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Toxic Effects of Spinosad on Predatory Insects

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Spinosad (Dow AgroSciences) is a mixture of tetracyclic-macrolide compounds produced by a soil actinomycete and has been classified as a bioinsecticide. Spinosad is highly active against Lepidoptera but is reported to be practically nontoxic to insect natural enemies. We assessed the impact of Spinosad in a granular maize-flour formulation on a selection of insect predators over periods of 2–14 days. In all cases, the quantities of Spinosad used were less than the maximum recommended rates given on the product label. Adults of *Aleochara bilineata* Gyllenhal (Coleoptera: Staphylinidae) suffered a high prevalence of mortality following consumption of 1000 or 2000 ppm Spinosad active ingredient (a.i.), but little mortality at 200 ppm. Larvae of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) did not consume the granular formulation and suffered little overall mortality. After 14 days of exposure, the earwig, *Doru taeniatum* (Dohrn) (Dermaptera:Forficulidae), suffered 48% mortality in the 1.2 ppm Spinosad treatment increasing to 98% in the 1200 ppm Spinosad treatment compared to 20% in controls. Earwigs suffered 86% mortality/intoxication 72 h after feeding on Spinosad-contaminated *Spodoptera frugiperda* J. E. Smith (Lepidoptera:Noctuidae) larvae. A field trial was performed to compare applications of commercial granular chlorpyrifos and Spinosad in maize-flour granules (200 and 2000 ppm a.i.; 4.8–48 g a.i./ha, respectively) or as an aqueous spray (160 ppm a.i.; 48 g a.i./ha) on earwigs held inside gauze bags. Mortality of earwigs on control plants was less than 15% at 2 days postapplication compared to 33% on plants treated with granular chlorpyrifos, 83% on plants sprayed with 160 ppm Spinosad, and 91–95% on plants treated with 200–2000 ppm Spinosad granules, respectively. Further mortality in the 24-h period postsampling ranged from <5% in control treatments, to 9% in the chlorpyrifos treatment, and to 55–65% in the Spinosad spray and granule treatments. We conclude that Spinosad cannot be considered to have an environmental safety profile similar to most established biological insecticides.

Key Words: **Spinosad; toxicity; natural enemies; formulation; Forficulidae.**

INTRODUCTION

The impact of synthetic pesticides on beneficial arthropods and the human health risks posed by exposure to these chemicals are issues of growing concern (National Research Council, 1996; Casida and Quistad, 1998). This has prompted the development of new compounds, such as imidacloprid, oxamyl, and cyfluthrin, with reduced environmental persistence and low mammalian and avian toxicity but a fairly broad spectrum of insecticidal activity (Harris, 2000). An example is Spinosad (Dow AgroSciences), a mixture of spinosyns A and D that are tetracyclic-macrolide compounds produced by an actinomycete, *Saccharopolyspora spinosa* Mertz and Yao, isolated from a Jamaican soil sample (Sparks *et al.*, 1998). As these products are created by biosynthesis during fermentation of *S. spinosa*, Spinosad has been classified as a bioinsecticide (Copping and Menn, 2000).

Spinosad is primarily a stomach poison with some contact activity and is particularly active against Lepidoptera and Diptera. It is a neurotoxin with a novel mode of action involving the nicotinic acetylcholine receptor and apparently the GABA receptors as well (Salgado, 1997, 1998). Exposure results in cessation of feeding followed, some 24 h later, by paralysis and death. Conventional toxicity tests indicate that Spinosad has virtually no toxicity to birds and mammals. With a contact LC_{50} value of $\geq$200 ppm, Spinosad has also been reported to be practically nontoxic to insect natural enemies such as *Orius* spp., *Chrysopa* spp., coccinelids, and the predaceous mite *Phytoseiulus persimilis* Athias-Henriot (Bret *et al.*, 1997). Additional studies in which Spinosad-treated aphids were fed to coccinelid and chrysopid larvae reported no predator mortality (Schoonover and Larson, 1995).

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Spinosad is classified by the U.S. Environmental Protection Agency as an environmentally and toxicologically reduced risk material (Saunders and Bret, 1997). As a result, the marketing strategy for Spinosad has focused heavily on its favorable environmental profile, reflected in the trade name "Naturalyte" used for this group of insect control products. Indeed, the safety profile of Spinosad has been described as similar to that of benign biological pesticides (Thompson and Hutchins, 1999).

Formulation can have a marked impact on the biological activity of a pesticide toward both target and nontarget arthropod species (Dahl and Lowell, 1984; Croft, 1990). The formulation of microbial insecticides with baits or phagostimulants can result in a substantial increase in the degree of control achieved (Burges and Jones, 1998). Feeding stimulants based on nixtamalized maize flour and corn oil have shown promise for the formulation of *Bacillus thuringiensis* and nucleopolyhedroviruses for control of maize pests (Tamez-Guerra *et al.*, 1996, 1998, 2000a,b).

