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# Lethal and sublethal effects of the naturally derived insecticide spinosad on parasitoids of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

DORA I. PENAGOS[1], JUAN CISNEROS[1], OLIVIA HERNÁNDEZ[1], & TREVOR WILLIAMS[1,2]

[1]*ECOSUR, Chiapas, Mexico, and* [2]*Depto. Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain*

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**Abstract**
Laboratory studies were performed on the lethal and sublethal effects of spinosad on three important species of parasitoids attacking *Spodoptera frugiperda* (J.E. Smith) in Mexico. Reproduction of the braconid *Chelonus insularis* (Cresson), on treated egg masses was completely eliminated at 200 parts per million (ppm) and reduced by ~70% at 20 ppm compared to the controls. Adult *C. insularis* did not avoid contact with residues on maize (200 ppm), but suffered a 7-day reduction in longevity after contact with residues. Initial toxicity of spinosad applied to a natural host of *S. frugiperda* was concentration dependent and resulted in 23 to 100% mortality of the eulophid *Euplectrus plathypenae* Howard at 25 to 200 ppm, respectively. The survival of *Eu. plathypenae* was initially reduced, especially in males, following contact with field weathered residues on maize (200 ppm). However, survival of both sexes rapidly returned to control values on foliage sampled after rainfall. A similar effect was observed in the mortality response of female *Eu. plathypenae* exposed to residues on sorghum. The ichneumonid *Eiphosoma vitticolle* Cresson did not avoid reproduction in *S. frugiperda* larvae that were externally contaminated with 200 ppm spinosad, although all spinosad-treated hosts died before the parasitoid progeny could develop. We use these results to predict the impact of spinosad applications on the foraging and reproduction of these parasitoids in the field. Such predictions require validation by field studies.

**Keywords:** *Spinosad, parasitoids, toxicity, mortality, reproduction, residues, repellency, maize,* Spodoptera frugiperda, Chelonus insularis, Eiphosoma vitticolle, Euplectrus plathypenae

## Introduction

Spinosad (Dow Agrosciences LLC) is a naturally derived insecticide produced by fermentation of the actinomycete, *Saccharopolyspora spinosa* Mertz & Yao (Bret et al.,

Correspondence: Trevor Williams, Depto. de Producción Agraria, Universidad Pública de Navarra, Pamplona 31006, Spain. Tel: +34-948-16-8913. Fax: +34-948-16-9732. E-mail: trevor.williams@unavarra.es

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1997). It is a neurotoxin comprising a mixture of spinosyns A and D which are tetracyclic-macrolide compounds that act upon the post-synaptic nicotinic acetylcholine receptor and the GABA receptors in a unique manner (Salgado, 1998; Watson, 2001). Spinosad is highly active by ingestion and to a lesser degree by contact. Exposure results in cessation of feeding followed later by tremors, paralysis and death. Spinosad-based products have been registered in more than 30 countries for control of pest Lepidoptera, Diptera, some Coleoptera, ants and thrips (Thompson et al., 2000).

Spinosad has very little mammalian toxicity (Breslin et al., 2000) and is classified by the United States Environmental Protection Agency as an environmentally and toxicologically reduced risk material (Thompson et al., 2000). 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 & 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 (Miles & Dutton, 2000). 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 laboratory and field studies in natural and semi-natural conditions report moderately harmful or harmful effects on populations of hymenopteran parasitoids (Pietrantonio & Benedict, 1999; Bernardo & Viggiani 2000; Hill & Foster, 2000; Tillman & Mulrooney, 2000; Nowack et al., 2001; Mason et al., 2002). Sublethal effects on parasitoid longevity and reproduction are also more commonly observed in insect parasitoids compared to predator species (Williams et al., 2003).

Spinosad is currently being evaluated for control of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) on maize in southern Mexico (Cisneros et al., 2002; Méndez et al., 2002; Williams et al., 2004). Parasitoids represent very important agents contributing to the biological control of this pest throughout the growing season (Andrews, 1988; Wheeler et al., 1989).

The parasitoids used in this study represent three of the most prevalent parasitoid species attacking *S. frugiperda* on maize and sorghum in southern Mexico. The braconid *Chelonus insularis* (Cresson) oviposits in eggs of noctuid hosts. The solitary progeny develop in the larval host, emerging from late instars to pupate in a cocoon external to the dead host. The ichneumonid *Eiphosoma vitticolle* Cresson is a large solitary larval endoparasitoid that parasitizes larvae in the second and third instar, emerging from the fifth instar host to pupate in a large silk cocoon. In contrast, the ectoparasitic progeny of the eulophid *Euplectrus plathypenae* Howard develop gregariously on the dorsal segments of middle instar noctuid larvae, eventually pupating in small cocoons on the host corpse. We selected these species for study due to their importance as natural enemies and the marked differences in their reproductive biology.

