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Mortality and food consumption in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae treated with spinosad alone or in mixtures with a nucleopolyhedrovirus

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Spinosad (a mixture of spinosyns A and D produced during fermentation of the soil actinomycete *Saccharopolyspora spinosa* Mertz and Yao; Actinomycetales: Pseudonocardiaceae) and the *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV) (Baculoviridae) provide alternatives to conventional insecticides for control of the major pest of maize in the Americas, the fall armyworm, *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) (Williams et al. 1999; Méndez et al. 2002; Martínez et al. 2003; Barrera et al. 2011; Ríos-Velasco et al. 2011). Méndez et al. (2002) showed that *S. frugiperda* larvae that consumed mixtures of spinosad and SfMNPV in semi-synthetic diet bioassays had a significant increase in mortality compared with insects that consumed either of these 2 components alone.

In this study, we evaluated the activity of spinosad on 3rd instars of *S. frugiperda* fed on maize leaves and determined the effects of spinosad–SfMNPV mixtures on larval mortality. We also evaluated the effects of spinosad alone and spinosad–SfMNPV mixtures on food consumption. The *S. frugiperda* colony was maintained in a growth chamber (26 ± 2 °C, 16:8 h L:D photoperiod, 70 to 80% RH) and fed a semi-synthetic diet (Poitout & Bues 1974). SfMNPV was isolated in Nicaragua and was previously characterized by Escribano et al. (1999). Virus occlusion bodies (OBs) were produced in 4th instars of *S. frugiperda*.

Larvae used in the bioassay to evaluate spinosad activity were reared in ventilated plastic cages (25 × 17.5 cm, 4 cm high) and fed from the time of emergence with true maize leaves surface-sterilized with 0.01% sodium hypochlorite and rinsed twice with sterile distilled water. Third-instars were starved for 8 to 12 h and then allowed to feed on maize-leaf pieces (20 × 10 mm), which were obtained from surface-sterilized expanding true maize leaves. Groups of 12 maize-leaf pieces had been dipped as described by Evans & Shapiro (1997) for 15 s in 1 of 5 concentrations of spinosad (ranging from 0.001 to 3.16 mg/L) (Tracer®, 48% spinosad, suspension concentrate; Dow AgroSciences, Zamora, Michoacán, Mexico). To enhance the wetting of the maize-leaf pieces, the surfactant sodium dodecyl sulfate (SDS) was used at 0.001% (w/v). Control pieces were dipped in distilled water plus 0.001% SDS. Treated maize-leaf pieces were allowed to dry for 1 h and then individually placed into cylindrical wells (each with a 22.1 mm bottom diameter) of 12-well Costar® tissue culture plates (Corning®, New York, New York, USA). To delay leaf dehydration, treated maize-leaf pieces were placed on a wet paper towel. Treated maize-leaf pieces were removed 3 d later, and surviving larvae were individually transferred into cylindrical wells (each with a 15.6 mm bottom diameter) of a 24-well Costar® tissue culture plate containing semi-synthetic diet. The larvae were checked for mortality at 24 h intervals. Each bioassay was performed 4 times. A separate bioassay was performed to simultaneously evaluate the effects of an ultra-low concentration of spinosad alone (0.001 mg/L) and spinosad–SfMNPV (1×10^6 OBs/mL) mixture on mortality and food consumption of 3rd instars of *S. frugiperda*. The bioassay was performed in the same way as described above, i.e., we added 0.001% SDS to the SfMNPV solutions to guarantee the virus dispersion, and into this solution we dipped pieces of maize leaves.

The LC_{50} for spinosad was calculated using probit regression analysis against log [spinosad concentration] using the POLO PC program (LeOra Software 1987), whereas for virus alone or in a mixture with the lowest concentration of spinosad, the expected mortality (E) was determined as described later in the text. The food consumption of 3rd instars of *S. frugiperda* was determined on each maize-leaf piece from activity of spinosad alone and spinosad–SfMNPV mixture bioassays described above. Images of these pieces were segmented using the software GIMP v.2.6.6 (GNU Image Manipulation Program, http://www.gimp.org) to distinguish between intact and consumed areas. The consumption rate was expressed as the average percentage of leaf area consumed per surviving larva, as described by Pineda et al. (2014), and by taking into account the number of larvae alive at 72 h. Food consumption was analyzed using 1-way ANOVA (MSD, $P < 0.05$).