As part of a program on biorational control of fall armyworm larvae, *Spodoptera frugiperda* J. E. Smith (Lepidoptera:Noctuidae), in Mesoamerican maize crops, we began to assess the efficiency of Spinosad in a granular maize-flour formulation. This represents an experimental formulation and does not appear on the product label. An important aspect of this study involved evaluating the impact of this formulation toward a range of insect predators. The aim of the study was not to determine precise concentration–mortality relationships, but rather to evaluate the likelihood of adverse effects on predator populations that were exposed to Spinosad in phagostimulant granules and in the corpses of *S. frugiperda* larvae that had died from exposure to Spinosad.

MATERIALS AND METHODS

Insects

Pupae of *Aleochara bilineata* Gyllenhal (Coleoptera: Staphylinidae) were obtained from De Groene Vlieg (The Netherlands). On emergence, adults were fed on minced beef and maintained at 24°C, 9 h:15 h (L:D) for up to 1 week prior to use in experiments. Eggs of *Chrysoperla carnea* (Stephens) (Neuroptera:Chrysopidae) were obtained from Bioplanet (Italy) and maintained in culture for one to two generations at the University of Southampton before use in experiments. Chrysopid larvae were reared in groups of 30–40 in 10-cm-diameter petri dishes with mesh lids and fed on eggs of *Sitotroga cereallella* (Olivier) *ad libitum*. Adults were provided with water and 25% honey solution, each on a cotton wool pad, and also with 0.5 ml of a diet mix (15 ml condensed milk, one entire hen's egg, yolk of one hen's egg, 30 g honey, 20 g fructose, 30 g brewer's yeast, 50 g wheatgerm, and 45 ml distilled water). Temperature and light regimes used in culture were as for *A. bilineata*. Adults and final-instar nymphs of the earwig *Doru taeniatum* (Dohrn) (Dermaptera:Forficulidae) were collected from maize fields in the vicinity of Tapachula, Chiapas, Mexico, and held in ventilated plastic containers at 22 ± 1°C, 12 h:12 h (L:D) for up to 1 week prior to their use in experiments. Earwigs were fed at 24- to 48-h intervals on larvae of *Galleria mellonella* (L.) (Lepidoptera:Pyralidae) obtained from a laboratory culture.

Preparation of Granules

A sample of the commercial formulation of Spinosad (Tracer Naturalyte insect control) was obtained as a gift from Dow AgroSciences, Mexico. Tracer contains 480 g of Spinosad active ingredient (a.i.) per liter. Phagostimulant granules were prepared by mixing 160 g nixtamalized maize flour, 34 g pregelatinized starch, 16 ml of corn oil, and 200 ml of distilled water to form a dough which was left to stand for 20 min before being passed through a wire gauze with a mesh aperture of 1.2 mm. During this procedure, the dough crumbled into irregular granules approximately 1 mm wide and 0.5 to 3 mm in length. These granules were placed next to a fan ventilator and allowed to air dry for 16 h at 25 ± 1°C prior to use. For granules containing Spinosad, the appropriate quantity of Tracer was added to the water component (appropriately adjusted to account for the volume of the product) and mixed thoroughly in to ensure homogeneous incorporation prior to being passed through the wire gauze.

Field trials with these granules have indicated that a suitable application rate is 24 kg/ha (J. Cisneros, unpublished data). Granules were therefore prepared that contained between 0.03 and 48 g Spinosad/24 kg of granule, representing concentrations from 1.2 to 2000 parts per million (ppm) Spinosad (Table 1). These quantities ranged from slightly less than product label recommended spray application rates down to >1000-fold less than might be applied in the field. These concentrations are hereafter referred to in terms of parts per million Spinosad a.i. in each experimental preparation (Table 1).

Mortality of Natural Enemies Exposed to Spinosad in Granules

Aleochara bilineata. Three adult female and three adult male *A. bilineata* were confined in a 5-cm-diameter petri dish with a mesh lid and maintained at 24°C, 9 h:15 h L:D. Each dish was lined with damp sand and sprinkled with 0.5 g of phagostimulant granules containing 0, 2, 20, 200, 1000, and 2000 ppm Spinosad. Raw minced beef was provided *ad libitum* as food. Mortality, taken as the absence of response to being

TABLE 1

Details of Quantities and Concentrations of Spinosad Recommended on the Product Label for Field Applications and Those Used in the Experiments Described in the Present Study

Type of application	Quantity of Spinosad (g a.i./ha)	Volume of spray or weight of granular application (/ha)	Concentration of Spinosad (ppm a.i.)
Product label recommendations for 1-ha application			
Ground spray	67–102[a]	56.8[b] liters	1180–1800
Aerial spray	67–102[a]	22.8[b] liters	2950–4480
Quantities used in our study			
Spray application			
Laboratory	60.00	300 liters	200.0
Field trial	48.00[c]	300 liters	160.0
Granular formulation[d]	48.00[c]	24 kg	2000.0
	29.00	24 kg	1200.0
	24.00	24 kg	1000.0
	4.80[c]	24 kg	200.0
	0.50	24 kg	20.0
	0.30	24 kg	12.0
	0.05	24 kg	2.0
	0.03	24 kg	1.2

[a] For control of armyworms in maize or sorghum, the Tracer product label recommends 140–213 ml/ha (Tracer contains 480 g Spinosad a.i./liter).
[b] Minimum recommended volumes of water (liters/ha).
[c] Quantities of Spinosad used in field trial; all other quantities were tested in the laboratory.
[d] The granular maize-flour formulation is not a product label recommended use for Spinosad.

gently touched, was monitored at 1, 3, 5, and 7 days. There were five replicates per treatment.