The aim of this study was to determine the importance of different routes of exposure on parasitoid survival, specifically contact with spinosad residues on the host and the host plant. We also assessed how rapidly toxicity declines in field-weathered residues. Finally, we examined sublethal effects that could affect the effectiveness of these parasitoids, including longevity and parasitoid reproduction on contaminated hosts. This information assists in the formation of specific hypotheses on the compatibility of spinosad use with the conservation of parasitoid populations in

maize and sorghum in Mexico. Once formulated, such hypotheses can be tested explicitly in field trials.

## Materials and methods

### *Preparation of spinosad solutions*

Experimental solutions of spinosad were prepared from a commercial formulation (Tracer®) containing 480 g/L of spinosad active ingredient (a.i.). Product label recommendations for control of *S. frugiperda* in maize are 67–102 g a.i./ha (Dow Agrosciences, 2003). For a nominal 300-L ground spray application volume, 67 g a.i./ha would represent a concentration of 223 mg/L or parts per million (ppm) spinosad. Consequently, we selected 200 ppm as being representative of a field application concentration and a range of dilute solutions (20–100 ppm) to examine the effects of exposure to lesser concentrations of the product.

### *Insect colonies*

Laboratory colonies of *C. insularis* and *Eu. plathypenae* were started using parasitoids that emerged from parasitized *S. frugiperda* larvae collected from maize plants grown within a 30-km radius of Tapachula, Chiapas, Mexico. Adult parasitoids were separated by species and placed in glass cages containing a source of 10% honey solution. Rearing cages were held at 26–28°C, 12:12 h L:D, 85–90% RH. Studies involving *Ei. vitticolle* were performed using adults that emerged from *S. frugiperda* larvae collected in maize.

Adult *C. insularis* were offered *S. frugiperda* egg masses (<24 h old) obtained from a laboratory culture maintained continuously on a semi-synthetic diet (adapted from Mihm, 1984) in ECOSUR, Tapachula, Mexico. Egg masses were removed after 24 h and replaced with newly laid egg masses. Larvae that emerged from exposed egg masses were reared individually on semi-synthetic diet until parasitoid pupation and adult emergence.

For *Eu. plathypenae*, adult parasitoids were offered third stage *S. frugiperda* larvae for periods of 48 h. Parasitized larvae were easily recognized by the presence of parasitoid eggs placed dorsally on the host. Parasitized larvae were removed and reared individually in 25-mL plastic cups containing semi-synthetic diet until parasitoid pupation and adult emergence. All experimental procedures involving parasitoids were performed at $26 \pm 1^{\circ}$C, 12:12 h L:D, 70–85% RH.

### *Effect of host contamination on* Chelonus insularis

Egg masses were laid by *S. frugiperda* females from the laboratory colony on the inside of white paper bags ($30 \times 14$ cm). Egg masses of similar size (approximately 100–200 eggs/mass) were carefully cut around and randomly assigned to one of the experimental treatments. Individual egg masses were immersed for 30 s in one of the following solutions (i) spinosad 200 ppm, (ii) spinosad 20 ppm, (iii) water control. All solutions included 0.01% (v/v) triton X-100 as a wetting agent. Egg masses were allowed to dry completely in a gentle air stream for 40 min before being placed in a 10-mL glass vial sealed with a piece of nylon gauze. The egg mass was placed with the paper side in contact with the side of the vial so that the parasitoid only came into

contact with the treated eggs. A single 3-day-old *C. insularis* mated female with no prior ovipositional experience was placed in each vial for a period of 1 h. After this time the female was carefully transferred to a clean glass vial with drops of 20% honey solution and was checked for mortality twice daily during the following 3 days. The experiment was performed with 39 different female parasitoids in each treatment.

Egg masses that had been exposed to female parasitoids from each treatment were selected at random ($n = 31$/treatment) for the following studies. Each egg mass was individually placed in a clean 10-cm diameter plastic Petri dish. The number of larvae that emerged from each egg mass was noted. Neonate larvae were individually transferred to plastic cups and reared on semi-synthetic diet. The number of larvae that died of parasitism was noted. Adult male parasitoids that emerged from spinosad-treated hosts were individually placed in glass vials at $20 \pm 1^{\circ}$C with drops of honey solution and checked daily for mortality. Adult female parasitoids were used for other purposes and are not considered further.

