

Ultralow Rates of Spinosad in Phagostimulant Granules Provide Control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Maize

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ABSTRACT Field trials in 2002 and 2003 were performed to determine the efficacy of maize flour-based granular formulations with ultralow rates of the naturally derived insecticide spinosad (0.1, 0.3, and 1.0 g [AI]/ha), for control of *Spodoptera frugiperda* (J.E. Smith) in maize, *Zea mays* L., in southern Mexico. Spinosad formulations were compared with a chemical standard, a commercial granular formulation of chlorpyrifos (150 g [AI]/ha). In both years, application of spinosad resulted in excellent levels of control, indicated by the number of living *S. frugiperda* larvae recovered from experimental plots. The efficacy of spinosad applied at 0.3 and 1.0 g (AI)/ha was very similar to that of chlorpyrifos. Natural reinfestation caused *S. frugiperda* numbers in insecticide treated plots to return to values similar to the control treatment by 10–15 d postapplication. Many spinosad-intoxicated larvae collected in the field died later in the laboratory in 2002, but not in 2003. Percentage mortality due to parasitoid emergence did not differ in any treatment in either field trial. The number of parasitoids that emerged from *S. frugiperda* collected in each treatment was significantly reduced after application of spinosad (all rates) or chlorpyrifos due to a reduction in the number of host larvae. Parasitoid numbers returned to control values by 9–15 d postapplication in all treatments. The most prevalent parasitoid was the braconid *Chelonus insularis* Cresson, which represented ≈80% of emerging parasitoids in both years. We conclude that appropriate formulation technology can greatly enhance the performance of this naturally derived, biorational insecticide.

KEY WORDS spinosad, fall armyworm, feeding stimulants, parasitoids, *Chelonus insularis*

SPINOSAD IS A NATURALLY DERIVED insecticide produced by the fermentation of the soil actinomycete *Saccharopolyspora spinosa* Mertz & Yao (Sparks et al. 1998). This product is a mixture of two tetracyclic macrolide molecules, spinosyn A and spinosyn D, which show neurotoxic properties to Lepidoptera, Diptera, Hymenoptera, and some Coleoptera (Bret et al. 1997). These compounds act upon the postsynaptic nicotinic acetylcholine and the GABA receptors in a unique manner (Salgado 1998, Watson 2001). Spinosad is highly active by ingestion and is less active by contact.

Spinosad has very little toxicity to birds and mammals (Bret et al. 1997, Breslin et al. 2000) and is classified by the United States Environmental Protection Agency as an environmentally and toxicologically reduced risk material (Saunders and Bret 1997). Different spinosad-based products have been registered in >30 countries for control of a broad range of foliar-feeding insect pests.

As a biorational pesticide, spinosad now represents an important option for pest control in a growing

number crops, produced under systems of integrated pest management (IPM) (Thompson and Hutchins 1999). The adoption of spinosad-based products by IPM practitioners is due to its effectiveness as an insecticide combined with its relatively low toxicity to a number of insect natural enemies (Copping 2001). A recent review of predator and parasitoid susceptibility to spinosad concluded that this product represented one of the most judicious insecticides available for the conservation of predator populations (Williams et al. 2003). However, the majority of field studies in natural and seminatural conditions reported harmful effects on populations of hymenopteran parasitoids, indicating that caution was required in the use of spinosad when conservation of parasitoid populations was of prime concern (Williams et al. 2003).

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a major pest of maize, *Zea mays* L., and sorghum *Sorghum bicolor* (L.) Moench in the Americas (Hruska and Gould 1997). In Mexico and Central America, this pest is usually controlled by several applications of organophosphate insecticides per season, applied in granular formulations directly into the developing leaf whorl, which is the feeding site of the larvae of this pest (Andrews 1988). However, lack of the use of protective measures results in alarming rates of chronic insecticide poi-

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soning in Mesoamerican crop producers (McConnell and Hruska 1993, Tinoco and Halperin 1998). This has stimulated a program of research on safe and reliable pest control measures for resource-poor maize producers in this region (Williams et al. 1999, Armenta et al. 2003).