Chrysoperla carnea. This experiment was conducted as for *A. bilineata,* except that there were 50 replicates per treatment, the petri dish was lined with damp filter paper, and 2- to 3-day-old larvae were used. The larvae were fed eggs of *S. cereallella ad libitum* throughout the experiment. Three granular treatments were used consisting of 0, 200, and 2000 ppm Spinosad. The insects were monitored at 3, 5, 7, 10, and 12 days, by which time all were either dead or had successfully pupated. Once adults emerged, they were confined in male–female pairs (both from the same treatment) in 9-cm-diameter × 5-cm-tall plastic pots. Adults were not further exposed to Spinosad. There were 14 replicates in the control treatment and 13 each for the 200- and 2000-ppm Spinosad treatments. Adult insects were provided with water, honey solution, and diet mix as described above. The number of eggs laid by each female was assessed over three 24-h periods, at 8, 10, and 12 days posteclosion.

Doru taeniatum. Adult earwigs were individually confined in 10-ml glass vials with 0.5 g of granules containing 0, 2, 20, or 2000 ppm Spinosad. Vials were sealed with a cotton wool plug that had been dampened with a few drops of water and placed horizontally on a plastic tray. Earwigs were held at 25 ± 1°C and checked for mortality at 48-h intervals over a period of 14 days. Dead earwigs did not respond when gently touched. Additional food items were not provided during the experimental period. The experiment was performed twice with 25 earwigs in each treatment (n = 200 total).

To provide data to support a subsequent field trail, an additional study was performed under identical conditions using granules containing 0, 200, or 2000 ppm Spinosad but with a 2-day exposure period. Earwigs were checked for mortality after 2 days of exposure. Living earwigs were individually transferred to a plastic cup with a small quantity of maize and soya-based semisynthetic diet. These individuals were checked for mortality 24 h later. Death was recorded when insects failed to respond to being gently touched or shaken. The experiment was performed twice with 50 earwigs in each treatment (n = 300 total).

Mortality of Earwigs That Consumed Spinosad-Treated S. frugiperda Larvae

A group of four third-instar *S. frugiperda* larvae were placed in sterile plastic petri dishes 9 cm in diameter with 0.5 g of granules containing 2000 ppm of Spinosad. After 24 h of exposure, *S. frugiperda* larvae were moribund and were offered to individual *D. taeniatum* adults in a new plastic petri dish. Earwigs that were observed to consume the corpses of *S. frugiperda* larvae were held in a 20-ml plastic cup with a small cube of semisynthetic diet and were checked at 24-h intervals for a period of 3 days. The condition of these earwigs was classified as unaffected, intoxicated, or dead. Dead earwigs did not respond to being touched. Intoxicated earwigs were moribund or showed typical signs of Spinosad poisoning, namely tremors and an inability to walk. Unaffected insects appeared to be as active and responsive as control earwigs. Control earwigs were treated identically except that *S. frugiperda* larvae were briefly chilled at 4°C to induce a moribund-like state and were not exposed to Spinosad. The experiment was performed 29 times for the controls and 35 times for Spinosad-treated larvae.

Field Trial

A plot of maize (40 × 120 m) close to the village of Morelos (14° N 52′, 92° W 22′) approximately 15 km southwest of the town of Tapachula, Chiapas, Mexico, was selected for the field trial. The climate in this region is hot (typically 35°C during the day and 23°C at night) and humid (85–95% relative humidity). The experimental site was approximately 50 m above sea level. No rainfall occurred during the experimental period. The maize was planted at a density of approximately 30,000 plants/ha and was occasionally irrigated by flooding. Plants were 40–60 cm high and had been subjected to no prior insecticide treatments. Eight counts were performed on 25 plants at 8 different locations within the maize plot to determine the degree of infestation by *S. frugiperda* larvae at the start of the experiment.

A total of 300 plants were selected at random and designated to one of the following six treatments: (i) Tracer spray at the rate of 100 ml/ha (equivalent to 160 ppm Spinosad delivered in a volume of approximately 7.5 ml of spray per plant), (ii) water control spray (7.5 ml/plant), (iii) granules containing 2000 ppm Spinosad, (iv) granules containing 200 ppm Spinosad, (v) a commercial mineral-based granular formulation of chlorpyrifos (Knocker 3G, Bravo S.A. de C.V., Mexico, 3% a.i.), and (vi) control granules. Each plant represented a single independent replicate ($n = 50$ per treatment).

Phagostimulant granules were sprinkled into the leaf whorl at the rate of 0.85 ± 0.04 g/plant (mean $\pm$ SE, $n = 20$), using a disposable plastic teaspoon which was found to be a reliable dosing technique with low variability in a previous study (Williams *et al.*, 1999). Granular chlorpyrifos was also sprinkled directly into the leaf whorl, in an identical manner, at the recommended rate of 10 kg/ha. Spray applications were made with a manual knapsack sprayer fitted with a cone nozzle, typical of those used by small-scale farmers in southern Mexico. To avoid risk of cross-contamination, all spray applications were made at least 20 m away from any plant that had been treated with granules.