*Repellency and effect of residues on longevity of* Chelonus insularis

Whorl stage maize plants, 1 m tall, planted in direct sunlight behind the laboratory of ECOSUR, Tapachula, Mexico, were sprayed to run-off with a solution of 200 ppm spinosad and 0.01% triton X-100 using a manual knapsack sprayer. Control plants were sprayed with a solution of triton X-100 alone. Leaves were randomly selected from the upper third portion of the plants at 1, 24, 48 and 72 h post-application. Rectangles approximately $5 \times 3$ cm were cut from these leaves and placed in 50-mL clear plastic centrifuge tubes (Corning, USA). Each tube contained a single leaf rectangle from the spinosad treated plants and one from the controls half of which were located in the upper or lower part of the tube alternately. Tubes were sealed with a piece of nylon gauze held in place by a rubber band. Additional tubes were prepared with two pieces of control leaves as an absolute control. Adult *C. insularis,* 7–10 days old, were obtained from the laboratory colony and were placed individually in each tube and left for 20 min to adjust. The position of parasitoids was observed at 15-min intervals and was recorded as being in contact with control or spinosad treated leaves. Observations were performed in a darkened laboratory from 08:30 until 15:00 h, a total of 27 observations/parasitoid, using the illumination of a 15-W red light bulb placed 50 cm above the centrifuge tubes. For analysis, the number of contacts with treated and control of leaf pieces was summed for the entire observation period. Contact with the plastic tube did not differ between tubes containing spinosad + control and control + control leaf pieces and was ignored for the purposes of analysis. Following the observation period, parasitoids were individually transferred to clean glass vials with a drop of 50% honey solution and incubated at $25 \pm 1^{\circ}$C. Mortality was recorded daily until all wasps had died. The entire procedure was performed with seven to 16 wasps for each timepoint for the spinosad + control treatment and five to eight wasps/timepoint in the control + control treatment (total $n = 74$). No rainfall occurred during the experimental period.

*Initial toxicity of spinosad residues to* Euplectrus plathypenae

Guinea grass, *Panicum maximum* Jacq. (Poaceae) is a non-cultivated host of *S. frugiperda*. Natural stands of the grass growing in the field opposite ECOSUR, Tapachula, Chiapas, Mexico were sprayed to run-off with solutions of 25, 50, 100 and

200 ppm spinosad or a water control. All solutions contained 0.01% triton X-100 as wetting agent. Due to the high temperature ($\sim$35°C), treated surfaces dried almost immediately after being sprayed. At 30 min post-application, 10-cm lengths of treated leaves were cut, individually placed in 50-mL polythene centrifuge tubes and immediately taken to the laboratory. The leaf covered the inside of the tube completely. In the laboratory a pair of *Eu. plathypenae* adult females were placed in each vial and maintained at 25°C and high humidity ($>$90%) for 12 h. After this time, parasitoids were placed in a clean tube with drops of 20% honey solution and checked daily for signs of intoxication such as tremors, inability to walk, paralysis (Bret et al., 1997), or mortality over the following 5-day period. The experiment was performed with between 24 and 52 parasitoids for each treatment ($n = 182$ in total).

*Toxicity of field weathered residues to* Euplectrus plathypenae

*Maize.* The trial was performed on 20–24 October 2003. Individual rows of maize plants (Tasca H-101), $\sim$60 cm tall, planted in a field 5 km from Tapachula, Chiapas, Mexico, were selected at random and sprayed to runoff with a solution of 200 ppm spinosad + a wetter-sticker 0.02% (v/v) Agralplus (Zeneca), using a manual knapsack sprayer. Control plants were sprayed with 0.02% Agralplus alone. No other insecticide treatments had been applied to the plants previously. Spray residues dried extremely rapidly. At 40 min post-application, two leaves were cut from half way up the stem of 10 maize plants, placed in plastic bags and immediately transported to the laboratory. All laboratory procedures were performed at 20 $\pm$ 1°C. Leaves from each plant were cut into three pieces (7.5 $\times$ 5 cm). Two of these pieces were individually placed in glass vials (7.5 cm tall $\times$ 1.5 cm diameter) containing a drop of 20% honey solution. Three adult *Eu. plathypenae* (one male + two females) recently emerged from the laboratory colony were placed in each tube which was sealed with nylon gauze. The parasitoids were incubated for 48 h and then placed in clean tubes with honey solution. The numbers of living and dead parasitoids were recorded daily until all parasitoids had died. The third piece of leaf was placed in a 50-mL plastic centrifuge tube together with 10 second instar *S. frugiperda* from the laboratory colony. The numbers of living and dead larvae were registered 48 h later. The entire procedure was repeated at 2 and 4 days after spraying. Only one sample (consisting of two leaves) was taken from a plant; plants were never re-sampled. Meteorological data were obtained from a computer logged weather station (Health EnviroMonitor, Davis Instruments Corp., Hayward, CA), located on the grounds of ECOSUR, Tapachula.

*Sorghum.* A similar but simpler experiment was performed on 11–15 September 2003 in a field planted with sorghum, $\sim$90 cm tall, located 8 km from the town of Tapachula. The procedures were identical to those described for maize with the following exceptions. All the parasitoids used were adult females and samples were taken at 0, 1, 2, 3, 4 and 5 days post-application. Parasitoid mortality was evaluated at 24, 48 and 72 h after being placed in contact with treated leaf pieces. Leaves were not offered to *S. frugiperda* larvae.

*Response of* Eiphosoma vitticolle *to contaminated hosts*

Third instar *S. frugiperda* were placed in a 10-cm diameter plastic Petri dish containing a filter paper disk moistened with 200 ppm spinosad solution. Larvae were allowed to move around freely on the filter paper for a period of 10 min. After this time they were placed on a paper towel and allowed to dry fully for 10 min. A pair of spinosad-treated and untreated control larvae of the same size and stage were placed at different ends of a 50-mL polythene centrifuge tube. A single mated female parasitoid, 3–7 days old, with no prior ovipositional experience, was carefully introduced to the centre of the tube, half way between each type of host, and her activity was observed. After stinging a host, the experimental larvae were replaced and the process repeated for a period of 10 min. The type of host stung by the wasp and the interval between stinging one host and stinging the next host were recorded. Larvae that had been stung were dissected under a binocular microscope within 24 h to detect the presence of parasitoid eggs. In total, the procedure was performed with 32 female parasitoids.

