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Use of the entomopathogenic nematode *Steinernema carpocapsae* in combination with low-toxicity insecticides to control fall armyworm (Lepidoptera: Noctuidae) larvae

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Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is one of the more severe pests in corn, *Zea mays* L. (Poaceae), worldwide (Belay et al. 2012). Application of insecticides is the most effective strategy to control fall armyworm larvae. However, the excessive use of carbamates, organophosphates, pyrethroids (all high-toxicity insecticides), and even *Bacillus thuringiensis* (*Bt*) (Bacillaceae) have caused issues of insecticide resistance. For instance, Zhu et al. (2015) reported multiple/cross resistance to organophosphates and *Bt* in fall armyworm. Moreover, Blanco et al. (2010) and Huang et al. (2014) reported resistance to Cry1Fa proteins of *Bt* in fall armyworm larvae from Puerto Rico and Florida, respectively. Similarly, resistance to Cry1F (Monnerat et al. 2015; Santos-Amaya et al. 2016), organophosphates, and pyrethroids were reported in fall armyworm populations from Brazil (Carvalho et al. 2013). In addition, the indiscriminate use of high-toxicity insecticides has occasioned harmful effects for human and environment (Malhat et al. 2015; Mostafalou & Abdollahi 2013).

The use of active ingredients with low toxicity and risk to human health and the environment offer new alternatives to control fall ar-

myworm in corn production regions. For instance, methoxyfenozide, spinetoram, and spinosad killed over 80% in a fall armyworm population from Puerto Rico (Belay et al. 2012). Furthermore, the entomopathogenic nematode *Steinernema carpocapsae* (Weiser) (Nematoda: Steinernematidae) also has been used to control fall armyworm, though with low levels of host infectivity (from 1–28%) (Epsky & Capinera 1994). As with other biological agents, adverse environmental conditions or the absence of enough fall armyworm larvae for the establishment and propagation of *S. carpocapsae* might be the major causes of lower percentages of mortality.

Use of insecticide combinations in the same spray tank is a common practice for control fall armyworm (Negrisoli et al. 2010). However, insecticide compatibility, synergistic or antagonistic effects, and optimum dosages need to be assessed by bioassays before their use in the field. Our objective was to evaluate the efficacy of *S. carpocapsae* in combination with low-toxicity insecticides at low and high dosages to control fall armyworm larvae in bioassays.

Fifth-instar larvae were collected from leaves and tassels of sweet corn cv. 'Suresweet 2011' planted in plots without insecticide applica-

Table 1. Active ingredients and laboratory dosages to evaluate the efficacy of 1 biological agent, 5 synthetic insecticides, and least toxic combinations of insecticides to control fall armyworm [*Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)] larvae.

Insecticides	Commercial name ^a Active ingredient and percentage	Low dosage		High dosage	
		g or mL per L	PPM	g or mL per L	PPM
Biological agent (nematode)					
<i>Steinernema carpocapsae</i> (SC) + rape seed oil 85% ^b	Capsanem + Addit	1.1 g + 2.5 mL	1,134 + 2,500	2.2 g + 2.5 mL	2,268 + 2,500
Low-toxicity insecticides					
<i>Bacillus thuringiensis</i> 23.7% (<i>Bt</i>)	Dipel® WG	1.2 g	1,200	4.8 g	4,800
Chlorantraniliprole 18.4%	Coragen®	0.4 mL	440	0.6 mL	640
Spinetoram 11.7%	Radiant® SC	0.7 mL	740	1.5 mL	1,480
High-toxicity insecticides					
β-cyfluthrin 12.7%	Baythroid® XL	0.2 mL	240	0.4 mL	420
Methomyl 90%	Lannate® SP	1.2 mL	1,200	2.4 mL	2,400
Combinations					
<i>Bt</i> + SC + oil	—	1.2 + 1.1 + 2.5	—	4.8 + 2.2 + 2.5	—
Chlorantraniliprole + SC + oil	—	0.4 + 1.1 + 2.5	—	0.6 + 2.2 + 2.5	—
Spinetoram + SC + oil	—	0.7 + 1.1 + 2.5	—	1.5 + 2.2 + 2.5	—

^aManufacturers: Koppert (Addit and Capsanem), Bayer (Baythroid and Dipel), DuPont (Coragen and Lannate), and Dow AgroSciences (Radiant).

^bThis formulation of the nematode is always applied with rape seed oil.

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tions in Lajas Research Substation at the University of Puerto Rico in May 2017. One larva was placed in a 20 ml plastic cup containing wheat germ-based artificial diet. Low and high registered label dosages (converted to lab dosages) of 1 biological agent, 5 synthetic insecticides, and the biological agent + low-toxicity insecticides were used (Table 1). Fifteen larvae were treated topically with 200 μ L of insecticide solution per dosage. The control was treated only with distilled water. Treated cups were held in a randomized complete block design with 4 replications (total $n = 60$ larvae per dosage) in the lab at 18 to 20 °C and photoperiod 12:12 h (L:D). Larval mortality was evaluated between 24 and 96 h after application. In separate assays insecticide dilutions of $\frac{1}{4}x$, $\frac{1}{2}x$, x , $\frac{1}{2}y$, and y were applied to 60 larvae per treatment to calculate the lethal dosages (LD_{50}) of chlorantraniliprole and spinetoram at 96 h ($x =$ low dosage and $y =$ high dosage equals maximum registered and described in Table 1). Abbott's formula (Fleming & Retnakaran 1985) was used to correct the data for control larval mortality in the bioassays and PROBIT analysis was conducted. Also, LSD ($P < 0.05$) values were calculated to differentiate means among treatments.