Four earwigs were placed in a nylon mesh bag 35 cm tall and 20 cm wide. This bag was then placed over the top third portion of the maize plant and firmly tied at the base to minimize insect movement. To ensure drying of spray residues, a 2.5-h interval was allowed to elapse between spray application of Spinosad and placing the bag containing earwigs. Two days after treatment, each experimental plant was cut and transported to the laboratory where the number of living and dead earwigs present in each bag was determined. Living earwigs were placed in plastic cups with a small piece of semisynthetic diet made of maize, soya, yeast, and vitamins, which was an acceptable foodstuff (J. Cisneros, personal observation). These insects were held at 25°C for 24 h following which the prevalence of mortality was determined. Death was recorded when earwigs failed to respond to being gently touched.

Statistical Analysis

For each experiment the proportions of insects that were alive on each date were analyzed according to the concentration of Spinosad using GLIM with a binomial error structure (Crawley, 1993). Numbers of eggs laid

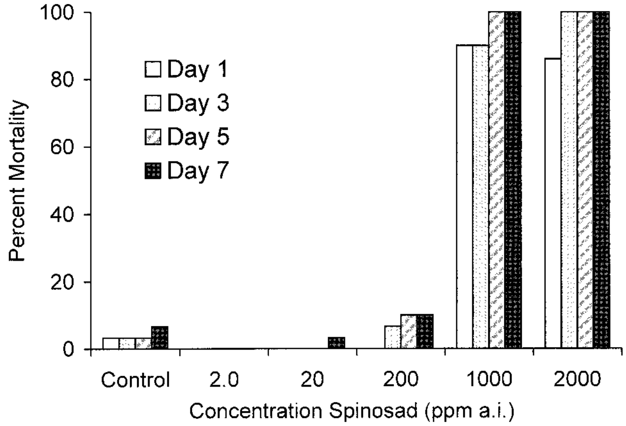

FIG. 1. Mortality of adults of *Aleochara bilineata* (Staphylinidae) at 1–7 days postexposure when offered 2.0–2000 ppm of Spinosad in maize flour granules.

by *C. carnea* were compared according to the concentration of Spinosad to which insects were exposed using linear regression (presented as *F* statistics). Data on recovery of earwigs and mortality of earwigs in the field trails were subjected to ANOVA in GLIM with normal or binomial errors. Where necessary, small degrees of overdispersion were corrected using the Williams' correction macro present in this program (Collett, 1991) or by scaling the error distribution and calculating the significance values in terms of *F* statistics (Crawley, 1993). The number of earwigs that died in the laboratory 24 h postcollection was subjected to a contingency table (χ^2) test.

RESULTS

Mortality of Natural Enemies Exposed to Spinosad in Granules

Aleochara bilineata. There was a highly significant effect of the concentration of Spinosad on staphylinid mortality at 1 day postexposure ($\chi^2 = 142$, $df = 1$, $P < 0.001$) increasing through to 5 days postexposure ($\chi^2 = 204$, $df = 1$, $P < 0.001$). Very little mortality occurred throughout the experimental period in control insects or in those exposed to 2 or 20 ppm of Spinosad. Just 10% mortality occurred in insects exposed to 200 ppm Spinosad, while concentrations higher than this resulted in high levels of mortality, with all insects dead after 3–5 days of exposure (Fig. 1). *Aleochara bilineata* adults were frequently seen feeding upon the Spinosad formulation, much of which was consumed by the end of the trial.

Chrysoperla carnea. Insects were not observed to feed upon the Spinosad granular formulation. Nonetheless, mortality increased significantly according to the concentration of Spinosad to which larvae were exposed from 3 days ($\chi^2 = 11.7$, $df = 1$, $P < 0.001$) to

12 days ($\chi^2 = 9.9$, $df = 1$, $P < 0.002$) after the start of the experiment. Mortality in control insects did not exceed 6% after 12 days, whereas mortality was moderately low in Spinosad treatments reaching 19% at 200 ppm and 34% at 2000 ppm of Spinosad after 12 days. Almost all larvae that successfully pupated subsequently emerged as adults (99 of 105), so effects of Spinosad treatment on eclosion were not examined. Fecundity did not vary significantly according to the concentration of Spinosad to which insects had been exposed on any of the 3 dates on which it was assessed [$F_{(1, 38)} = 0.38$, 0.04 and 2.79 for days 8, 10, and 12, respectively].

Doru taeniatum. In the experiment involving 1.2–1200 ppm Spinosad, earwigs confined in vials with granules containing Spinosad for a period of 14 days suffered a significantly higher mortality than earwigs confined with control granules ($\chi^2 = 81.0$, $df = 3$, $P < 0.001$). Earwig mortality ranged from 20% in the controls compared to 48, 68, and 98% in the 1.2-, 12-, and 1200-ppm Spinosad treatments, respectively. The quantity of granules consumed by each earwigs was not determined.