The use of granular phagostimulant formulations of biopesticides for control of noctuid pests increases the efficacy of these products, resulting in increased levels of pest control (Tamez-Guerra et al. 1998, Castillejos et al. 2002). Previous studies have indicated that *S. frugiperda* is highly sensitive to spinosad (Méndez et al. 2002). The product label-recommended rate for control of *S. frugiperda* in maize in the United States is 67–100 g (AI)/ha. Therefore, the aim of this study was to evaluate the feasibility of using phagostimulant granular formulations with ultralow rates of spinosad for control of *S. frugiperda* in maize in the State of Chiapas, in the southernmost region of Mexico. We also studied the impact of granular spinosad applications on the prevalence of hymenopteran parasitoids emerging from *S. frugiperda* larvae collected from the field.

Materials and Methods

Field Site and Insects. Field trials were performed in maize fields close to the village of Mazatán, Chiapas, Mexico (14° 52' 44" N, 92° 27' 45" W) at ≈20-m altitude, during July 2002 and June 2003. During this period, the weather was hot (daily range 23–36°C) with regular rainfall in the afternoon (≈300 mm/mo). Insecticides had not been applied to the crop before the experiments. Maize plants (a locally grown creole variety) were planted at a density of ≈35,000 plants per hectare and were 50–60 cm in height at the start of the trial. Natural *S. frugiperda* infestations were evaluated by examining 150 randomly selected plants 2–3 d before the start of the experiment. Natural infestations were augmented by manual infestation using *S. frugiperda* from a laboratory colony maintained on semisynthetic diet (adapted from Mihm 1984) in the laboratories of El Colegio de la Frontera Sur, Tapachula, Chiapas, Mexico.

Preparation of Granular Formulations. Phagostimulant granules were prepared as described by Castillejos et al. (2002). Briefly, this involved mixing 800 g of nixtamalized maize flour (Molinas Azteca de Chiapas, Villaflores, Mexico), 190 g of pregelatinized cornstarch (Productos de Maíz, Lerma, Mexico), 10 g of corn oil (Aceites La Central, Guadalajara, Mexico), and 1000 ml of distilled water to form a soft dough. The dough was left to stand for 30 min before being passed through a wire gauze with a mesh aperture of 1.2 mm. During this process, the dough crumbled into irregular granules ≈1 mm in width and 0.5–3 mm in length. The granules were placed next to a fan ventilator and allowed to air dry for 24 h at 25 ± 1°C before use. For granules containing spinosad, the appropriate quantity of Tracer (Dow Agrosciences, LLC, Indianapolis, IN) containing 480 g/liter (AI) was diluted as necessary, added to the water component and mixed thor-

oughly to ensure homogeneous incorporation, before being passed through the wire gauze.

Field Trial 2002. A maize field was divided into 40 experimental plots of 5 by 5 m with a barrier of 5 m of maize plants between plots. Plants within plots were manually infested, each with ≈3 second instars of *S. frugiperda* larvae from the laboratory culture. Two days later, each of the plots was randomly assigned to one of the following treatments: 1) control granules with no active ingredient, 2) granules containing 10 ppm (milligrams per kilogram) spinosad, 3) granules containing 30 ppm spinosad, 4) granules containing 100 ppm spinosad, and 5) commercial granular formulation of chlorpyrifos (3% [AI]; Knocker 3G, Bravo, Mexico). Granules were applied directly into the leaf whorl by using a plastic jar with a perforated lid at a rate of 25 g per plot, equivalent to 10 kg/ha. The spinosad treatments at 10, 30, and 100 ppm were therefore equivalent to application rates of 0.1, 0.3, and 1.0 g (AI)/ha, respectively. Chlorpyrifos granules were applied at the recommended rate of 5 kg/ha, equivalent to 150 g (AI)/ha. There were eight replicate plots assigned to each treatment.