In general, a higher mean percent mortality was noted at higher dosages ($F = 84.30$; $df = 1$; $P < 0.001$) (Table 2). However, *S. carpocapsae* and chlorantraniliprole caused 30 to 37% of larvae mortality with low and high dosages at 96 h. The use of spinetoram resulted in percentages of mortality up to 54.3% at 96 h at the higher dosage. These results are different from those reported by Belay et al. (2012), where chlorantraniliprole and spinetoram caused larvae mortality over 80% at the same period. In fact, the LD_{50} was 2,754 ppm for chlorantraniliprole and 851 ppm for spinetoram at 96 h in this study. Differences might be related to (1) the prolonged used of these active ingredients caused some level of resistance, (2) instar stage, third vs fifth used in this research, or (3) differences among fall armyworm populations on the island. *Bt* and β -cyfluthrin had the lowest percentages of mortality ($< 15\%$), as expected. In contrast, methomyl caused 81 and 95% of larvae mortality in low and high doses, respectively, at 48 h. Resistance to *Bt* and pyrethroids were reported in fall armyworm, as mentioned above, and to carbamates (e.g., carbaryl, methomyl, and thiodicarb) in fall armyworm populations from Florida (Yu 1991), and to methomyl in

some regions of Puerto Rico (Mota D, personal communication). Interestingly, a susceptible fall armyworm population to methomyl was identified in Puerto Rico in this study and might help to conduct genetic studies of resistance to carbamates in the future.

The use of *S. carpocapsae* in combination with chlorantraniliprole or spinetoram produced higher percentages of mortality at 24 h compared with the use of these insecticides alone regardless of the dosage used (Table 2). Furthermore, the highest percentages of larval mortality (over 90%) were noted with high dosages at 72 h. Likewise, the combination of *Bt* + *S. carpocapsae* was effective (81.3% of dead larvae) with the high dose at 96 h compared with the lowest mortality caused by *Bt* (6.7%), or *S. carpocapsae* (35%) applied alone. Apparently, larvae exposed to 2 different modes of action [septicemia (*S. carpocapsae*) + lysed midgut epithelial cells (*Bt*), impaired regulation of muscles (chlorantraniliprole), or abnormal neural transmission (spinetoram)] at the same time, caused their higher mortality. However, further research is required to corroborate this synergistic effect.

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Summary

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), has resistance to many groups of synthetic insecticides. Our objective was to evaluate the efficacy of *Steinernema carpocapsae* (Weiser) (Nematoda: Steinernematidae) in combination with low-toxicity insecticides at low and high dosages to control fifth-instar larvae in bioassays. The use of *S. carpocapsae* + chlorantraniliprole or spinetoram caused larvae mortality of over 90% at 72 h at the high dose and should be included as a least toxic strategy to control fall armyworm.

Key Words: dosages; efficacy; larvae mortality; *Spodoptera frugiperda* (J. E. Smith); synergistic effect

Table 2. Percentage of mortality of fall armyworm [*Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)] larvae in fifth-instar at low and high dosages of 6 insecticides, and least toxic combinations among them evaluated from 24 to 96 h in Lajas Research Substation at the University of Puerto Rico in 2017.

Insecticides	24 h		48 h		72 h		96 h	
	Low	High	Low	High	Low	High	Low	High
Biological agent (nematode)								
<i>Steinernema carpocapsae</i> (SC)	13.3	12.0	20.7	19.0	28.5	33.9	30.8	35.0
Low-toxicity insecticides								
<i>Bacillus thuringiensis</i> (<i>Bt</i>)	1.7	2.4	4.1	2.4	4.1	6.7	8.7	6.7
Chlorantraniliprole	6.7	3.3	7.5	9.4	18.4	18.4	30.4	37.0
Spinetoram	18.3	28.3	35.8	32.1	36.7	51.0	47.8	54.3
High-toxicity insecticides								
β -cyfluthrin	1.9	10.0	1.9	12.0	2.2	12.0	2.2	12.0
Methomyl	76.7	81.7	81.0	94.8	83.9	94.8	83.9	96.4
Combinations								
<i>Bt</i> + SC	20.0	26.7	20.7	53.4	35.7	75.0	45.8	81.3
Chlorantraniliprole + SC	50.0	65.0	50.0	73.2	75.0	91.7	89.7	94.9
Spinetoram + SC	41.7	88.3	80.4	94.6	91.7	100.0	97.0	100.0
Mean	25.6	35.3	33.6	43.4	41.8	53.7	48.5	57.5
LSD ($P < 0.05$)	12.2	12.6	12.2	15.4	17.7	20.1	18.3	19.2

Sumario

El gusano cogollero [*Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)] tiene resistencia a diferentes grupos de insecticidas sintéticos. El objetivo fue evaluar la eficacia de *Steinernema carpocapsae* (Weiser) (Nematoda: Steinernematidae) en combinación con insecticidas de baja toxicidad a dosis bajas y altas para el control de larvas de quinto estadio en bioensayos. El uso de *S. carpocapsae* + chlorantraniliprole o spinetoram causó un porcentaje de mortalidad de larva de más del 90% a las 72 h en la dosis alta, y debería ser incluido como una estrategia menos tóxica para el control del gusano cogollero.

Palabras Clave: dosis; eficacia; mortalidad de larvas; *Spodoptera frugiperda* (J. E. Smith); efecto sinérgico

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