*Statistical analyses*

The prevalence of mortality in parasitoids exposed to spinosad residues on egg masses was compared by contingency tables ($\chi^2$ test). Comparison of mortality in tests involving multiple concentrations of spinosad was performed by log likelihood ratio test ($G$ test) (Sokal & Rohlf, 1981). Comparisons of mean progeny production, mean number of eggs/host and the mean interval between attacks were determined by $t$-test in the GLIM program (Numerical Algorithms Group, 1993). GLIM was also used to compare the prevalence of mortality, and the proportion of contacts with leaf pieces, between control and spinosad treatments with a binomial error distribution specified. GLIM employs a logistic model to handle binomially distributed data, so that the results are given as a log odds ratio ($\log_e[p/q]$). Consequently, the standard errors of binomial means become increasingly asymmetrical as the mean approaches the upper (1) and lower (0) limits of the distribution (Aitkin et al., 1989). Visually, this asymmetry is only evident at proportions of less than 0.20 or more than 0.80. Overdispersion was corrected for by scaling or using Williams' procedure, available as a macro in the GLIM package (Crawley, 1993). The results of scaled or corrected analyses are given as $F$ values. Parasitoid longevity was compared by two-way analysis of variance in GLIM with a normal error distribution and treatment and timepoint as factors. Survival of *Eu. plathypenae* exposed to spinosad residues was subjected to Weibull analysis (Crawley, 1993). To estimate the mean time to death, Weibull model means and associated errors are given as natural logarithms that have to be back-transformed and raised to the power of $1/\alpha$, the shape parameter, which describes the relationship between the risk of dying (hazard) and insect age. This process generates asymmetrical error estimates. In all cases the validity of models was checked by examination of the distribution of residuals and fitted values.

## Results

*Effect of host contamination on* Chelonus insularis

No adult parasitoid mortality was observed in any treatment at 24 h after exposure to egg masses that had been treated with spinosad. At 72 h post-exposure, 69% (27/39)

of the parasitoids exposed to egg masses treated with 200 ppm spinosad had died whereas none of the wasps from the 20 ppm spinosad or control treatments had died ($\chi^2 = 14.2$, df = 1, $P < 0.001$, for comparison of 200 ppm spinosad treatment and control).

All *S. frugiperda* from egg masses treated with 200 ppm spinosad died in the egg stage, during eclosion or immediately thereafter. As a consequence, no parasitoids were reared from this treatment (Table I). A total of 5809 larvae were reared from egg masses exposed to *C. insularis* in the control and spinosad 20 ppm treatments. Host eclosion was reduced by approximately two-thirds in the 20 ppm spinosad treatment compared to the control ($t = 5.20$, df = 60, $P < 0.001$). The prevalence of mortality of host larvae in the 20 ppm spinosad treatment was almost twice that of the controls ($F_{1,44} = 47.4$, $P < 0.001$, following Williams' correction for overdispersion). The mean number of *C. insularis* progeny produced by each female parasitoid was reduced by approximately two-thirds in the 20 ppm spinosad treatment compared to the controls ($t = 3.32$, df = 60, $P = 0.002$) (Table I).

Adult males that successfully developed in hosts from the 20 ppm spinosad treatment suffered no adverse effects on longevity. The mean (±SE) adult longevity of male *C. insularis* that developed in hosts from the 20 ppm spinosad treatment was 48.3±1.5 days ($n = 85$) compared to 48.2±1.1 days ($n = 161$) for control males ($F_{1,244} = 1.13$, $P = 0.29$).

### *Repellency and effect of residues on longevity of* Chelonus insularis

Spinosad residues were not repellent to adult *C. insularis.* Parasitoids were observed in contact with spinosad-treated leaves on 54.3, 46.2, 45.5 and 52.3% of occasions for maize leaves treated 1, 24, 48 and 72 h prior to testing. These differences were not significant ($F_{3,43} = 2.50$; $P = 0.07$, scale parameter = 1.54). Control parasitoid longevity varied among timepoints with parasitoids used for the 48-h residue treatments having, on average, shorter lives (16.8 days) than in other timepoints that varied between 23.0 and 26.5 days) ($F_{3,72} = 3.09$; $P = 0.032$). The longevity of parasitoids was consistently reduced in parasitoids exposed to spinosad-treated leaves compared to controls ($F_{1,73} = 9.04$; $P < 0.004$). The average reduction of longevity in wasps exposed to spinosad residues was 7.0±2.16 days. The interaction between treatment and timepoint was not significant ($F_{3,69} = 0.748$; $P = 0.52$) indicating that the treatment effect was similar at all timepoints.