At 3, 7, 10, and 15 d postapplication, 12 randomly selected plants from each plot were cut, placed in plastic bags, and transported to the laboratory, where living *S. frugiperda* larvae were transferred to individual plastic cups containing semisynthetic diet and reared through to pupation. The number of larvae that died postcollection from presumed spinosad intoxication (nonspecific mortality) was noted. The total number of larvae from which parasitoids emerged was also recorded.

Field Trial 2003. The field trial in 2003 was identical to that performed in 2002, except for the following aspects. The plots used were 6 by 6 m with a distance of 6 m between plots. At 1 d before the applications, plants were artificially infested with second instars of *S. frugiperda* from the laboratory colony at a rate of two larvae per plant. Sampling occurred at 2, 5, 9, and 14 d postapplication.

Statistical Analysis. The results of each field trial were analyzed separately. The number of living *S. frugiperda* larvae collected from each plot at each time point was subjected to univariate repeated measures analysis of variance (ANOVA) by using the SAS statistical package (SAS Institute 1992). Tests of sphericity were performed using Mauchly's criterion (Crowder and Hand 1990). The number of parasitoids that emerged from field-collected larvae was also subjected to univariate repeated measures ANOVA. The prevalence of nonspecific mortality in field-collected *S. frugiperda* larvae and parasitoid emergence observed in the laboratory was analyzed in GLIM (Numerical Algorithms Group 1993) with a binomial error structure. The means of binomial data have asymmetrical standard errors. Where necessary, scaling was performed to adjust for minor overdispersion in the data. The results of scaled analyses are given in terms of *F* statistics. The accuracy of models was determined by examination of the distribution of observed and fitted values (Crawley 1993).

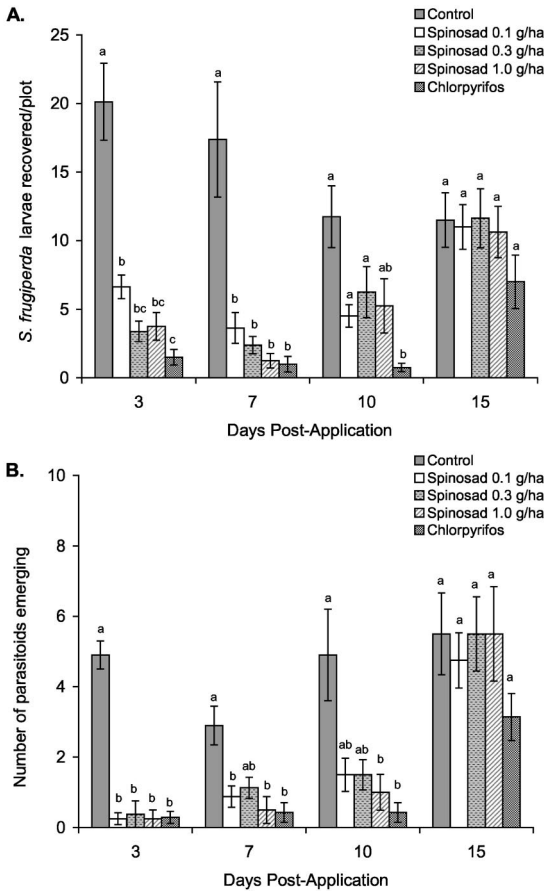


Fig. 1. (A) Mean number of living *S. frugiperda* larvae recovered per plot (12-plant sample) and (B) mean number of hosts from which parasitoids emerged in the laboratory, after application of three rates of spinosad in granular formulation or chlorpyrifos granules in field trial performed in 2002. Bars represent SEM. Columns headed by identical letters are not significantly different for comparisons among treatments within each time point (repeated measures ANOVA, $P > 0.05$).