In contrast, in the experiment with just a 2-day exposure period, prevalence of earwig mortality was 5% in the controls compared to 68% in the 200-ppm Spinosad and 81% in the 2000-ppm Spinosad treatments ($\chi^2 = 149$, $df = 2$, $P < 0.001$). Earwigs consumed an average of 9.8 ± 1.07 mg of granules in 24 h (mean ± SE, $n = 60$). When checked 24 h later, mortality had increased to 10% in the controls, compared to 80% in the 200-ppm Spinosad and 91% in the 2000-ppm Spinosad treatments ($\chi^2 = 161$, $df = 2$, $P < 0.001$).

Mortality of Earwigs Fed Spinosad-Treated S. frugiperda Larvae

Earwigs that fed upon Spinosad-treated *S. frugiperda* larvae suffered significantly higher intoxication and mortality than earwigs that fed upon untreated larvae ($\chi^2 = 46.9$, $df = 1$, $P < 0.001$). Within 24 h of feeding upon *S. frugiperda* larvae that had died from consumption of Spinosad granules, 17% of earwigs were dead and 49% were intoxicated or moribund (Fig. 2). The prevalence of mortality increased until 72 h postconsumption by which time, 72% of earwigs were dead and 14% were intoxicated. No mortality or evidence of intoxication was observed in control earwigs.

Field Trial

The maize plot had a moderately heavy infestation of *S. frugiperda*, with 62% of plants showing current fall armyworm feeding damage. Overall recovery of earwigs (living and dead) did not differ significantly between treatments ranging from a mean (±SE) of 3.50 ± 0.14 earwigs per plant (88% recovery) in the control granule treatment to 3.88 ± 0.04 earwigs/plant (97% recovery) in the 200-ppm Spinosad granule treatment [$F_{(5, 294)} = 1.54$, $P = 0.18$].

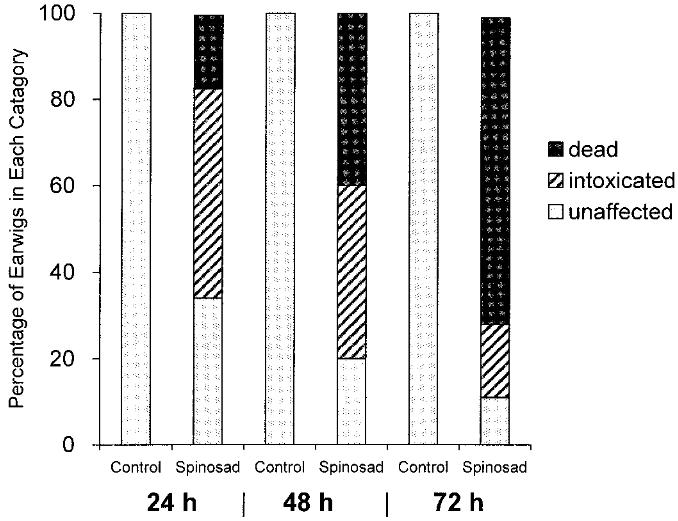

FIG. 2. Prevalence of mortality or intoxication of *Doru taeniatum* at 24–72 h after consuming *Spodoptera frugiperda* larvae that had died from consumption of maize flour granules containing 2000 ppm of Spinosad. Intoxicated individuals did not recover.

Mortality of earwigs on the control granule and control spray plants was less than 15%. Earwig mortality was significantly higher on plants treated with chlorpyrifos or Spinosad compared to control treatments [$F_{(5, 294)} = 104.9$, $P < 0.001$). In the Spinosad treatments, earwig mortality was significantly higher in the granule treatments than in the Spinosad spray treatment (Fig. 3). There was no significant difference in earwig mortality between the 2000-ppm Spinosad and the 200-ppm Spinosad granule treatments, in which mortality at 2 days postapplication ranged from 90.7 to 95.2%, respectively ($\chi^2 = 2.94$, $df = 1$, $P = 0.085$).

Living earwigs were maintained in the laboratory and checked for mortality at 24 h postcollection. The 24-h mortality of postcollection earwigs from control plants was 1.8–4.5% for the control spray and the control granule treatments, respectively. In contrast, 24-h mortality postcollection ranged from 9% in the chlorpyrifos treatment, to 55% in the Spinosad granule treatments (200 and 2000 ppm), to 65% in the Spinosad spray treatment (160 ppm), but did not differ significantly among Spinosad treatments ($\chi^2 = 0.61$, $df = 2$, $P = 0.73$).