Table I. Reproduction of *Chelonus insularis* on fall armyworm egg masses treated with spinosad (values are means±SE)

| Treatment | Number parasitoids tested | Number hosts emerged/egg mass | Percentage mortality of host larvae | Number of parasitoid progeny/female |
|---|---|---|---|---|
| Water control | 31 | 142.1±16.8 a | 24.7 (20.9–29.1) a | 51.6±10.2 a |
| Spinosad 20 ppm | 31 | 45.3±7.9 b | 43.5 (36.1–51.1) b | 15.7±3.5 b |
| Spinosad 200 ppm | 31 | 0* | – | – |

*All hosts died during or immediately after eclosion. Means followed by identical letters are not significantly different for comparisons between treatments within each column (*t*-test of means, $P > 0.05$; data on mortality in larval stage were subjected to Williams' correction for overdispersion, SEs are asymmetrical).

*Initial toxicity of spinosad residues to* Euplectrus plathypenae

Spinosad resulted in a significant increase in mortality of female *Eu. plathypenae,* which rose steadily in all treatments in the 5 days following exposure to residues on guinea grass ($G = 38.6$, df $= 4$, $P < 0.001$, for comparison of cumulative mortalities among treatments at 5 days), whereas no mortality was observed in the control (Figure 1). At 100 and 200 ppm, parasitoid mortality was 100% by 4 days post-exposure. Mortality in the 25 and 50 ppm treatments was 23 and 82%, respectively. Such low concentrations are unlikely to be used for *S. frugiperda* control in field crops but may occur through spray drift or uneven spray residues. Clear signs of poisoning were seen in parasitoids in the 48-h period following exposure to spinosad (Figure 1). These were characteristic of a neurotoxin and included tremors, loss of coordinated movement, and a twitching paralysis (Salgado, 1998).

*Toxicity of field weathered residues to* Euplectrus plathypenae

*Maize*. The mean time to death of female and male *Eu. plathypenae* exposed to control leaf pieces was approximately 35 and 15 days, respectively (Figure 2). Contact with fresh (day 0) spinosad residues on maize leaves reduced the longevity of female parasitoids by ~60%, whereas male parasitoids were more severely affected, 80% of which were dead 48 h after contact with fresh residues. The toxicity of residues declined rapidly; the mean times to death of both sexes exposed to 2- and 4-day-old residues were similar to those of control insects (Figure 2).

Mortality of *S. frugiperda* larvae that were placed on pieces of spinosad-treated maize leaves confirmed that the presence of toxic residues was initially high (100% mortality at 0 days) but this rapidly declined to 19% mortality on 2-day-old residues

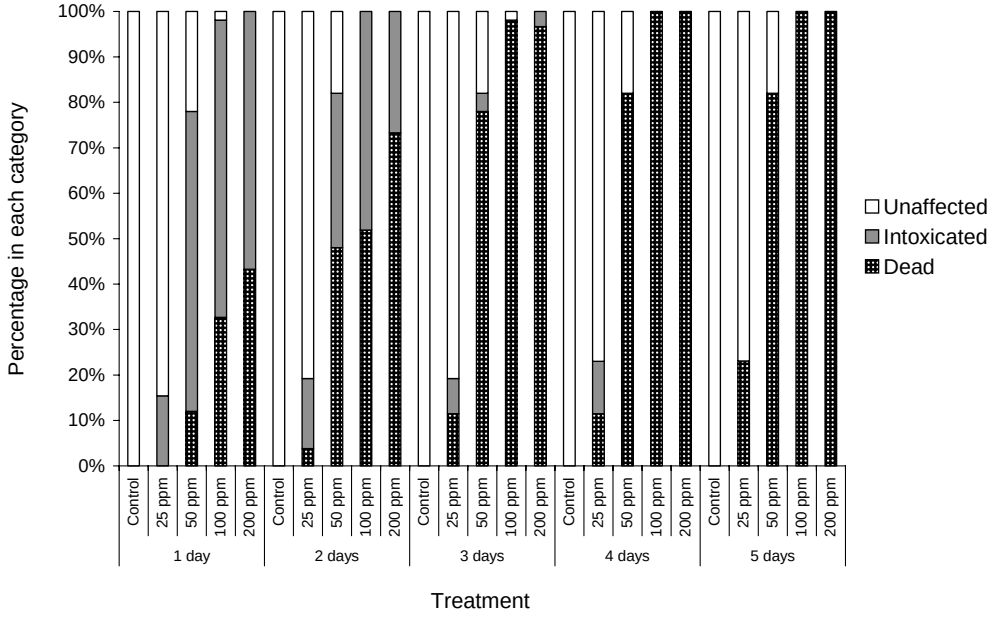


Figure 1. Cumulative prevalence of intoxication and mortality observed in female *Euplectrus plathypenae* at 1–5 days after being exposed to guinea grass treated with 25, 50, 100 and 200 ppm spinosad.