Results

Field Trial 2002. At the start of the field trial, 42% of plants were naturally infested with *S. frugiperda* larvae. The number of living *S. frugiperda* larvae recovered from control plots declined steadily during the course of the trial (Fig. 1A). Application of all rates of spinosad resulted in significant reductions in the number of *S. frugiperda* larvae recovered at 3 and 7 d postapplication, but by 10 d postapplication larval recovery had returned to control values (Table 1; Fig. 1A), presumably as a result of natural reinfestation. The efficacy of chlorpyrifos in controlling *S. frugiperda* exceeded that of spinosad only at the lowest rate (0.1 g [AI]/ha) at 3 d postapplication, was similar to all rates of spinosad at 7 d postapplication, and was more effective than 0.1 and 0.3 g (AI)/ha but not 1.0 g (AI)/ha spinosad at 10 d postapplication. By 15 d

Table 1. Univariate repeated measures ANOVA of field trials involving treating maize with three rates of spinosad in phagostimulant granular formulation or chlorpyrifos granules

Source	Sum of squares	df	Mean square	F value
Field trial 2002				
Recovery of larvae				
Among subjects				
Treatment	54.87	4	13.72	31.3***
Error	15.32	35	0.44	
Within subjects				
Time	24.01	3	8.00	23.4***
Time × treatment	14.75	12	1.23	3.59***
Error	35.98	105	0.34	
Parasitoid emergence				
Among subjects				
Treatment	35.00	4	8.75	17.5***
Error	17.49	35	0.50	
Within subjects				
Time	2.41	3	0.81	2.78*
Time × treatment	15.80	12	1.32	4.54***
Error	30.44	105	0.29	
Field trial 2003				
Recovery of larvae				
Among subjects				
Treatment	73.28	4	18.32	43.1***
Error	14.87	35	0.42	
Within subjects				
Time	27.04	3	9.01	36.9***
Time × treatment	25.46	12	2.12	8.70***
Error	25.59	105	0.24	
Parasitoid emergence				
Among subjects				
Treatment	21.41	4	5.35	17.9***
Error	10.16	34 ^a	0.30	
Within subjects				
Time	31.13	3	10.38	42.4***
Time × treatment	8.79	12	0.73	2.99**
Error	24.95	105	0.24	

The recovery of *S. frugiperda* larvae and eclosion of adult parasitoids in the laboratory were analyzed. Samples were taken at 3, 7, 10, and 15 d postapplication in 2002 field trial and 2, 5, 9, and 14 d postapplication in 2003 field trial.

Probability given in terms of * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Mauchley's criterion for sphericity values were as follows: 2002 trial, larval recovery $W = 0.9694$; $\chi^2 = 1.05$; $df = 5$; $P = 0.96$; parasitoid emergence $W = 0.7394$; $\chi^2 = 9.88$; $df = 5$; $P = 0.08$. 2003 trial, larval recovery $W = 0.8044$; $\chi^2 = 7.34$; $df = 5$; $P = 0.20$; parasitoid emergence $W = 0.8867$; $\chi^2 = 4.05$; $df = 5$; $P = 0.54$.

^a No host larvae were recovered in a single observation from the chlorpyrifos treatment, 2-d sample that was excluded from the analysis.

postapplication, recovery of *S. frugiperda* larvae was similar in all treatments (Fig. 1A).

In the 3-d sample, nonspecific mortality of field-collected larvae in the laboratory differed markedly among treatments (Fig. 2). Nonspecific mortality was not observed in larvae from the chlorpyrifos treatment (although the sample size was very small, $n = 12$), and these insects were excluded from the analysis. Nonspecific mortality, was significantly higher in the spinosad 0.3 and 1.0 g (AI)/ha treatments compared with control insects, presumably a consequence of spinosad intoxication ($F = 23.2$; $df = 3, 28$; $P < 0.001$; scale parameter = 2.0). There were no clear patterns in nonspecific mortality in the remaining samples.