The expected mortality, E, from spinosad–SfMNPV mixture bioassays was considered an independent variable (Finney 1964) and determined according to Equation 1.

$$E = [O_{NPV} + O_{SPIN}(1 - O_{NPV})] \times 100 \qquad (1)$$

where O_{NPV} is the proportional mortality produced by SfMNPV alone and O_{SPIN} is the proportional mortality produced by spinosad alone.

The effects were classified as antagonistic, additive, or synergistic using χ^2 comparisons according to Trisyono & Whalon (1999) and Hummelbrunner & Isman (2001).

The effectiveness of spinosad against lepidopteran pests has been recognized widely (Salgado 1997; Pineda et al. 2004; Sántis et al. 2012). In our study, the LC_{50} value for spinosad was calculated as 0.39 mg/L at 48 h after leaf consumption (range of 95% C.I.: 0.14 to 1.43 mg/L) ($y = 0.78 \pm 0.08$) ($\chi^2 = 6.2$, df = 4, $P < 0.05$). This value was 7.6- and 5.4-fold lower than the values previously obtained using diet surface contamination (2.98 ppm) and diet incorporation bioassays (2.1 mg/kg), respectively, against 2nd instars of *S. frugiperda* and 3rd instars of

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Table 1. Interaction of nucleopolyhedrovirus (SfMNPV) and the insecticide spinosad in feeding bioassays with *Spodoptera frugiperda* 3rd instars.

Hours after treatment	Conc. SfMNPV (OBs/mL)	Conc. spinosad (mg/L)	Number dead	Number tested	Percentage mortality observed	Percentage mortality expected	χ^2	Effect
144	0	0	0	180	0.0	n/a	n/a	n/a
	10^6	0	12	48	25.0	n/a	n/a	n/a
	0	0.001	18	48	37.5	n/a	n/a	n/a
	10^6	0.001	33	48	68.8	53.1	4.60	Synergy
168	10^6	0	19	48	39.6	n/a	n/a	n/a
	0	0.001	18	48	37.5	n/a	n/a	n/a
	10^6	0.001	33	48	68.8	62.2	0.86	Additive

Tabular $\chi^2 = 3.84$ with df = 1 and $P = 0.05$. n/a = not applicable.

Spodoptera littoralis Boisduval, respectively (Méndez et al. 2002; Pineda et al. 2004), and 4.5-fold higher than the value previously obtained using diet incorporation bioassays (0.081 mg/kg) against 3rd instars of *Spodoptera exigua* Hübner (Osorio et al. 2008). Differences in toxicity caused by spinosad between semi-synthetic and natural diet bioassays may simply reflect differences in the bioassay methods and species tested. In addition, maize is the principal host plant of *S. frugiperda*, which may result in a greater consumption of insecticide-treated plant surface than on other host plants (Pineda et al. 2014).

When conducting diet surface contamination assays, Méndez et al. (2002) observed that a high spinosad concentration (3 ppm) plus SfMNPV at 20 or 70 OBs/mm^2 had a synergistic effect on mortality of *S. frugiperda* 2nd instars. Here, we used an ultra-low spinosad concentration (0.001 mg/L) and also found that the application of spinosad–SfMNPV mixtures caused higher mortality than treatments with the individual components of the mixture (Table 1); however, this observed mortality was significantly greater than expected only at 144 h after treatment, i.e., a synergistic effect. The biological explanation for this synergistic effect is currently unknown, but the cause may be an initial response to spinosad that subsequently enhanced the deadly effects of the virus. In this regard, we observed that when larvae were treated with spinosad alone or spinosad–SfMNPV mixtures, larval mortality with clear symptoms of spinosad toxicity began at 48 h after treatment, whereas symptoms of virus-induced mortality in larvae treated with spinosad–SfMNPV mixtures began at approximately 96 h after treatment.