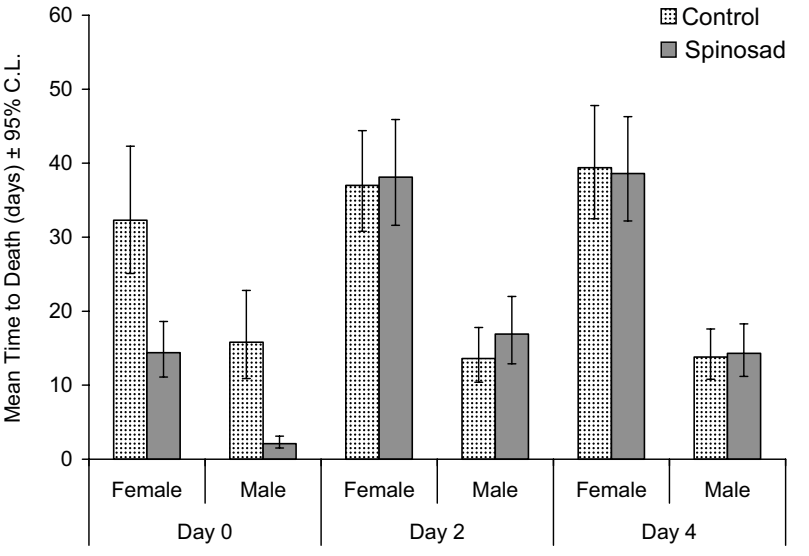


Figure 2. Mean time to death of adult *Euplectrus plathypenae* of both sexes exposed to fresh (day 0), 2 or 4 day old residues of spinosad applied at 200 ppm to maize plants and water controls. Survival times and 95% C.L. were estimated by Weibull analysis.

and 1.0% on 4-day-old residues. Both treatment ($F_{1,59} = 68.7$, $P < 0.001$) and interval between spraying and sampling ($F_{2,58} = 71.2$, $P < 0.001$) were therefore highly significant. Mortality of control larvae varied between 2.0 and 7.0% in the same period, resulting in a significant treatment × interval interaction term ($F_{2,56} = 16.6$, $P < 0.001$, scale parameter = 1.3 in all cases).

These results can be understood in relation to meteorological conditions during the trial. Day temperatures were high (>35°C), with high humidity and cloudless skies every day (Table II). A small quantity of rain fell in the afternoon following the application (7.6 mm) but a much greater quantity fell the following day (day 1, 32 mm), so that spinosad residues sampled on day 2 would have been washed off experimental plants. Additional rainfall on the afternoon of day 2 (also 32 mm) washed the plants once more prior to the final sample being taken on the morning of day 4 (Table II).

*Sorghum.* In sorghum, the average mortality in control wasps was 2.5%; control mortality did not exceed a maximum of 6.6% at any timepoint in any sample. The 24-h mortality of female *Eu. plathypenae* was significantly greater than control mortality ($\chi^2 = 46.8$, df = 1, $P < 0.001$) and declined significantly in residues over 2 days old compared to younger residues ($\chi^2 = 84.9$, df = 1, $P < 0.001$). The 48- and 72-h mortalities of parasitoids were significantly affected by age of the deposits ($P < 0.001$ in all cases). Fresh deposits (day 0) were toxic to female parasitoids (98% mortality at 72 h) whereas 1–2-day-old residues had intermediate toxicity (50–70% mortality). In contrast, 3- and 4-day-old residues did not result in more than 15% mortality (Figure 3).

As in the maize trial, these results should be viewed in relation to meteorological conditions (Table II). The weather conditions were similar to those of the maize trial with high day temperatures, high humidity and sunny, with cloudless skies most of the

Table II. Meteorological conditions during the experiments on the decay of spinosad residues applied at 200 ppm (a.i.) to maize and sorghum plants in southern Mexico

| | Meteorological parameters | | | |
|---|---|---|---|---|
| | Temperature (°C) Mean (min–max) | Precipitation (mm) | Relative humidity (%) Mean (min–max) | Solar radiation (W/m$^2$) Mean ± SE (peak) |
| *Maize trial* | | | | |
| Day 0 | 26.3 (22.3–36.4) | 7.6 | 80 (48–92) | 420 ± 29 (1172) |
| 1 | 27.7 (22.3–37.9) | 32.0 | 75 (39–94) | 548 ± 32 (1067) |
| 2 | 29.6 (22.6–38.2) | 32.0 | 67 (42–90) | 592 ± 28 (1098) |
| 3 | 29.3 (23.4–37.2) | 0 | 67 (41–85) | 546 ± 27 (1082) |
| 4 | 29.7 (23.8–37.5) | 0 | 65 (37–83) | 521 ± 26 (1051) |
| *Sorghum trial* | | | | |
| Day 0 | 27.2 (21.7–36.9) | 0.0 | 72 (49–90) | 486 ± 42 (1159) |
| 1 | 28.3 (23.2–36.8) | 0.0 | 69 (45–80) | 388 ± 22 (1054) |
| 2 | 27.7 (24.1–36.8) | 1.8 | 77 (47–93) | 396 ± 29 (1320) |
| 3 | 26.9 (23.9–37.1) | 15.8 | 79 (43–96) | 438 ± 32 (1204) |
| 4 | 26.4 (22.9–36.3) | 0.0 | 82 (48–94) | 324 ± 24 (1020) |

day. Appreciable rainfall (15.8 mm) only occurred on day 3 of the trial, which appears to be correlated with the decrease in toxic activity of spinosad on sorghum leaves observed in 3- and 4-day-old residues.