Percentage of mortality due to parasitoid emergence (data pooled for all time points) was 31.8%

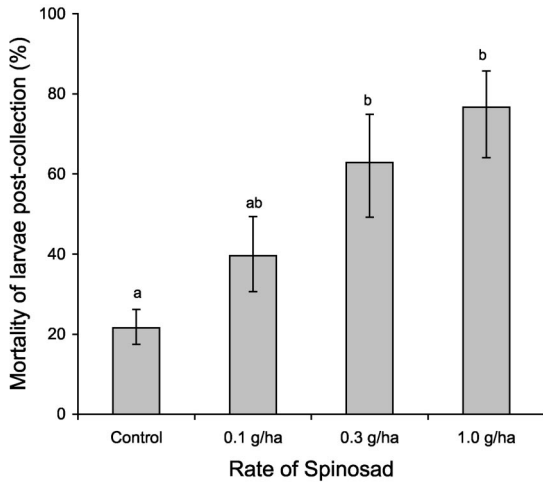


Fig. 2. Percentage of nonspecific mortality in *S. frugiperda* larvae collected from field plots in 2002 field trial at 3 d postapplication and incubated in the laboratory. Nonspecific mortality was not observed in larvae from the chlorpyrifos treatment. Columns headed by identical letters are not significantly different for comparisons among treatments within each time point (factorial analysis in GLIM with binomial errors, $P > 0.05$).

(range of SE, 30.0–33.8) and did not differ significantly among treatments ($F = 2.59$; $df = 4, 35$; $P = 0.053$; scale parameter = 1.86). There was a significant difference in the number of parasitoids that emerged in the laboratory from *S. frugiperda* larvae collected in each treatment ($F = 17.9$; $df = 4, 35$; $P < 0.001$). Numbers of emerging parasitoids were reduced in all treatments compared with the control, although the magnitude of the reduction tended to be greater at the highest rate of spinosad and the chlorpyrifos treatment, compared with the other spinosad treatments (Table 1; Fig. 1B). Recovery of parasitoid numbers to control values was observed in the samples taken at 10 and 15 d postapplication with the low rates (0.1 and 0.3 g [AI]/ha) of spinosad showing faster recovery than the high rate (1.0 g [AI]/ha) and the chlorpyrifos treatments. The most prevalent parasitoid species was *Chelonus insularis* Cresson, which represented 77.0% of emerging parasitoids ($n = 344$). The other species in order of abundance were *Eiphosoma vitticolle* Cresson (11.0%), *Euplectrus plathypenae* Howard (7.6%), and *Ophion flavidus* Brullé (2.6%), followed by a few individuals of *Pristomerus spinator* (F.), *Meteorus* sp., and the tachinid *Lespesia aechippivora* (Riley).

Field Trial 2003. At the start of the field trial, 69% of plants were naturally infested with *S. frugiperda* larvae. In many respects, the results in 2003 closely resembled those seen in the previous year. The number of living *S. frugiperda* larvae recovered from control plots declined during the 14 d of the experiment (Fig. 3A). Application of all rates of spinosad resulted in significant reductions in the number of *S. frugiperda* larvae recovered at two and 5 d postapplication, similar to the efficacy observed in the chlorpyrifos-treat-

