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SUBLETHAL EFFECTS OF SPINOSAD (TRACER®) ON THE COTTON LEAFWORM (LEPIDOPTERA: NOCTUIDAE)

Gamal Elsayed Abouelghar[1], Hanem Sakr[2], Hager Ali Ammar[1], Adel Yousef[1], Moustafa Nassar[1]*

[1] Department of Pesticides, Faculty of Agriculture, Menoufia University, Shebin El-Kom 32511, Egypt
[2] Department of Zoology, Faculty of Science, Menoufia University, Shebin El-Kom 32511, Egypt

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Abstract: The effects of sublethal concentrations of spinosad (Tracer®) on development, fecundity, and food utilization, in the cotton leafworm, *Spodoptera littoralis* (Boisd.) were investigated. The fourth-instar larvae were fed on castor bean leaves treated with LC_{25} (13.9 ppm) or LC_{50} (57.8 ppm) of spinosad. Pupation and pupal weight were significantly reduced in both LC_{25} and LC_{50} treatments, compared with those of the controls. The fecundity rates of females in either LC_{25} or LC_{50} treatment were also reduced, compared with the controls. The residual activity of spinosad, applied on cotton at labeled field- and subfield-rates (200 and 70 g active substance (a.s.)/ 200 l water, respectively), was examined against the fifth-instar larvae of *S. littoralis*. Feeding deterrent effects were significantly demonstrated in larvae that fed on leaves collected from field plots with residual deposits of spinosad at 3 and 7 days old after application (DAA). The residual activity of spinosad on feeding and other metabolic parameters was decreased after 21 DAA indicating that the chemical started to degrade under field conditions. A histological study on midgut from larvae that previously fed on leaves treated with a concentration corresponding to the labeled-field rate of spinosad showed some alterations occurred after 48 and 96 h of treatment, compared to the normal midgut from the controls. The histological alterations included degeneration in the epithelial lining of the midgut and in the peritrophic matrix. Such histopathological effects are presumed to be responsible for the reduction in growth and food utilization caused by spinosad. It is, therefore, concluded that spinosad has sublethal effects on *S. littoralis* that may affect population dynamics in the field via reductions in survival and reproduction.

Key words: biological activity, midgut histology, residual activity, spinosad

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisduval), has long been a major polyphagous pest, widely distributed throughout Africa, Mediterranean Europe, and several parts of Asia (Azab *et al*. 2001). Larvae of this pest can feed on ~90 economically important plant species belonging to 40 families (Brown and Dewhurst 1975). In Egypt, the insect is one of the most destructive pests of cotton which is considered the most valuable crop in the country. Over the past 25 years, the intensive use of broad-spectrum insecticides against *S. littoralis* has led the development of insect resistance to many registered pesticides (Aydin and Gurkan 2006). In this scenario, using new types of insecticides, originated from natural agents or products that disrupt the physiological processes of the target pest, could be useful alternatives in the integrated management approach (Dhadialla *et al*. 1998; Thompson *et al*. 2000; Smagghe *et al*. 2003). Spinosad is one of these biologically derived insecticides. It is comprised primarily of two macrocyclic lactones, spinosyn A and D, which are secondary metabolites produced by the actinomycete *Saccharopolyspora spinosa* under natural fermentation conditions. Although superficially similar to avermectins in structure, the modes of action, toxicological profiles, and cross-sensitivity spectra of the two are quite different (Thompson *et al*. 1997). The mode of action of spinosad is two-fold, the primary target site is the nicotinic acetylcholine receptor, but the gamma-aminobutyric acid (GABA) receptor is also affected to some degree (Salgado 1997). Routes of entry include both topical and ingestion (Thompson *et al.* 1995). Signs of spinosad poisoning include initial flaccid paralysis, followed by tremors and eventual death (Thompson *et al.* 1995). Spinosad has been applied to over 200 different crops. It has been used to control caterpillars in cotton, loopers in cabbage, leafminers in various crops, leafrollers on apples, thrips in citrus, *etc*. (Bret *et al.* 1997; Thompson *et al*. 2000). Use of spinosad in conventional agriculture started with applications of the Tracer® formulation on cotton in 1997. It was applied for caterpillars in cotton, especially in situations where the caterpillars were resistant to pyrethroids or other broad-spectrum materials (Bret *et al.* 1997). In addition, its good environmental performance (quick degradation, low toxicity to humans, and low doses of use)

*Corresponding address:
gabouelghar@gmail.com

makes spinosad a choice for Integrated Pest Management (IPM) programs in vegetables and ornamentals (Pineda *et al*. 2004).

Thus, this study was conducted to investigate the effects of sublethal concentrations of spinosad on some biological characteristics of the cotton leafworm, *S. littoralis*. We also determined the residual activity of spinosad on the food consumption and utilization by larvae that fed on leaves with residues of spinosad. Additionally, the histomorphological alterations probably caused in the midgut of *S. littoralis* larvae due to feeding on spinosad were also investigated.

MATERIALS AND METHODS

Rearing of insect

Experiments were conducted on *S. littoralis* taken from the laboratory-stock culture established from eggs obtained from the Institute of Plant Protection Research, Ministry of Agriculture, Dokki, Egypt. The larvae were reared in the laboratory on castor bean (*Ricinus communis*) leaves as described by El-Defrawi *et al.* (1964). The rearing conditions were 27±2ºC, 60±5% relative humidity (RH), with a photoperiod of 14:10 (L:D) h. Adults were fed on a 10% honey solution. The Defla (*Nerium oleander*) leaves, for the oviposition of adults, were placed in the cage. Egg-masses were collected daily and kept in 90-cc plastic until hatching.

