Feb and Aug 2018 affecting ears in Lajas (Table 2). This species previously was reported attacking sugarcane and corn in Puerto Rico (Martorell 1976), the Caribbean region, and the southern United States (Joyce et al. 2014).

With respect to the insecticide susceptibility, *Chromobacterium subtsugae* Martin et al. (Neisseriales: Neisseriaceae) caused low levels of mortality (< 10%) for both species, and nucleopolyhedrovirus did not cause larval mortality in Juana Díaz and Lajas in corn earworm larvae. *Bacillus thuringiensis* caused higher mortality in corn earworm compared to fall armyworm (Table 1). These *B. thuringiensis* results are not new, due to the reported resistance to Cry proteins, especially in fall armyworm populations from Puerto Rico (Blanco et al. 2010; Zhu et al. 2015). In contrast, methomyl was highly effective (mortality > 80%) in fall armyworm, whereas it was < 35% in corn earworm. Chlorantraniliprole and spinetoram induced low levels of larval mortality for both species in Lajas (Table 1). Differences might be related to the prolonged use of these 2 insecticides or others having the same mode of action (e.g., spinosad and spinetoram) in corn winter nurseries planted in Lajas. Belay et al. (2012), and this study, reported higher larval mortality in Santa Isabel, Isabela, and Juana Díaz, where other active ingredients are used frequently in their integrated pest management programs. Larvae of corn earworm were highly susceptible to chlorpyrifos and the entomopathogenic nematode *S. carpocapsae* + oil (mortality > 95%) at 96 h. In fact, the LC₅₀ for chlorpyrifos was 248 ppm at 120 h, while 312,500 *S. carpocapsae* nematodes per L + 625 ppm of oil caused 53% larval mortality in the same period of time. These dosages are equivalent to 1/8 of the low

field dosages used for chlorpyrifos, 1/4 for *S. carpocapsae*, and 1/8 of the standard dosage used for the oil. Thus, repeated treatments of the entomopathogenic nematode might be applied directly to ears, either alone or in combination with chemical insecticides. Inside the ears, *S. carpocapsae* may be more efficacious for control of larvae, because the nematodes are not exposed to ultraviolet light, which affects their multiplication, propagation, and levels of infectivity (Shapiro-Ilan et al. 2006). Furthermore, combinations of *S. carpocapsae* with low-toxicity insecticides were reported to be highly effective in bioassays (Viteri et al. 2018) and field evaluations (D Viteri, personal communication) to control lepidopterans. Likewise, although high-toxicity insecticides are not recommended due to the adverse effects in the environment and human health (Mostafalou & Abdollahi 2013; Ding et al. 2015; Malhat et al. 2015), chlorpyrifos may be an option to decrease corn earworm and fall armyworm populations in severe infestations, which are common in tropical environments.

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Summary

Corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), and fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), are important pests in sweet corn. Our objectives were to assess the occurrence of the Lepidopteran species affecting sweet corn in Puerto Rico, and to evaluate the efficacy of 9 insecticides to control larvae of corn earworm and fall armyworm. *Spodoptera frugiperda* was observed in all plant stages, whereas *H. zea* and *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae) affected only ears. Larvae of corn earworm and fall armyworm were susceptible (mortality > 80% at 96 h) to *Steinernema carpocapsae* (Weiser) (Nematoda: Steinernematidae) + oil and to methomyl, respectively, whereas both species were susceptible to chlorpyrifos. The LC₅₀ values for chlorpyrifos was 248 ppm, whereas 312,500 *S. carpocapsae* nematodes per L + 625 ppm of rapeseed oil caused 53% of larval mortality at 120 h post-treatment for corn earworm larvae.

Key Words: *Helicoverpa zea* (Boddie); larval mortality; lethal concentrations; *Spodoptera frugiperda* (J. E. Smith); vegetative and reproductive stages

Table 2. Occurrence (expressed as relative %) of corn earworm (*Helicoverpa zea* (Boddie); Lepidoptera: Noctuidae), fall armyworm (*Spodoptera frugiperda* (J. E. Smith); Lepidoptera: Noctuidae), and sugarcane borer (*Diatraea saccharalis* (F.); Lepidoptera: Crambidae) in ears of sweet corn, *Zea mays* L. (Poaceae), in 3 locations at Puerto Rico during 2017 and 2018.

Date	Isabela		Juana Díaz		Lajas		
	Corn earworm	Fall armyworm	Corn earworm	Fall armyworm	Corn earworm	Fall armyworm	Sugarcane borer
Oct 2017	—	—	—	—	77.6	12.6	9.8
Nov 2017	—	—	—	—	59.9	26.1	14.0
Jan 2018	—	—	—	—	96.1	3.9	0.0
Feb 2018	98.5	1.5	—	—	97.2	1.4	1.4
Mar 2018	99.2	0.8	48.8	51.2	99.2	0.8	0.0
Apr 2018	—	—	—	—	—	—	—
May 2018	94.6	5.4	—	—	—	—	—
Jun 2018	—	—	71.7	28.3	—	—	—
Jul 2018	97.9	2.1	—	—	100.0	0.0	0.0
Aug 2018	—	—	—	—	75.1	24.7	0.2
Sep 2018	—	—	—	—	92.2	7.8	0.0
Oct 2018	90.8	9.2	57.2	42.8	55.8	44.2	0.0
Nov 2018	54.3	45.7	45.9	54.1	—	—	—
Mean	89.2	10.8	55.9	44.1	83.7	13.5	2.8

Sumario

El gusano de la mazorca del maíz, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), y el gusano cogollero, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), son plagas de importancia económica en el maíz dulce. Los objetivos de esta investigación fueron identificar las especies de Lepidópteros que afectan el maíz dulce y evaluar la eficacia de 9 insecticidas para el control de las larvas del gusano de la mazorca y el gusano cogollero. *Spodoptera frugiperda* fue observado en todos los estados fenológicos del maíz mientras que *H. zea* y *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae) afectaron solo las mazorcas. Las larvas del gusano de la mazorca y cogollero fueron susceptibles (mortalidad sobre el 80%) a *Steinernema carpocapsae* (Weiser) (Nematoda: Steinernematidae) + aceite y methomyl, respectivamente; mientras que las 2 especies fueron susceptibles a chlorpyrifos. Las CL₅₀ para chlorpyrifos fue de 248 ppm, mientras que 312,500 nemátodos de *S. carpocapsae* por L + 625 ppm de aceite de colza causó 53% de mortalidad en larvas a las 120 h después de la aplicación para el gusano de la mazorca.

Palabras Clave: *Helicoverpa zea* (Boddie); mortalidad larval; concentraciones letales; *Spodoptera frugiperda* (J. E. Smith); estados vegetativos y reproductivos

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