*Response of* Eiphosoma vitticolle *to contaminated hosts*

Each female parasitoid stung an average (±SE) of 7.8 ± 0.6 hosts during the 10-min exposure period. Overall, 45.2% (57/126) of the hosts stung had been previously treated with spinosad. This was not significantly different from a random distribution of attacks ($\chi^2 = 1.14$, df = 1, $P = 0.28$). The mean interval between stinging was similar for control (131 ± 18 s) and spinosad treated hosts (116 ± 15 s) ($t = 0.65$, df = 30, $P = 0.52$). Dissection revealed that spinosad treated hosts received a similar mean number of eggs (2.23 ± 0.24 eggs/host) to control hosts (1.72 ± 0.26) ($t = 1.42$, df = 30, $P = 0.17$). Hosts had to be dissected within 24 h post-parasitism as host mortality reached 100% by 72 h after contact with filter paper impregnated with 200 ppm spinosad.

## Discussion

Assessment of the impact of spinosad on non-target insects is particularly relevant now that synthetic spinosyn analogues (spinosoids) are being developed for increased environmental stability and an altered spectrum of insecticidal activity (Crouse et al., 2001; Sparks et al., 2001). Natural enemy responses to spinosad can be classified using the International Organization for Biological Control of Noxious Animals and Plants (IOBC) laboratory and field scales that run from 1 (not harmful) to 4 (harmful). Exposure to treated egg masses resulted in a 69% mortality of *C. insularis* at 200 ppm in the laboratory, representing a class 2 (slightly harmful) effect. Contact with residues on maize leaves reduced the longevity of *C. insularis* by an average of 7 days, irrespective of the interval between application and testing (0–3 days). Spinosad

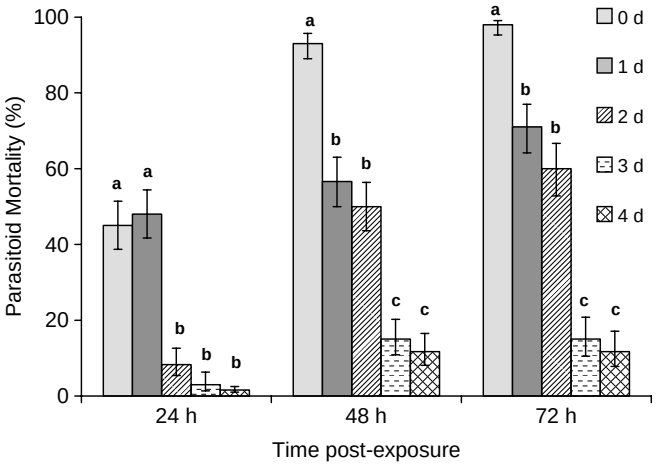


Figure 3. Mortality of *Euplectrus plathypenae* females at 24, 48 and 72 h after exposure to 0–4 day old residues of spinosad (200 ppm) on sorghum leaves. Vertical bars represent SEs and are asymmetrical. Columns headed by identical letters are not significantly different for comparisons between residues ages within each response time (ANOVA, $P > 0.05$, with binomial error distribution. SE values of 72-h responses have been scaled to account for overdispersion, scale parameter 1.3).

is usually reported to degrade quickly following application in the field with residues no longer toxic by ~7–10 days post-application (McLeod et al., 2002; Thompson et al., 2002a,b), although particularly dry conditions may result in greater persistence of residues (Williams III et al., 2003). Parasitoid populations may therefore recover in a relatively short period of time assuming a suitable degree of crop reinfestation by the host population (Scholz et al., 2002; Viñuela et al., 2002). Recovery in parasitoid populations has been observed in 9–15 days in maize plots in southern Mexico treated with ultra-low rates (0.1–1.0 g a.i./ha) of spinosad in a novel phagostimulant granule formulation (Williams et al., 2004). However, even after recovery, parasitoid populations may suffer sublethal effects from spinosad residues that may decrease their effectiveness as natural enemies. Unfortunately, it is difficult to quantify such effects in the field (Stark et al., 2004).

Adult *C. insularis* did not avoid contact with spinosad residues indicating that spinosad does not exhibit repellent properties, unlike pyrethroids, for example. It is possible that cut maize leaves released volatile compounds that could affect the behaviour of this parasitoid (Turlings et al., 1995), but as treated and control leaves were handled in the same way, we believe that leaf volatiles did not represent a serious source of error in our experiment. Foraging of *Trichogramma pretiosum* in maize plots in Australia was reduced by approximately two-thirds on spinosad-treated egg masses compared with control egg masses, but was completely eliminated on deltamethrin-treated egg masses (Scholz & Zalucki, 2000). Microparasitoids, such a *Trichogramma* spp. tend to be more sensitive to spinosad residues than robust species such as *C. insularis*. This indicates that *C. insularis* are unlikely to avoid foraging on spinosad treated plants in the field.