DISCUSSION

The earwig *D. taeniatum* is abundant in maize in Mesoamerica and is a generalist predator (Cañas and O'Neil, 1998). It is considered to be an important predator of *S. frugiperda* eggs and larvae (Van Huis, 1981; Jones *et al.*, 1989; Castillejos *et al.*, 2001). Moreover,

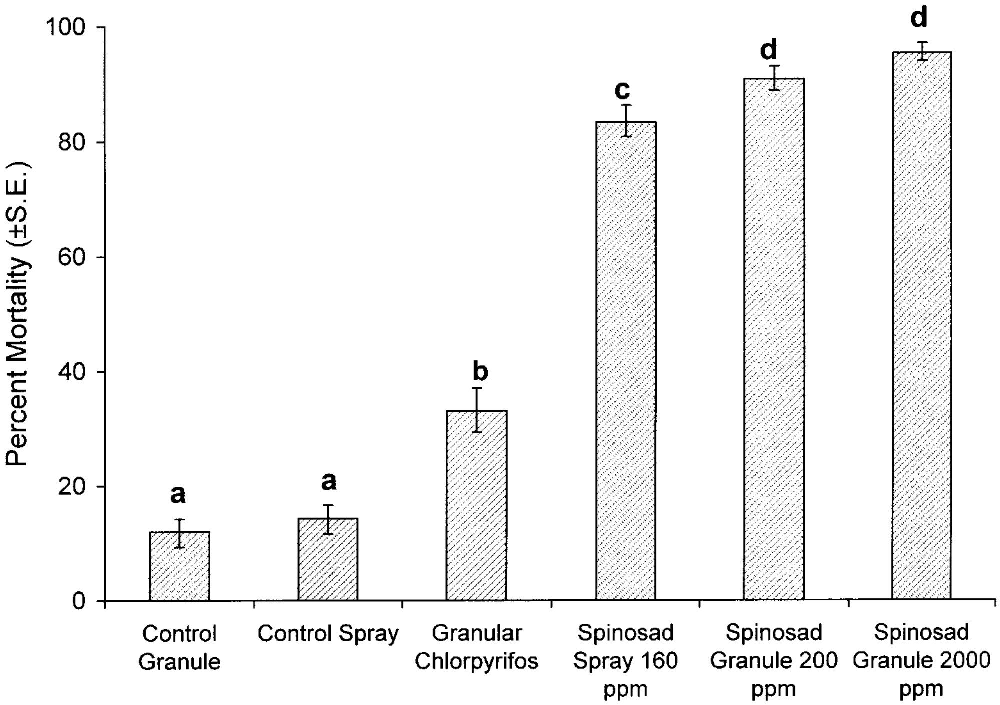

FIG. 3. Mortality of *Doru taeniatum* collected after 2 days of exposure to a commercial mineral-based granular formulation of chlorpyrifos or Spinosad in spray and maize-flour formulation. The field trial was performed on maize plants and in all cases insecticide applications were made directly into the leaf whorl. Control insects were exposed to water or maize-flour granules without insecticide. Identical letters above columns indicate no significant differences between treatments ($P > 0.05$, analysis in GLIM with binomial errors).

densities of *D. taeniatum* may be positively correlated with the presence of *S. frugiperda* feeding damage (Chapman *et al.*, 2000), suggesting that this predator is attracted to plants infested by fall armyworm larvae. *Aleochara* spp. are unusual in being staphylinid endoparasitoids of dipteran pupae and have a global distribution. They are important biocontrol agents of a number of serious pests, including onion fly, *Delia antiqua* (Meigen), and cabbage root fly, *Delia radicum* (L.). *Chrysoperla* spp. are similarly widespread and are major predators of hemipteran pests as larvae and as adults. They are considered to be important natural enemies in a broad range of crops, including maize. Thus the natural enemies chosen for study span a range of life histories and are from diverse taxonomic groups (Dermaptera, Coleoptera, and Neuroptera).

Laboratory feeding tests indicated that consumption of a maize-flour-based granular formulation of Spinosad resulted in little mortality of the staphylinid *A. bilineata* at concentrations between 2 and 200 ppm but a high prevalence of mortality was observed at higher concentrations. Mortality of *C. carnea* larvae was moderately low, presumably because this species did not consume or consumed very little of the granular formulation. In contrast, the earwig *D. taeniatum* was highly sensitive to Spinosad suffering high mortality at concentrations between 12 and 200 ppm. Moreover, earwigs fed *S. frugiperda* larvae that had died from consumption or contact with Spinosad suffered a high prevalence of mortality or intoxication. Once insects show symptoms of Spinosad poisoning, they do not recover (Bret *et al.*, 1997).

The use of phagostimulant formulations allows the active ingredient to be delivered to the pest in concentrated form; the use of feeding stimulants therefore shows considerable promise for the delivery of many bioinsecticides (Hostetter *et al.*, 1982; Bartlet *et al.*, 1990; Burges and Jones, 1998). Presently, the application of Spinosad in the granular maize-flour formulation we have studied represents a nonlabeled use. The Tracer product label recommendations for control of armyworms in maize range from 67 to 102 g a.i./ha. For a minimum recommended volume ground spray (56.8 liters/ha), this is equivalent to 1180–1800 ppm Spinosad or 2950–4480 ppm Spinosad for a minimum volume aerial application (22.8 liters/ha) (Table 1). Spray volumes of 300 liters/ha are probably more usual for ground spray applications in maize, representing a Spinosad concentration of around 200 ppm.

Considerable insect predator mortality was observed following Spinosad application in the field. In the 160 ppm Spinosad spray treatment (48 g a.i/ha) and the 200 ppm Spinosad granule treatment (4.8 g a.i./ha) earwig mortality was between 94 and 96% at 3 days postapplication. Moreover, laboratory feeding tests indicate that concentrations of less than 12 ppm of Spinosad (0.3 g a.i./ha) are toxic to earwigs.