Bioassay of insecticide

Spinosad 24% wt/vol SC (Tracer®); Dow AgroSciences Indianapolis, IN, USA, was used in the experiment. To determine the LC_{50} value of spinosad against *S. littoralis* larvae, six to nine concentrations of the compound were prepared by diluting in tap water from stock solution. Newly moulted fourth-instar larvae (≤ 1-day-old) were fed on castor bean leaves which had been treated with each test concentration using a leaf-dipping technique. At least forty larvae were used for each concentration in three replications with 10–15 larvae per replicate. The larvae were placed in clear glass jars (400 ml) provided with treated leaves and covered by pieces of muslin. Larval mortality was scored daily; if no movement was observed, larvae were considered as dead. Larvae fed on leaves treated only with tap water served as the control. Mortality percentages were examined after 48 and 72 h of treatment, and corrected using Abbott's formula (Abbott 1925). Data were analyzed by Probit analysis (POLO-PC; LeOra Software 1987) to estimate LC_{50}, LC_{25} and 95% confidential limits (CL).

Biological activity of sublethal concentrations

In this experiment, the biological activity of the sublethal concentrations (LC_{25} and LC_{50}) of spinosad was evaluated. Newly moulted fourth-instar were obtained from the stock culture and starved for 2 h prior to exposure to the treated leaves. Castor bean leaves were dipped in each diluted concentration for ~10 sec, and into only water for the control treatment. At least three hundred larvae were used for each concentration, in three replications with 100 larvae each. The larvae of each replicate were reared in a glass jar (2-liters volume, 10 cm in diameter, 21 cm in depth). The larvae were provided with leaves treated with either LC_{25} or LC_{50}, and then allowed to feed on the treated leaves for 72 h. The larvae were maintained at 25±2°C, 65±5% RH, and a long 14:10 (L:D) h photoperiod in a climate incubator. For each treatment, the survivors were transferred into clean jars and provided daily with fresh untreated leaves until larvae either died or successfully pupated. Pupae were placed individually into a small tube, and those aged two days were weighed individually. Since not all larvae pupated on the same day, the early pupae were temporarily preserved at 4°C. When all the larvae had pupated, pupae were placed at room temperature for eclosion. Means of larval period, pupation, pupal weight, and adult emergence were recorded for each treatment.

For the fecundity assay, ten pairs of moths that emerged on the same day from each treatment (LC_{50}, LC_{25}, and the control) were collected and put into a glass jar (2-liters volume, 10 cm in diameter, 21 cm depth) in five replications (2 ♀ x 2 ♂ per replicate) and provided with fresh leaves of Defla for oviposition and absorbent cotton soaked in a 10% honey solution for moth nutrition. The egg-masses laid were counted daily and removed individually to a Petri dish for further observation. The leaves were replaced every two days until the death of the females. The egg-masses were examined using a binocular and number of eggs for each mass was counted using a glass-micrometer slide. To evaluate the fertility, five to seven egg-masses obtained from each treatment were observed daily for hatching, then the hatch percent was counted. Number of eggs hatching was assessed after 4 d when the egg hatch was completed in the control.

Residual activity of spinosad on food utilization

In this experiment, the residual activity of spinosad applied under field conditions, at the recommended field- and sub-field rates, on feeding utilization in *S. littoralis* larvae was assessed. Cotton plants (*Gossypium barbadense* L.) were treated with spinosad at the rate of 200 g active substance (a.s.)/ 200 liters water that equal 476 g a.s./ha (field recommended rate based on Dow AgroSciences (Eger and Lindenberg 1998) and sub-field rate (70 g active substance (a.s.)/200 liters water). Cotton plants in the field plots were sprayed with selected rates of spinosad solutions using a knapsack sprayer on both sides until runoff. The plants in the control plots were sprayed with water only. Leaves from the cotton plants were collected at 0, 3, 7, and 21 days after application (DAA). At each time point, 15–20 leaves from each treated and untreated plot were collected and brought to the laboratory for feeding experiments.

For the feeding assay, twenty newly moulted fifth-instar larvae (≤ 1-day-old) were collected from the stock culture and placed separately in Petri dishes (12 cm in diameter by 3 cm in height) for each treatment. Each dish was provided with two leaf discs, and wet filter paper was placed on the bottom of the dish to avoid early drying of the leaf discs. Each treatment was replicated three times. Larvae were fed for 72 h on treated leaves and then

provided daily with fresh untreated leaves until larvae either died or successfully pupated. The means of larval period, pupation, pupal weight, and adult emergence were recorded for each treatment. For each dish, larva and the introduced leaf discs were weighed. Food utilization parameters, including weight of consumed food (CF), consumption index (CI), relative growth rate (RGR), efficiency of the conversion of ingested food to body tissues (ECI), efficiency of the conversion of digested food to body tissues (ECD), and approximate digestibility (AD), were measured gravimetrically according to the method described by Waldbauer (1968). The index of feeding deterrency was calculated as:

$$(C–T)/(C+T) \times 100$$

where:
C – the consumption of the control leaf,
T – the treated leaf (Nathan *et al*. 2005).