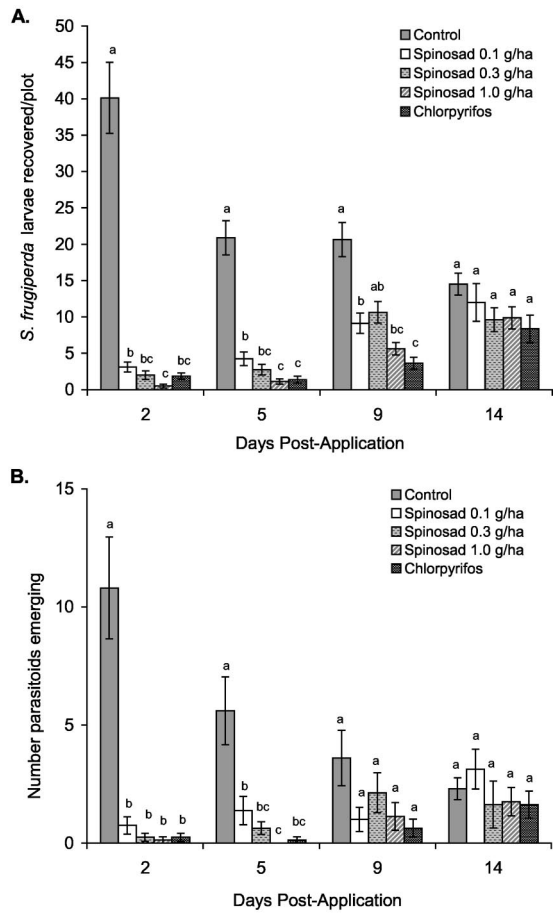


Fig. 3. (A) Mean number of living *S. frugiperda* larvae recovered per plot (12-plant sample) and (B) mean number of hosts from which parasitoids emerged in the laboratory, after application of three rates of spinosad in granular formulation or chlorpyrifos granules in field trial performed in 2003. Bars represent SEM. Columns headed by identical letters are not significantly different for comparisons among treatments within each time point (repeated measures ANOVA, $P > 0.05$).

ment (Table 1; Fig. 3A). By 9 d postapplication, larval recovery had returned to control values in the 0.3 g (AI)/ha spinosad treatment. All the remaining treatments had returned to control values in the sample taken at 14 d postapplication.

Unlike the previous year, nonspecific mortality of field-collected larvae in the laboratory did not exceed 5% in any treatment and was not considered further. The overall percentage of parasitism (data pooled for all time points) was 21.3% (range of SE, 19.7–23.0) and did not differ significantly among treatments ($F = 2.24$; $df = 4, 35$; $P = 0.085$; scale parameter 2.26). Numbers of parasitoids that emerged in the laboratory were severely reduced in both spinosad and chlorpyrifos treatments, but all recovered to control values in the sample taken at 9 d postapplication (Table 1; Fig. 3B). The most

prevalent species was again *C. insularis*, which represented 77.4% of emerging parasitoids ($n = 195$). The other species in order of abundance were *Ei. vitticollis* (7.2%), *Eu. plathypenae* (5.6%), *P. spinator* (5.6%) and *O. flavidus* (4.1%).

Discussion

Studies with insect pathogens have demonstrated that phagostimulant formulations can markedly improve the efficacy of microbial insecticides as biocontrol agents (Bell and Kanavel 1977, Bartelt et al. 1990). This is because such formulations increase the feeding activity of the pest, which increases the likelihood of consuming a lethal dose, resulting in improved levels of pest control. The delivery of spinosad in phagostimulant granules for control of fall armyworm in maize therefore seemed to present a particularly intriguing opportunity for study because of the high activity of spinosad by ingestion. The favorable environmental profile, the low impact on natural populations of predators in the maize crop, and the minimal risk that this material represents to human health also attracted us to study this product.

The product label recommended rate for control of *S. frugiperda* in maize in the United States is 67–100 g (AI)/ha in ground spray application, although other publications indicate lower rates (25 or 50 g [AI]/ha) are effective for *S. frugiperda* control by ground spray application (Thompson and Hutchins 1999, Thompson et al. 2000). Therefore, we were surprised to find that extremely low rates of spinosad in phagostimulant granules gave excellent control of *S. frugiperda* infestations in maize. The efficacy of the formulation was clearly related to the rate of spinosad that it contained (Figs. 1A and 3A). Even so, the degree of insect control at 0.3 and 1.0 g (AI)/ha was close to that of chlorpyrifos applied at a much higher rate (150 g [AI]/ha) in a nonphagostimulant, mineral-based, commercial granular formulation.