Earwigs were not offered a choice of food items in the laboratory tests and may have chosen to feed from items that did not contain Spinosad if they had been available. However, when earwigs were placed on maize plants in the field, alternative food items were available, including thrips, aphids, and *S. frugiperda* larvae and their feces, which are consumed by *D. taeniatum* (J. Cisneros, personal observations). Indeed, many insect predators are omnivorous scavengers that are likely to consume dead or dying insects that have been treated with Spinosad. This may explain the high prevalence of mortality in earwigs placed on plants previously sprayed with Spinosad as all potential foodstuffs would have been contaminated by the Spinosad spray. These results contrast with those reported by Schoonover and Larson (1995) in which coccinelid adults and chrysopid larvae were fed aphids previously treated with 200 ppm Spinosad with no reported predator mortality (Bret *et al.*, 1997). Moreover, with product label maximum application rates exceeding 4000 ppm, the value of relatively low concentration toxicity studies may be questioned.

Earwigs were confined on experimental maize plants in the field trial and may have left Spinosad-treated plants under normal conditions. However, we observed no evidence of contact repellency and granules containing Spinosad were readily consumed by earwigs and staphylinid parasitoids. In contrast, the mineral-based granular formulation of chlorpyrifos was probably unattractive or repellant to earwigs and caused only moderate mortality.

The fact that Spinosad is obtained from a naturally occurring soil organism does not automatically mean that it is safe and innocuous. In addition, Stark *et al.* (1995) have pointed out the need for caution when making assumptions on pesticide impact on beneficial organisms based solely on laboratory-generated toxicity data. For example, contact bioassays of Spinosad at the recommended field rate caused 19–65% mortality in the parasitoid *Catolaccus grandis* (Burks) (Hymenoptera:Pteromalidae) compared to 56–73% mortality from methyl parathion, 38–83% from endosulfan, and 90–92% from malathion. However, both Spinosad and malathion completely inhibited parasitoid reproduction when present at one-fourth of their respective recommended field rates (Elzen *et al.*, 2000).

The effectiveness of Spinosad in granular maize-flour formulations is currently being tested against *Spodoptera* pests in Mexico. Bait formulations of Spinosad are also being developed for large-scale use against fruit flies (Montoya-Gerardo *et al.*, 2001) and for the control of ants (Blewett and Cooper, 1998). More importantly, Spinosad is being evaluated for use against insect pests in delicate forest ecosystems (Wanner *et al.*, 2000). Judging by the results of the present study, in which low to moderate concentrations of Spinosad caused substantial mortality to insect natural enemies, we disagree with the assertion, by representatives of Dow AgroScience (Thompson and Hutchins, 1999), that Spinosad has a safety profile similar to benign biological pesticides.

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REFERENCES

Bartlet, R. J., McGuire, M. R., and Black, D. A. 1990. Feeding stimulants for the European corn borer (Lepidoptera:Pyralidae): Additives to a starch-based formulation for *Bacillus thuringiensis*. *Environ. Entomol.* **19,** 182–189.

Blewett, T. C., and Cooper, R. B. 1998. Control of the red imported fire ant with Spinosad. *Down to Earth* **53,** 27–31.

Bret, B. L., Larson, L. L., Schoonover, J. R., Sparks, T. C., and Thompson, G. D. 1997. Biological properties of Spinosad. *Down to Earth* **52,** 6–13.

Burges, H. D., and Jones, K. A. 1998. Formulation of bacteria, viruses and protozoa to control insects. *In* "Formulation of Microbial Biopesticides: Beneficial Microorganisms, Nematodes and Seed Treatments" (H. D. Burges, Ed.), pp. 33–127. Kluwer, Dortrecht.

Cañas, L. A., and O'Neil, R. J. 1998. Applications of sugar solutions to maize, and the impact of natural enemies on fall armyworm. *Int. J. Pest Manag.* **44,** 59–64.

Casida, J. E., and Quistad, G. B. 1998. Golden age of insecticide research: Past present or future? *Annu. Rev. Entomol.* **43,** 1–16.

Castillejos, V., García, L., Cisneros, J., Goulson, D., Caballero, P., Cave, R. D., and Williams, T. 2001. The potential of *Chrysoperla rufilabris* and *Doru taeniatum* as agents for dispersal of *Spodoptera frugiperda* nucleopolyhedrovirus in maize. *Entomol. Exp. Appl.* **98,** 353–359.

Chapman, J. W., Williams, T., Martínez, A. M., Cisneros, R., Caballero, P., Cave, R. D., and Goulson, D. 2000. Does cannibalism in *Spodoptera frugiperda* reduce the risk of predation? *Behav. Ecol. Sociobiol.* **48,** 321–327.

Collett, D. 1991. "Modelling Binary Data." Chapman & Hall, London.

Copping, L. G., and Menn, J. J. 2000. Biopesticides: A review of their action, applications and efficacy. *Pest Manag. Sci.* **56,** 651–676.

Crawley, M. J. 1993. "GLIM for Ecologists." Blackwell, Oxford, UK.

Croft, B. A. 1990. "Arthropod Biological Control Agents and Pesticides." Wiley, New York.