Reproduction of *C. insularis* was completely eliminated at 200 ppm because all hosts died prior to, or in the act of eclosion, or immediately afterwards. Ovicidal properties of spinosad have been reported for lepidopteran species (Bret et al., 1997; Peterson et al., 1998) although the magnitude of the ovicidal activity is lower in water

compared to that observed when using organic solvents (Pineda et al., 2000). At 20 ppm, the average number of adult progeny produced by each female *C. insularis* was reduced by ~70% compared to the control. This was due to lower host eclosion combined with increased mortality of parasitized hosts prior to parasitoid emergence. Spinosad acts more slowly than most synthetic insecticides (Thompson et al., 2000). Consequently, the effects of spinosad poisoning were evaluated at 3–5 days post-exposure. Evaluations of mortality performed prior to 48 or 72 h post-exposure are unlikely to reflect accurately the proportion of the population that has acquired a lethal dose of spinosad (Viñuela et al., 2001; Mason et al., 2002).

For *Eu. plathypenae*, contact with fresh dried residues on leaf surfaces resulted in 100% mortality at 100 and 200 ppm, representing a class 4 toxicity rating (harmful). For both *Eu. plathypenae* and *C. insularis*, exposure to lesser concentrations of spinosad resulted in a lower prevalence of mortality. However, the toxicity of spinosad residues declined rapidly and rainfall was clearly implicated in this process; crops that received rain became non-toxic to *Eu. plathypenae* in 1–3 days. In contrast, the lack of rainfall in the tests with *C. insularis* resulted in a detectable toxic effect that reduced parasitoid longevity in all samples taken during the 3-day trial.

The ichneumonid *Ei. vitticolle* showed no aversion to spinosad-treated hosts, despite the fact that these hosts had acquired a lethal dose of the compound. The act of parasitism by this wasp is very rapid and the host responds by wriggling aggressively in the presence of the parasitoid. Previous studies demonstrated that diseased *S. frugiperda* larvae became sluggish and were more readily parasitized by *Ei. vitticolle* than healthy larvae, presumably due to a reduction in the ability to defend themselves from this parasitoid (López et al., 2002). In contrast, the larvae employed in the present study were recently treated and had not developed signs of spinosad poisoning, which may have influenced the result that we observed.

Recently, published studies on the impact of spinosad on 52 species of natural enemies were reviewed in detail (Williams et al., 2003). Mortality responses to spinosad were classified using the IOBC toxicity scales. Overall, 71% of laboratory studies and 79% of field-type studies on predators gave a class 1 result (not harmful). In contrast, hymenopteran parasitoids were observed to be significantly more susceptible to spinosad than predatory arthropods with 78% of laboratory studies and 86% of field-type studies returning a moderately harmful or harmful result. Compared to predators, parasitoids are also more likely to suffer debilitating sublethal effects including loss of reproductive capacity and reductions in adult longevity and foraging ability (Elzen et al., 2000; Scholz & Zalucki 2000; Suh et al., 2000; Consoli et al., 2001; Viñuela et al., 2001; Schneider et al., 2003; Williams III et al., 2003). In the present study, sublethal effects were observed in the reproduction and longevity of *C. insularis* and on the survival of *Eu. plathypenae*. In contrast, *Ei. vitticolle* oviposited normally in spinosad-contaminated host larvae although all hosts subsequently died from spinosad intoxication.

Laboratory dose-mortality studies are of limited use in predicting the impact of a toxicant on non-target invertebrate populations in the field (Stark et al., 1995; Wright & Verkerk, 1995; Longley & Jepson, 1996). Species or stage-related differences in biology and behaviour can significantly influence the susceptibility of natural enemies to pesticides (Longley & Jepson, 1997; Verkerk et al., 1998). This was particularly evident when considering the impact of spinosad on immature parasitoids developing in *S. frugiperda* eggs and larvae. Similar effects, including an inability to spin a cocoon

for pupation or spinosad-induced mortality at the moment of adult emergence have been observed in parasitoids of other taxa (Suh et al., 2000; Gahbiche, 2001; Mason et al., 2002).

These observations lead us to formulate several hypotheses for field testing. First, we predict that spinosad contamination of host egg masses is likely to adversely affect the reproduction of *C. insularis*. However, as *S. frugiperda* egg masses are usually found on the underside of leaves close to the ground, they may be protected from heavy contamination by spray droplets directed at larvae feeding in the developing leaf whorl. Second, contact with dry residues on leaves appears unlikely to seriously affect parasitoid foraging behaviour or reproduction, particularly if several days have passed since the application. Third, we can predict that spinosad applications made during periods with frequent rainfall are likely to be less harmful to foraging parasitoids than applications made during extended dry periods as toxic residues are likely to be rapidly eliminated from crop leaf surfaces by rainfall. Such predictions can only be validated by performing field studies looking at parasitoid abundance and performance in the presence of insecticide residues.

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