In both field trials, there was a trend for larval recovery to increase over time as insects reinfested spinosad- and chlorpyrifos-treated plants, whereas larval recovery decreased over time in control plots. We attribute this to natural mortality of initially high infestations in control plots from natural enemies and due to cannibalism, which normally results in no more than one large *S. frugiperda* larva infesting the leaf whorl of each plant (Chapman et al. 2000). The effect of predation and self-thinning by cannibalism would have been less important in spinosad- and chlorpyrifos-treated plots until larval numbers increased by reinfestation to about one larva per plant. In this respect, the mean recovery at 14–15 d postapplication, in all treatments and in both years, was ≈ 8 –14 larvae from a sample of 12 plants, i.e., ≈ 1 larva per plant.

In the case of the spinosad treatments, the initial efficacy estimates were conservative in the first field trial, because a high prevalence (40–77%) of non-specific mortality was observed in *S. frugiperda* larvae sampled at 3 d postapplication and incubated in the laboratory. We presume that most of this mortality was

due to spinosad intoxication, although $\approx 20\%$ mortality was also observed in the control insects. Spinosad is relatively slow-acting, such that insects that have received a lethal dose may not die until several days later. This delayed effect, however, was not seen in the 2003 trial.

In addition to being highly attractive to *S. frugiperda* larvae, the granular maize flour-based formulation has an additional advantage, compared with simple aqueous formulations, in that it facilitates the persistence of the active ingredient in the field (Castillejos et al. 2002). This effect arises from two sources; the opaque maize flour matrix protects the product from UV degradation (Tamez-Guerra et al. 1996), and the granules turn into a paste when it rains and therefore resist being washed off the leaf surfaces (McGuire et al. 1996). The principal factors affecting the duration of pest control after foliar application of synthetic insecticides on maize are twofold. First, the rapid growth of the plant in the whorl stage means that treated leaf surfaces in the whorl quickly develop into external leaves, carrying the active ingredient out of the whorl and diluting it during the process of leaf expansion (Andrews 1980). Second, first instar *S. frugiperda* disperse on silk threads and may rapidly recolonize treated plants once toxic residues have degraded or been washed away by rainfall (Harrison 1986).

The effect of spinosad applications on parasitoid communities was an issue of concern given that many species of parasitic Hymenoptera are sensitive to spinosad (Hill and Foster 2000, Suh et al. 2000, Elzen et al. 2000). The percentage of parasitism in field-collected *S. frugiperda* did not differ among treatments in either field trial. The number of parasitoids that emerged from each treatment was, therefore, directly related to the number of host larvae recovered in each sample, as is apparent in Figs. 1B and 3B. This is probably a consequence of the biology of the dominant parasitoid, *C. insularis*, which accounted for $\approx 80\%$ of parasitism observed in each field trial. This braconid parasitizes the eggs of *S. frugiperda* but develops and emerges in the larval stages. The spinosad and chlorpyrifos treatments were therefore unlikely to have directly affected the host searching and parasitism behavior of this wasp. As a result, the emergence of parasitoids in the laboratory reflects the probability that the parasitized host larva survives the insecticidal treatments. *C. insularis* is highly susceptible to contact with spinosad (D.I.P., unpublished data). However, the use of the granules applied directly into the leaf whorl allows the product to be applied directly to the feeding site of the pest larvae, thus avoiding contamination of other parts of the plant where parasitized egg masses and searching parasitoids may be located.

The development of spinosad formulations and application strategies that mimic those of synthesized insecticides suggest that the exploitation of this product is based upon the chemical paradigm (Gaugler 1997), i.e., this naturally derived product is designed to be used in the same way as a synthetic. It has been

stated many times that biological and naturally derived pesticides do not behave and do not have the same properties as synthetic insecticides (Gaugler 1997; Waage 1997, 1999). Particular attention should be paid to the development of suitable formulations that take advantage of their unique characteristics and mode of action. The fact that phagostimulant granules permit the control of fall armyworm larvae with ultralow rates of spinosad seems a particularly pertinent example of how appropriate formulation technology can enhance the performance of a naturally derived, biorational insecticide.

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