Histological assay

Another experiment was conducted to determine the histological effects of spinosad on the larval midgut, fat body, and Malpighian tubules. Twenty newly moulted fifth-instar larvae (≤ 1-day-old) were allowed to feed on cotton leaves treated as described above with a concentration (1.0 g a.s. spinosad/1 liter water) corresponding to the labeled-field rate for 48 h. The survivors were transferred into clean jars and allowed to feed on fresh untreated leaves for two more days. The larvae in the control treatment were fed on leaves treated with only water. Larvae from both the spinosad and control treatments were removed after 48 and 96 h of treatment for histology assay. Larval-gut samples were dissected from the middle of the midgut and fixed in Bouin's solution. Routine steps of histopathological processing were used. Transverse sections (5 mm) were stained with Hematoxylin-Eosin (HE). The morphological alterations of the midgut cell structure and organization of each specimen were analyzed by microscopic examination and compared to the tissues taken from the control group. Also, the histological changes of the fat bodies and Malpighian tubules were examined and compared to the controls.

Data analysis

Results are expressed as means ±SE. All data obtained from the experiments were subjected to analysis of variance (ANOVA) using CoStat (ver. 6.311, CoHort Software, Monterey, CA). Treatment means were compared by Tukey's studentized range test, by the least significant difference (LSD) at p = 0.05.

RESULTS

Biological activity of sublethal concentrations

Toxicity data of spinosad against the fourth-instar *S. littoralis* larvae showed that LC_{25} and LC_{50} values, at 72 h of treatment, were 13.95 ppm (95% CL, 5.06–25.46) and 57.84 ppm (95% CL, 37.80–93.77), respectively. The data in table 1 indicated that sublethal concentrations (LC_{25} and LC_{50}) of spinosad resulted in a significant prolongation of the larval period in comparison with that of the control. Pupation rates were also significantly decreased by both LC_{25} and LC_{50} indicating 1.6- and 2.63-fold lower than that of the control, respectively. The data also indicated that mean weights of pupae that developed with LC_{25} and LC_{50} were significantly decreased with 15.2 and 22.3%, respectively, than those of the controls. The rate of adult emergence was also significantly decreased at $LC_{50,}$ compared to the control. In addition, the effect of sublethal concentrations on fecundity rate was considerable. The means of cumulative eggs/female from LC_{25}- and LC_{50}-treatments were significantly decreased with 1.46- and 2.30-fold lower than those of the controls, respectively. The egg hatch percentages were also decreased at LC_{25} and LC_{50} with 1.11- and 1.22-fold lower than the control, respectively.

The residual activity of spinosad

Effects on food consumption and other nutritional indices

The data presented in tables 2 and 3 show the deterrent effect of spinosad on food consumption and other nutritional indicies of *S. littoralis* larvae after feeding on leaves collected from cotton field plots treated previously with the recommended field and sub-field rates. It was obvious that feeding on spinosad-treated leaves resulted in a significant decrease in the mean weights of food consumed by larvae fed on leaves collected at 0, 3, 7, and 21 days following insecticide application. Feeding on leaves from field plots, at 0-day-old DAA, showed that both field- and sub-field rates significantly reduced the mean weights of consumed food with 44.07 and 41.12%, respectively, in comparison with those fed on untreated leaves

Table 1. Sublethal effects of spinosad, at LC_{25} and LC_{50}, on larval period, pupation rate, pupal weight, adult emergence, fecundity, and fertility of *S. littoralis* after feeding the fourth-instar larvae on treated leaves for 72 h

Treatment	Concentration [ppm][a]	Larval period [days]	Pupation [%]	Pupal weight [mg][a]	Emergence [%]	Fecundity[b] Eggs/♀	Hatch [%]
Control	–	7.16±0.16 c	87.7±0.63 a	278.8±2.07 a	88.5±0.74 a	706.75±8.2 a	95.6±0.18 a
Spinosad	13.9	8.75±0.18 b	54.7±1.25 b	236.3±3.72 b (15.2%)	85.7±0.70 ab	526±3.6 b (25.6%)	85.7±0.09 b
Spinosad	57.84	9.9±0.14 a	33.3±1.81 c	216.7±1.56 c (22.3%)	81.2±0.43 b	501.08±14.3 b (29.1%)	78.0±2.79 c

Data are the means ±SE of three replicates of 100 fourth-insatr larvae each. Means within the same column followed by the same letter(s) are not significantly different (LSD mean separation: p ≤ 0.05)
[a]sublethal concentrations used were equal to LC_{25} and LC_{50}.
[b]values between parentheses represent reduction percent relative to the control

(Table 2). Similar deterrent effects of food consumption were also observed for larvae fed on leaves from treated plots at 3 DAA. However, it was obvious that the deterrent effect of residual activity of spinosad decreased after 7 and 21 days old following application, indicating that the chemical started to degrade under field conditions. In general, the recommended-field rate caused higher deterrent activity of larval feeding than the sub-field rate.

The metabolic parameters in *S. littoralis* larvae fed on spinosad-treated leaves are provided in table 3. Feeding on treated leaves, at zero-time of field application caused a CI values at both field rates tested. The mean values of RGR in larvae that fed on treated leaves were in negative values as a result of larval weight loss, compared to that of the control. For the digestibility parameter, our data also showed a significant reduction in AD of larvae fed on leaves treated with both field rates. In addition, both ECI and ECD to body tissues were also in negative values for the larvae that fed on treated leaves. The data also demonstrated that all nutritional indices determined for larvae fed on treated leaves at 3 DAA were significantly decreased. Data also showed a significant reduction in RGR, ECI and ECD values of larvae fed on leaves with residues of spinosad, applied at either field- or sub-field-rate, up to 21 days old following insecticide application, in comparison with those in the control larvae.

Effects on biology of S. littoralis

Data of the residual activity of spinosad indicated that the fifth-instar larvae fed on leaves collected from field plots treated with the higher concentration (200 g a.s./200 l), at 0 or 3 DAA, completely failed to reach the pupal stage (Table 4). The results also showed that feeding on leaves with spinosad residues at 7 and 21 DAA resulted in a significant decrease in pupation percentages for both tested concentrations. Similarly, the means of pupal weights were significantly decreased at the low and high concentrations. Generally, it seems that the residual activity of spinosad was decreased at 21 DAA indicating that the chemical started to degrade under field conditions.