Dahl, G. H., and Lowell, J. R. 1984. Microencapsulated pesticides and their effects on non-target insects. *In* "Advances in Pesticide Formulation Technology" (H. B. Scher, Ed.), pp. 141–150. Amer. Chem. Soc., Washington, DC.

Elzen, G. W., Maldonado, S. N., and Rojas, M. G. 2000. Lethal and sublethal effects of selected insecticides and an insect growth regulator on the boll weevil (Coleoptera:Curculionidae) ectoparasitoid *Catolaccus grandis* (Hymenoptera:Pteromalidae). *J. Econ. Entomol.* **93,** 300–303.

Harris, J. G. 2000. "Chemical Pesticide Markets, Health Risks and Residues." CABI, Wallingford, UK.

Hostetter, D. L., Smith, D. B., Pinnell, R. E., Ignoffo, C. M., and McKibben, G. H. 1982. Laboratory evaluation of adjuvants for use with *Baculovirus heliothis* virus. *J. Econ. Entomol.* **75,** 1114–1119.

Jones, R. W. Gilstrap, F. E., and Andrews, K. L. 1989. Dinámica poblacional de la tijereta, *Doru taeniatum* (Dohn) (Dermaptera: Forticulidae) en maíz y sorgo en Honduras. *Ceiba* **30,** 67–80.

National Research Council. 1996. "Ecologically Based Pest Management: New Solutions for a New Century." National Academy Press, Washington, DC.

Montoya-Gerardo, P. J., Flores-Breceda, S., and Hernández-Ortiz, E. 2001. Efecto de la aplicación del spinosad en poblaciones de *Ceratitis capitata* y *Anastrepha ludens* (Diptera:Tephritidae). *In* "Memoirs of the 36th National Congress of Entomology." Sociedad Entomol. Mex., Querétaro, Mexico.

Salgado, V. L. 1997. The modes of action of Spinosad and other insect control products. *Down to Earth* **52,** 35–43.

Salgado, V. L. 1998. Studies on the mode of action of Spinosad: Insect symptoms and physiological correlates. *Pestic. Biochem. Physiol.* **60,** 91–102.

Saunders, D. G., and Bret, B. L. 1997. Fate of Spinosad in the environment. *Down to Earth* **52,** 14–20.

Schoonover, J. R., and Larson, L. L. 1995. Laboratory activity of Spinosad on non-target beneficial arthropods. *Arthr. Manag. Tests* **20,** 357.

Sparks, T. C., Thompson, G. D., Kirst, H. A., Hertlein, M. B., Larson, L. L., Worden, T. V., and Thibault, S. T. 1998. Biological activity of the spinosyns, new fermentation derived insect control agents, on tobacco budworm (Lepidoptera:Noctuidae) larvae. *J. Econ. Entomol.* **91,** 1277–1283.

Stark, J. D., Jepson, P. C., and Mayer, D. F. 1995. Limitations to use of topical toxicity data for predictions of pesticide side effects in the field. *J. Econ. Entomol.* **88,** 1081–1088.

Tamez-Guerra, P., Castro-Franco, R., Medrano-Roldán, H., McGuire, M. R., Galán-Wong, L. J., and Luna-Olvera, H. A. 1998. Laboratory and field comparisons of strains of *Bacillus thuringiensis* for activity against noctuid larvae using granular formulations (Lepidoptera). *J. Econ. Entomol.* **91,** 86–93.

Tamez-Guerra, P., McGuire, M. R., Behle, R. W., Hamm, J. J., Sumner, H. R., and Shasha, B. S. 2000a. Sunlight persistence and rainfastness of spray-dried formulations of baculovirus isolated from *Anagrapha falcifara* (Lepidoptera:Noctuidae). *J. Econ. Entomol.* **93,** 210–218.

Tamez-Guerra, P., McGuire, M. R., Behle, R. W., Shasha, B. S., and Galán Wong, L. J. 2000b. Assessment of microencapsulated formulations for improved residual activity of *Bacillus thuringiensis*. *J. Econ. Entomol.* **93,** 219–225.

Tamez-Guerra, P., McGuire, M. R., Medrano-Roldan, H., Galán-Wong, L. J., Shasha, B. S., and Vega, F. E. 1996. Sprayable granule formulations for *Bacillus thuringiensis*. *J. Econ. Entomol.* **89,** 1424–1430.

Thompson, G., and Hutchins, S. 1999. Spinosad. *Pestic. Outlook* **10,** 78–81.

Van Huis, A. 1981. Integrated pest management in the small farmer's maize crop in Nicaragua. *Meded. Landbou. Wageningen* **81**(6), 221 pp.

Wanner, K. W., Helson, B. V., and Harris, B. J. 2000. Laboratory and field evaluation of Spinosad against the gypsy moth, *Lymantria dispar*. *Pest Manag. Sci.* **56,** 855–860.

Williams, T., Goulson, D., Caballero, P., Cisneros, J., Martínez, A. M., Chapman, J. W., Roman, D. X., and Cave, R. D. 1999. Evaluation of a baculovirus bioinsecticide for small scale maize growers in Latin America. *Biol. Contr.* **14,** 67–75.