In our study, all concentrations of spinosad in the range of 0.001 to 0.316 mg/L reduced food consumption of larvae between 2- and 12-fold at 2 d after treatment compared with the control ($\chi^2 = 90.6$, $P < 0.01$; Kruskal Wallis test) (Fig. 1). Similarly, Sántis et al. (2012) observed that the consumption rate by 3rd instars of *S. exigua* of pepper leaves treated with spinosad (60 to 120 mg/L) decreased between 4- and 6-fold at 2 d after treatment compared with the control. In another study, spinosad caused reductions up to 98% in the consumption rates of 3rd and 5th instars of *S. exigua* (Yee & Toscano 1998). In the case of the spinosad–SfMNPV mixture, food consumption (20.8 ± 3.1%) was significantly reduced by approx. 2-fold compared with the control (34.7 ± 2.9%) ($F_{2,94} = 10.25$, $P < 0.001$). However, no significant difference in food consumption was observed between the spinosad–SfMNPV mixture and spinosad alone (0.001 mg/L) (19.2 ± 2.4%), which, also, may indicate a faster response to spinosad than to SfMNPV. The reason for this reduction may be related directly to the mode of action of the neurotoxin spinosad. Neurotoxic insecticides cause insect paralysis (Salgado 1997) and, consequently, a cessation of feeding. In general, reduction in food consumption is very important from a practical point of view, because it reduces the damage caused to crops by larval feeding. We conclude that *S. frugiperda* is highly susceptible to spinosad. Also, we recognize that the effects of spinosad–SfMNPV mixtures require validation in field trials to determine if such mixtures may offer a consistent and efficient means for control of this pest.

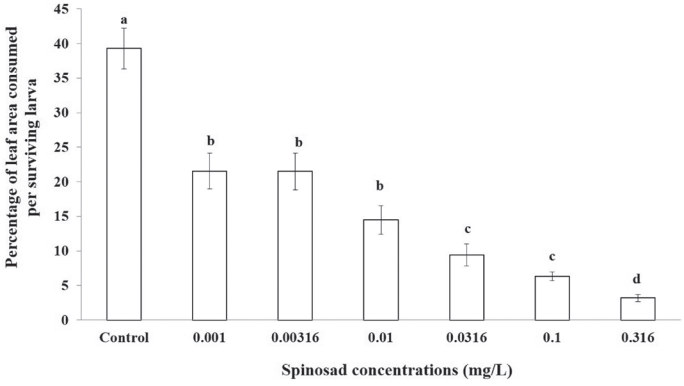

Fig. 1. Percentage (mean ± SE) of leaf area consumed per surviving *Spodoptera frugiperda* 3rd instar feeding either on untreated maize-leaf pieces or on maize-leaf pieces treated with spinosad (mg/L). Mortality was recorded at 72 h after treatment. Different letters above the error bars indicate statistically significant differences based on the Kruskall-Wallis test ($P < 0.05$).

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Summary

The combined and individual effects of spinosad and *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV) (Baculoviridae) on the mortality and food consumption of 3rd instars of *S. frugiperda* were evaluated using maize-leaf bioassays. The mortality data from spinosad (0.001 mg/L)–SfMNPV (10^6 OBs/mL) mixtures showed a low synergistic effect at 144 h after treatment. The consumption rate was significantly reduced by 2- to 12-fold in the spinosad–SfMNPV mixture treatment compared with the control, but no difference in consumption rate was observed between this treatment and the treatment with spinosad alone. Laboratory observations on the efficiency of spinosad–SfMNPV mixtures require validation in field studies under commercial growing conditions.

Key Words: baculovirus; fall armyworm; synergism

Sumario

El efecto combinado y solo de spinosad con el nucleopoliedrovirus múltiple de *Spodoptera frugiperda* (SfMNPV) (Baculoviridae) se evaluaron sobre sobre la mortalidad y tasa de consumo de larvas de tercer estadio de *S. frugiperda* en condiciones de laboratorio mediante

bioensayos con hojas de maíz. Los datos de mortalidad de la mezcla de spinosad (0.001 mg/L)–SfMNPV (10^6 OBs/mL) provocó un efecto sinérgico a las 144 h después del tratamiento. La tasa de consumo se redujo significativamente de 2- a 12-veces en la mezcla spinosad–SfMNPV comparado con el testigo, pero no se observaron diferencias comparado con el spinosad solo. Las observaciones de laboratorio sobre la mezcla de spinosad–SfMNPV requieren ser validadas en estudios de campo bajo condiciones de cultivos comerciales.

Palabras Clave: baculovirus; gusano cogollero; sinergismo

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