Table 2. Food consumed and feeding deterrence of the fifth-instar *S. littoralis* larvae after 48 h feeding on leaves collected from field plots sprayed with spinosad

Treatment	Field rate [g a.s./ 200 l]	Mean wt. of consumed food [mg/larva] and feeding deterrence index [%] after 48h feeding on leaves collected at indicated days after application (DAA)[a]			
		0-DAA	3-DAA	7-DAA	21-DAA
Control	–	1,172.1±0.13 a	1,480.2±0.27 a	775.1±0.17 a	1,191.1±0.28 a
Spinosad	70	445.0±0.12 b (44.96%)	544.1±0.20 b (46.25%)	688.2±0.18 b (26.77%)	542.0±0.09 b (17.69%)
Spinosad	200	489.2±0.22 b (41.12%)	436.0±0.28 c (54.49%)	629.1±0.20 b (30.88%)	566.2±0.15 b (15.59%)

Mean (±SD) values followed by the same letter(s) within each column, are not significantly different based on the LSD test at $p \leq 0.05$. Fifteen replications with individual fifth-instar for each assay

[a]numbers between parentheses represent the feeding deterrence index (FDI) % (Nathan *et al*. 2005)

Table 3. Nutritional indices of the fifth-instar *S. littoralis* larvae after a 48 h feeding on cotton leaves collected from field plots sprayed with spinosad

Treatment	Field rate [g a.s./ 200 l]	CI [mg/mg/day]	RGR [mg/mg/day]	AD [%]	ECI [%]	ECD [%]
Feeding on 0-day-old residues						
Control	–	1,502.0±22.2 a	371.0±30 a	79.0±4.9 a	25.3±4.7 a	32.4±7.7 a
Spinosad	70	794.1±21.7 c	–28.0±12 b	69.4±1.6 b	–4.1±4.3 b	–6.4±12.5 b
Spinosad	200	955.0±39.9 b	–145.0±14 c	72.4±19.4 ab	–17.8±3.8 c	–29.8±16.9 c
Feeding on 3-day-old residues						
Control	–	1,494.1±21.1 a	508.1±40.0 a	76.8±5.9 a	34.8±6.34 a	46.3±12.9 a
Spinosad	70	922.0±33.3 b	153.0±60.0 b	63.1±12.9 b	17.4±6.59 b	30.1±15.0 b
Spinosad	200	926.1±65.5 b	-56.0±9.0 c	73.1±9.6 a	-19.1± 15.4 b	-49.1±21.4 c
Feeding on 7-day-old residues						
Control	–	961.0±18.5 a	359.1±6.6 a	66.5±7.8 a	38.2±8.11 a	59.1±17.9 a
Spinosad	70	801.1± 21.2 b	218.0±5.5 b	60.5±10.9 a	29.3±9.8 b	51.5±18.0 a
Spinosad	200	898.2±22.3a b	123.0±6.6 c	59.4±11.7 a	14.7±8.11 c	28.1±15.8 b
Feeding on 21-day-old residues						
Control	–	1,727.2±38.8 a	467.1±9.1 a	78.9±8.77 a	29.6±7.55 a	39.3±13.9 a
Spinosad	70	1,233.1±32.2 a	205.1±8.8 b	74.2±6.93 a	18.7±6.63 b	28.3±10.5 b
Spinosad	200	1,225.2±37.0 a	158.0± 8.9 c	74.8±7.43 a	15.3±7.74 b	25.6±119 b

CI – consumption index; RGR – relative growth rate; AD – approximate digestibility; ECI – efficiency of conversion of ingested food to body tissues; ECD – efficiency of digested food to body tissues

Values in each column within each interval followed by the same letter are not significantly different based on the LSD test at $p \leq 0.05$. Fifteen replications with individual fifth-instar for each treatment

Table 4. Biological effects of the fifth-instar *S. littoralis* larvae after a 48 h feeding on cotton leaves collected from field plots sprayed with spinosad

Treatment	Field rate [g a.s./ 200 l]	Pupation [%]	Pupal weight [a] [mg]	Adult emergence [%]
Feeding on 0-day-old residues				
Control	–	93.3±0.25 a	229.1±27.45	92.9± 0.25
Spinosad	70	– [b]		
Spinosad	200	– [b]		
Feeding on 3-day-old residues				
Control	–	93.3±0.25 a	256.7±17.48 a	100 a
Spinosad	70	26.7±0.44 b	175.0±5.0 b (31.8%)	66.7±0.47 b
Spinosad	200	– [b]		
Feeding on 7-day-old residues				
Control	–	100±0 a	241.1±21.83 a	100 a
Spinosad	70	23.0±0.44 b	166.7±9.42 b (30.8%)	66.7± 0.47 b
Spinosad	200	20.0±0.40 b	163.3±4.71 b (32.2%)	55.0±0.43 c
Feeding on 21-day-old residues				
Control	–	93.3±0.25 a	289.1±27.12 a	100 a
Spinosad	70	40.0±0.49 b	218.0±27.86 b (24.5%)	100 a
Spinosad	200	46.7±0.56 b	211.7±12.13 b (26.8%)	100 a

Values (means ±SD) in each column within each interval followed by the same letter are not significantly different based on the LSD test at $p \leq 0.05$. Fifteen replications with individual fifth-instar for each treatment

[a] values between parentheses are reduction percent relative to the control

[b] all larvae failed to reach the pupal stage

Histological analysis of spinosad

It is evident that spinosad administered to the fifth-instar larvae by feeding on leaves treated with a concentration corresponding to the labeled-field rate caused some cytological changes in the midgut, fat bodies, and Malpighian tubules. The first noticeable histopathological signs appeared in the larval midgut after 48 h of treatment and became more obvious after 96 h (Figs. 2a, b), compared to that of the control (Figs. 1a, b). Some epithelial cells showed apparent histolysis and cytoplasmic vacuolation and some cells have pyknotic nuclei (Fig. 2a). Also, it appeared that the apical brush border of the epithelial cells was destroyed. The muscle fibers of treated individuals were separated from each other leaving a degenerated area in-between (Fig. 2b). The peritrophic membrane completely disappeared (Fig. 2a), and the regenerative cells were dissolved (Fig. 2b). The histological examination also showed some fat bodies in the larval midgut sections after 96 h of treatment with spinosad (Fig. 3b). The fat bodies appeared to be dissolved as a result of the treatment compared with the fat bodies of the control larvae (Fig. 3a). In addition, the Malpighian tubules of larvae exposed to spinosad were highly affected after 96 h of treatment (Fig. 4b). The lumen of the Malpighian tubules seemed to be filled with secretion and the cells had pyknotic nuclei (Fig. 4b) compared with those in the control larvae (Fig. 4a).

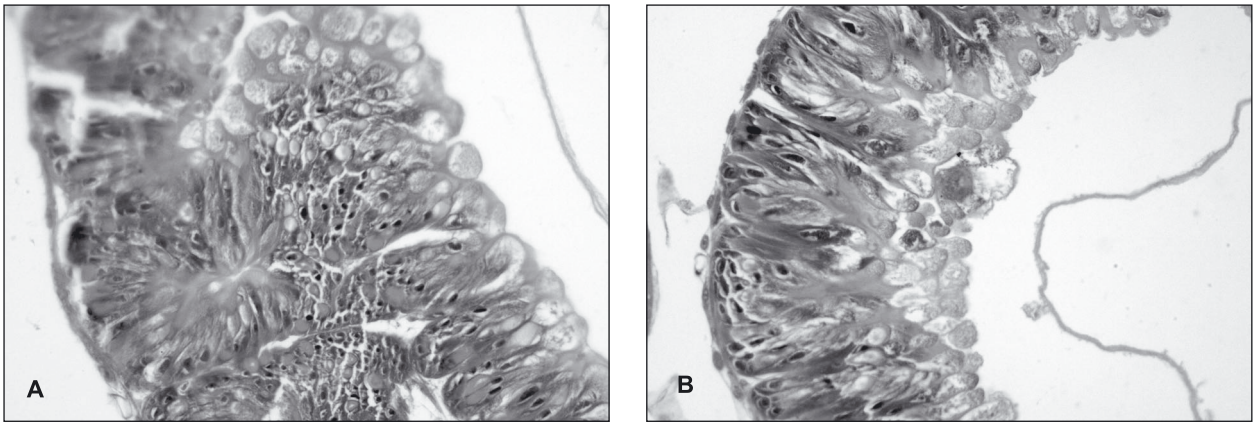

Fig. 1a–b. Transverse section through the midgut of healthy (the control) fifth-instar *S. littoralis* larvae stained with Hematoxylin-Eosin (HE-200x)

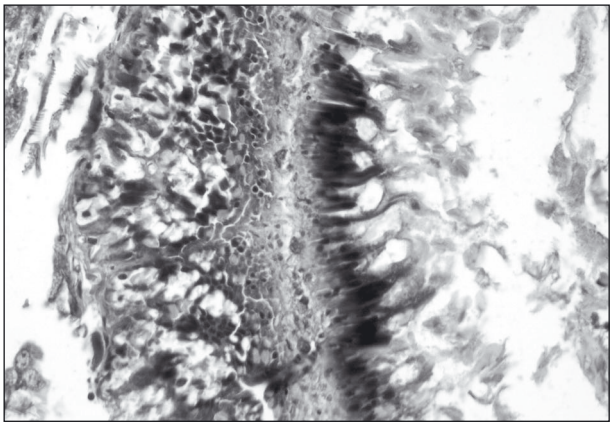

Fig. 2a. Transverse section through the midgut of fifth-instar *S. littoralis* larvae treated with spinosad (1 g a.s./ liter) after a 48 h post-treatment. Staining was done using Hematoxylin-Eosin (HE-200x). Some epithelial cells show apparent histolysis, cytoplasmic vacuolation. Also, some cells have pycknotic nuclei and the apical brush border of the epithelial cells is destroyed. The peritrophic membrane has completely disappeared

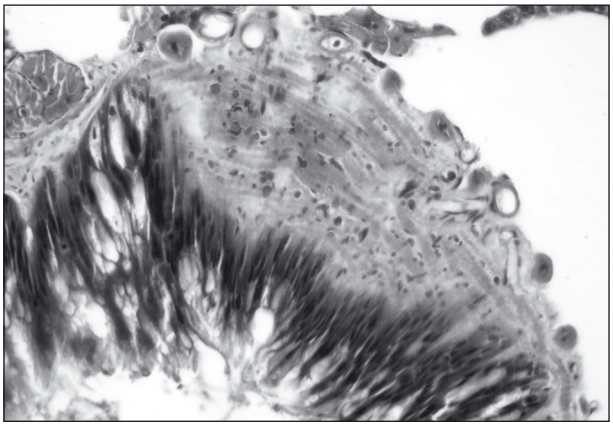

Fig. 2b. Midgut of fifth- instar *S. littoralis* larvae after a 96 h post-treatment with spinosad (1.0 g a.s./liter) – (HE-200x). The muscle fibers are separated from each other leaving a degenerated area in between. The regenerative cells are dissolved

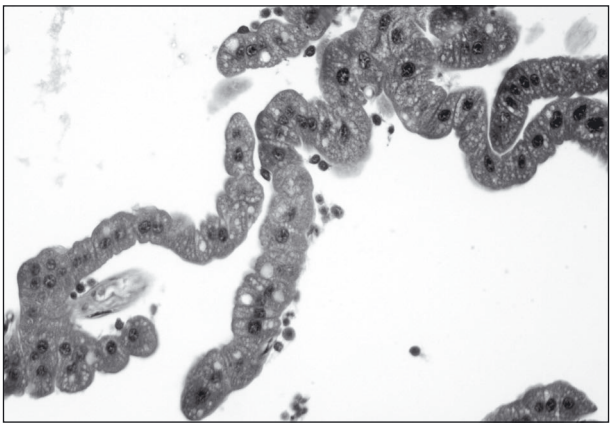

Fig. 3a. Transverse section through the fat-body of healthy fifth-instar *S. littoralis* larvae. Staining was done using Hematoxylin-Eosin (HE-200x)

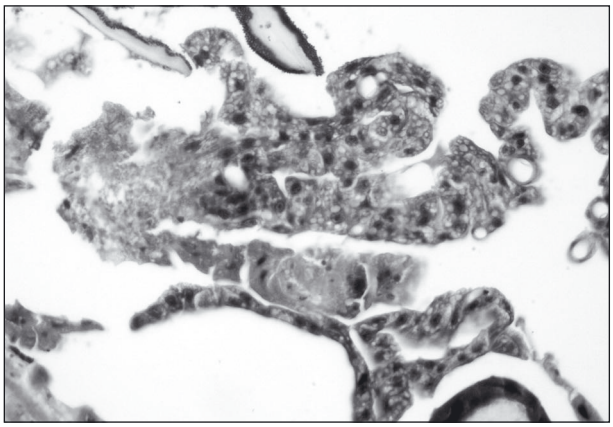

Fig. 3b. Fat-body of fifth-instar *S. littoralis* larvae treated with spinosad (1.0 g a.s./liter) after a 96 h post-treatment (HE-200x). The fat body seems to be dissolved as a result of the treatment

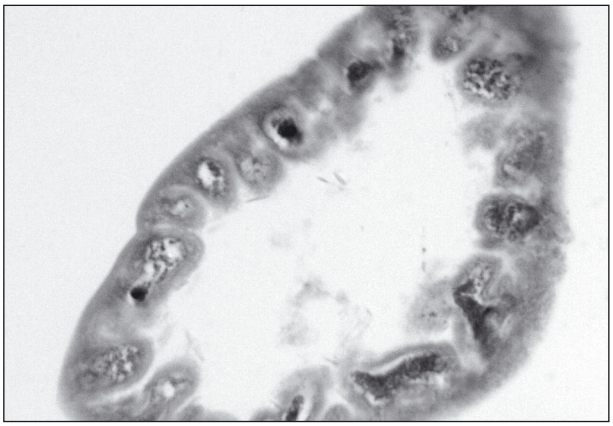

Fig. 4a. Transverse section through the malpighian tubule of healthy fifth-instar *S. littoralis* larvae (HE-40x)

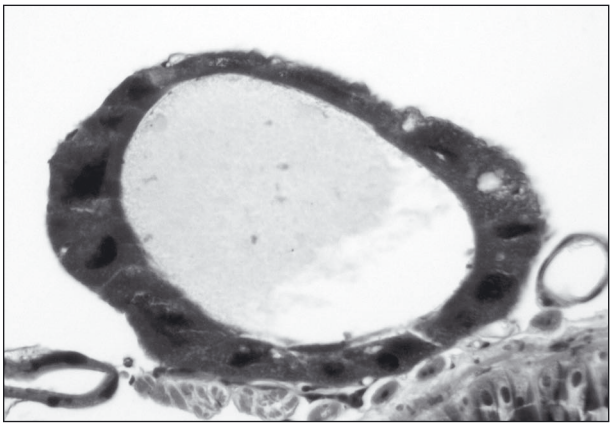

Fig. 4b. Malpighian tubules of fifth-instar *S. littoralis* larvae treated with spinosad (1.0 g a.s./liter) after a 96 h post-treatment (HE-40x). The lumen in the malpighian tubule is filled with secration and the cell has pyknotic nuclei

DISCUSSION

Activity of sublethal concentrations of spinosad

Spinosad has been shown to be an effective pest control agent (Brickle *et al*. 2001), particularly for control of Lepidopteran insect pests (Wanner *et al*. 2000). It was reported by Galvan *et al*. (2005) that spinosad decreased the survival of first instars, extended the time it took first instars to become adults, decreased weight gain, and reduced the fertility of female *Harmonia axyridis.* Schneider *et al.* (2004) found that a sublethal dose of spinosad also affected the life history parameters, such as a delay in development, a reduction in the rate of pupae formation, pupal mortality, adult longevity and adult emergence in third-instar larvae of the endoparasitoid *Hyposoter didymator.* Also, the sublethal effects of spinosad on the biological characteristics of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae), were investigated (Yin *et al.* 2008). Most of the previous studies focused on the effects on beneficial insects but few on the major target pests. The current study demonstrated that spinosad had considerable effects on the biological characteristics of the cotton leafworm, *S. littoralis*, when the fourth-instar larvae were fed on leaves treated with the sublethal concentrations (LC_{25} and LC_{50}) for only 72 h. Both the sublethal concentrations prolonged the larval period. Pupation rate and pupal weight were also lower than those in the control. Similar results were demonstrated by Yin *et al*. (2008) on the diamondback moth, *P. xylostella,* when the third-instar larvae were treated with spinosad at LC_{25} and LC_{50}.

Research on sublethal effects was undertaken to discover the negative, non-lethal impacts of insecticides on various life-history parameters that might affect population dynamics (Stark and Banks 2003). Sublethal effects such as suppression of larval weight, insect malformations, and reproductive capacity reduction observed in the survivors, could have a negative impact on insect population dynamics (Knight 2000; Pineda *et al*. 2004). On the other hand, insect pests can acquire tolerance toward insecticides in a short time and may even develop resistance after a relatively longer period of exposure by increasing the specific activities of metabolic enzymes (Yin *et al*. 2008). Insecticide hormoligosis was known to occur in some insects and mites, which may result in pests resurging. Fujiwara *et al.* (2002) reported that fecundity in *P. xylostella* females increased when the larvae were treated with fenvalerate at LC_{25}. In the present study, hormoligosis was not observed in *S. littoralis* treated with sublethal concentrations of spinosad. The fecundity and egg hatchability of *S. littoralis* females decreased significantly when the fourth-instar larvae were fed on leaves treated with spinosad at LC_{25} or LC_{50}. It is unclear whether and to what degree the sublethal effects of spinosad affect the next generation. The endpoint of a sublethal effect depends on the insecticide class, the application dose, and the insect species (Yin *et al.* 2008). The larval development time of *P. xylostella*, adult preoviposition period, and total preoviposition period tended to be longer by sublethal concentration of spinosad than in the control (Yin *et al.* 2008). Therefore, the mean generation times of *P. xylostella* were prolonged. It is possible that spinosad has sublethal effects on *P. xylostella* that may affect population dynamics in the field via reductions in survival and reproduction. Pineda *et al.* (2006) demonstrated that spinosad and methoxyfenozide negatively affected the reproduction of *S. littoralis*. These effects are very important from a practical point of view, because offspring can then be reduced and as a consequence, the insect population can be maintained below a level of economic loss. Spinosad had sublethal effects on the biotic performance of both parent and offspring of *P. xylostella*. For this reason, spinosad may provide more benefits in an integrated pest management program for *P. xylostella* than conventional pesticides that have high acute toxicity (Galvan *et al.* 2005; Michaud and Grant 2003).

It is obvious, that most studies about the toxicity of spinosad on Lepidoptera pests have been conducted on larval stages, and little has been published about its sublethal effects on adults of target species (Sparks *et al*. 1998; Pineda *et al*. 2004). However, Pineda *et al*. (2007) found some effects on the reproduction of *S. littoralis* when adults were exposed to both residual and ingestion exposure. Such effects may be due to the costs of fighting the intoxication involving the transfer of energy reserves to combat the neurotoxic effect. Egg fertility was reduced in *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) when females were fed with spinosad at a concentrations ≤ 1 mg a.s./liter and then paired with untreated males (López and Latheef 1999). The mechanism by which these effects might be exerted are poorly understood and they merit further study.

Residual activity of spinosad on feeding deterrence and nutritional indices

Our current study clearly showed the deterrent effects of spinosad on the consumption and utilization of food by *S. littoralis* larvae after feeding of the fifth-instar on leaves collected from cotton field plots sprayed with the recommended field and sub-field rates at different times after application. It was obvious that spinosad applied at both selected rates strongly suppressed feeding and growth rates of larvae that fed on leaves with spinosad residues up to 3-days-old DAA. The residual activity of spinosad was decreased after 7 and 21 DAA, indicating that the chemical started to degrade under field conditions. Spinosad diminished the ability of the larvae to convert both ingested and digested nutrients into growth, *i.e.* ECI and ECD, particularly immediately after feeding on treated leaves collected at 0-day after application. This effect declined with time, when larvae were fed on leaves with spinosad residues up to 7 DAA. Growth was more severely reduced than food intake. The reduction in growth also occurred during periods when food intake was not affected, possibly due to post-ingestive effects. Spinosad degrades quickly, and generally shows little residual insecticidal activity 3–7 days after application (Williams *et al*. 2004). Pineda *et al*. (2006) found that spinosad and methoxyfenozide significantly suppressed weight gain of neonates and fourth instars of *S. littoralis* which were continuously fed with an artificial diet containing the insecticides. The sup-

pression of weight gain caused by spinosad has not been studied thus far. Yee and Toscano (1998) reported a decrease in the weight of third- and fifth-instars of *S. exigua* exposed to lettuce leaf disks contaminated with spinosad. Similar results were demonstrated in our present study in which a clear suppression of weight gain in the fifth-instar of *S. littoralis* was observed after feeding on leaves treated with spinosad. The reason for this is not immediately apparent, but we speculate that it is directly related to the mechanism of spinosad action. Neurotoxic insecticides cause paralysis of insects (Haynes 1988), and consequently, a cessation of feeding. A decrease in the consumption rate and feeding duration was previously demonstrated in the beet armyworm, *S. exigua* after treatment with the Naturalyte® (Yee and Toscano 1998). Even though feeding cessation and malformation of the mouthparts in larvae of *S. littoralis* fed with leaves treated with sublethal concentrations of spinosad were not measured in the current study, both effects were visually observed. These effects are very important from a practical point of view because the larval-feeding-damage to crops would be lessened (Pineda *et al*. 2006).

However, the effects of spinosad on food metabolism are not yet clear. Nasiruddin and Mordue (1993) reported the adverse effects of azadirachtin on midgut epithelial cells, which might disrupt enzyme secretion and nutrient absorption. The ability to convert food into biomass was reduced after third instar *S. littoralis* larvae were fed on diet treated with azadirachtin for two days, and this could have affected growth (Martinez and van Emden 1999). Timmins and Reynolds (1992) attributed a similar reduction in the efficiency of food utilization following *Manduca sexta* treatment with azadirachtin to increased energetic costs arising from a reduced ability to utilize dietary nitrogen, which would not necessarily interfere with absorption from the gut (digestibility). They hypothesized that, in the absence of an adequate supply of amino acids, other nutrients then in excess for growth, might be diverted into other metabolic pathways. The reductions in food intake and in the ability to convert food into biomass would eventually have extended the development time of the larvae. The effects on the nutritional responses of *S. littoralis* were more pronounced in the instars immediately after the treatment, than later when a recovery of normal behavior occurred (Martinez and van Emden 1999). Thus, besides the lack of adequate food intake and impaired metabolism following spinosad treatment, it is possible that sublethal concentrations fed to *S. littoralis* affected the endocrinal events to a small extent manifested by delays in insect development. There are clearly many advantages associated with insecticides which have an extended residual effect, even if they are slow-acting. At the same time, it is important to have available a variety of insecticides with an effective immediate knockdown effect on target pests. Such knockdown insecticides are particularly important as "rescue treatments" in pest-cropping systems in which there are no reliable monitoring devices and economic action thresholds. Information about residual toxicity of an insecticide is important for optimized scheduling of applications to obtain prolonged protection and to reduce costs associated with insect pest control.

Histomorphological alterations of the midgut by spinosad

In this study, alterations were found in the midgut of fifth-instar *S. littoralis* larvae fed on leaves treated with spinosad at a concentration corresponding to the labeled-field rate with a consequent deterrent activity of food utilization and prolongation of the larval and pupal periods. This fact indicates that the spinosad insecticide promotes morphology alterations and consequently affects nutrient absorption efficiency. The midgut is the middle portion of the insect digestive tract where food digestion and absorption occur. Some epithelium cells produce enzymes and others absorb the digested food (Terra and Ferreira 1994). Since most nutrient absorption occurs in this region, the cellular modifications are more intense here since the digestive process is intensified in the midgut, and it is, therefore, the most vulnerable region to the action of foreign substances. The verification of the location and form of action on the insect has great importance for the development of an efficient and safe insecticide (Barreto *et al*. 2006). Morphological studies are an important tool when trying to understand the form of action of natural products may take (Dequech *et al*. 2007). The deleterious physiological effects can be measured by growth reduction and presence of abnormalities (Mordue and Nisbet 2000). The present study showed that regenerative cells of the midgut from larvae fed on spinosad-treated leaves seemed to be sensitive to the treatment and thus, these cells were unable to replace the damaged cells of the midgut. The midgut epithelial cells showed signs of apoptosis manifested as shrinkage of the cells and the presence of condensed chromatin, with some vacuolization. This process is generally considered as an important mechanism by cells against pathogenic and toxic agents (Dougherty *et al.* 2006; Sakr 2007).

The histomorphological alterations observed in the midgut of *S. littoralis* larvae after feeding with spinosad – treated leaves were similar to these obtained in the midgut of larvae treated with other biopesticides, *e.g.* γ-endotoxins from *Bacillus thuringiensis* (Quesada-Morga and Santiago-Alvarez 2001), *B. thuringiensis* (Vip3A protein) products (Abdelkefi-Mesrati *et al.* 2011), exotoxin protein extracted from *Metarhizum anisopliae* (Quesada-Morga *et al.* 2006), and toxins from *Streptomyces lavendulae* culture filtrate (Sakr 2007). The toxicity action of pathogenic toxins was due to paralysis in the midgut of these larvae. For instance, feeding *S. littoralis* larvae on an artificial media mixed with soluble protein extracted from *M. anisopliae* caused deterioration and destruction of the midgut epithelium (Quesada-Morga *et al.* 2006). Roel *et al*. (2010) also investigated the effect of sub-lethal doses of neem oil, *Azadirachta indica* (Meliaceae), on the midgut of *S. frugiperda.* They indicated that histo-physiological alterations such as degeneration of the epithelial lining of the midgut and in the peritrophic matrix were found at all concentrations of the neem oil (0.006; 0.05; 0.4%). Also, neem oil action reflected on the insect development causing alterations to larval and pupal stage duration as well as reduction of pupal weight and an increasing death rate. These changes in insect development were a function of the neem oil concentration level.

The present results verified the biological effects of sublethal concentrations (LC_{25} and LC_{50}) of spinosad against the cotton leafworm, *S. littoralis*. The residual activity of spinosad on feeding and other metabolic parameters was markedly revealed. Some histo-physiological alterations such as degeneration of the epithelial lining of the midgut and in the peritrophic matrix were also found after 48 h-feeding on spinosad-treated leaves. We also demonstrated that spinosad negatively affected the reproduction of this pest. These effects are very important from a practical point of view, because offspring can be reduced and as a consequence, the insect population can be maintained below a level of economic loss. We concluded that spinosad represents an important choice to be used in integrated pest management where *S. littoralis* is a major pest. However, further studies on such unconventional insecticides will be needed to determine the relation between the progression of toxicity symptoms and the histological changes in the midgut and other tissues, in an attempt to understand the nature, cause, and significance of such changes.

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