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Is emamectin benzoate effective against the different stages of *Spodoptera exigua* (Hübner) (Lepidoptera, Noctuidae)?

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The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera, Noctuidae), is a major polyphagous pest in greenhouses and open fields worldwide and also a main problem in sweet pepper greenhouses. The effectiveness of the pesticide emamectin benzoate was tested in the laboratory on different stages of *S. exigua* using different concentrations and uptake routes. After dipping young (<24-h-old) and old (>48-h-old) *S. exigua* eggs in emamectin benzoate at 0.5, 1 and 1.5 mg/L a.i. the chemical did not exhibit any ovicidal activity. There was, however, progressive neonate mortality at all concentrations, culminating at 72 hours after hatching, when 100% of the larvae from the treated young eggs died. Second and fourth instar *S. exigua* larvae did not exhibit significant mortality when exposed to the inert surfaces which were treated. In contrast, ingesting a diet contaminated with 0.5 mg/L a.i. of emamectin benzoate caused 100% mortality in L2 and L4 larvae 24 and 72 hours after ingestion, respectively. The LC_{50} value of the compound against L4 larvae that fed on sprayed sweet pepper leaves for 24 hours was 0.81 mg/L a.i.. When adults were fed on a solution of 0.5 mg/L a.i., there was a reduction in the female and male lifespan of 29.3% and 55.3%, respectively. Fecundity was reduced by more than 99%. These data suggest that emamectin benzoate is not only a useful insecticide when ingested by beet armyworm larvae but it also has ovolarvicidal and adult activity.

Keywords: Avermectins; beet armyworm; *Capsicum annuum* L.; effectiveness; emamectin benzoate; laboratory experiments; pepper; *Spodoptera exigua*

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Introduction

Spain is an important producer of sweet pepper (*Capsicum annuum* L.), with a total cultivated area of 18,100 ha (MAGRAMA 2012). The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera, Noctuidae), is a major polyphagous pest in numerous crops in greenhouses and open fields worldwide (Mohaghegh, De Clercq and Tirry 2001; Smagghe *et al.* 2003). It is also an important pest of sweet pepper in Spain (Van der Blom 2008). Females lay their eggs in batches that are often several layers deep and covered with a dense mass of hairs. Larvae feed both on the leaves and the sweet pepper fruit, damaging the value of the crop. The pupation itself takes place in the soil (Van der Linden 1996). Due to its polyphagous nature, this pest has a long history of exposure to a broad array of insecticides (Moulton, Pepper and Dennehy 1999; Smagghe *et al.* 2003). As a result, it has already evolved resistance to multiple classes of chemical insecticides (Van der Linden 1996; Moulton *et al.* 1999; Smagghe *et al.* 2003).

In light of the increased awareness of unwanted effects of conventional insecticides and the need for eco-friendly plant protections, avermectins have emerged as a promising new group of insecticides (Venkateswari *et al.* 2008). Avermectins, a family of 16-membered macrocyclic lactones produced by the soil microorganism, *Streptomyces avermitilis* MA-4680 (NRRL 8165), are an important tool in promoting animal and human health and crop protection. The major component of the fermentation, avermectin B_1 (abamectin), is a mixture of B_{1a} (≥80%) and B_{1b} (Lasota and Dybas 1991). Emamectin benzoate is a semisynthetic derivative of abamectin and has been developed for the purpose of controlling lepidopterous pests on a variety of vegetable crops worldwide (Jansson *et al.* 1997; Babu 1988). This pesticide stimulates the release of the neurotransmitter γ-aminobutyric acid (GABA) (Jansson *et al.* 1997). In general, the chloride ion flux produced by opening ion channels in neuronal cells as a result of GABA signalling results in the loss of cell function and the disruption of nerve impulses (Jansson *et al.* 1997). Consequently, invertebrates are irreversibly paralysed and starved. Emamectin benzoate is not a systemic compound, but it exhibits translaminar activity. Insecticide-treated leaves maintain a reservoir of the active substance, resulting in long-term residual pest control through larval feeding (Ishaaya, Barazani and Horowith 2002). The fast degradation of emamectin benzoate residues on the plant surface (López *et al.* 2011) allows, however, minimal chemical exposure to beneficial organisms (Ishaaya *et al.* 2002). In Spain, emamectin benzoate is currently registered for the control of several species of Lepidoptera in vegetable crops and vineyards at a maximum field recommended rate of 150 g commercial product/hl (MAGRAMA 2013).

The effect of emamectin benzoate on larvae has been examined both at the field level and in laboratory experiments. Emamectin benzoate has been shown to be effective against various Lepidoptera, such as the Noctuidae *S. exigua* (Eckel *et al.* 1996), *Spodoptera litura* (F.) (Ahmad, Saleem and Ahmad 2005; Venkateswari *et al.* 2008; Firake and Pande 2009), *Helicoverpa armigera* (Hübner) (Sontakke *et al*. 2007; Deshmukh *et al.* 2010; Rajesh, Bheemanna and Kumar 2010), the Yponomeutidae *Plutella xylostella* (L.) (Shelton *et al.* 2000; Barrera-Urzúa *et al.* 2006), the Tortricidae *Choristoneura rosaceana* (Harris) (Ahmad, Hollingworth and Wise 2002), *Grapholita lobarzewskii* (Nowiki) (Charmillot *et al.* 2007), and the Pyralidae *Maruca vitrata* (F.) (Chouraddi

et al. 2009). Although the product was originally developed for controlling lepidopteran pests, it also exhibits activity against insects from different orders. For example, when applied to cotton leaves, emamectin benzoate has been demonstrated to control *Phenacoccus solenopsis* Tinsley (Hemiptera, Pseudococcidae) (Dhawan *et al.* 2008). Similarly, this chemical is also efficient in controlling *Bactrocera zonata* (Saunders) (Diptera, Tephritidae) adults when mixed into their diet (Badr El-Sabah, Amani and Hoda 2009).

Although it is well-known that emamectin benzoate ingestion leads to larval toxicity, there is comparatively less information concerning contact toxicity. Moreover, there are also few data on toxicity in other pest lifecycle stages, and the results that do exist are contradictory and inconclusive. Therefore, this study aimed to evaluate the lethal and sublethal effects of different uptake routes of emamectin benzoate at low concentrations [0 to 2 mg/L active ingredient (a.i)] on *S. exigua* eggs, L2 and L4 larvae, and adults.

Materials and Methods

Insect rearing

Insect rearing and laboratory bioassays were carried out in a controlled environment chamber (25 ± 2 °C, 75 ± 10% relative humidity and 16:8 h (light:dark) photoperiod).

Spodoptera exigua were reared in our laboratory from eggs obtained from the Public University of Navarra (Pamplona, Spain). At least three generations were reared prior to the assays to guarantee colony health. These insect lines do not have any history of insecticide exposure. The larvae were fed with an artificial diet (Poitout and Bues 1974), while the adults were fed *ad libitum* on a 15% solution of honey in distilled water kept in small glass vials (15 mm in diameter and 22 mm high), covered with Parafilm® with a piece of Spontex® wiper leading out of the solution to provide a wick for the insects to use for drinking. Filter paper was provided to females as an oviposition substrate and was periodically replaced.

Chemicals

Emamectin benzoate (Affirm®, 0.95% SG) was obtained from Syngenta Agro S.A. (Madrid, Spain). Distilled water was used as a control.

Egg treatment and neonate mortality

Egg batches that contained at least 100 eggs were collected from 7-day-old females (corresponding with the fourth oviposition day). The batches were chosen according to the following requirements: a) deposited in a single layer, b) apparently healthy when observed through a binocular lens, and c) not covered by female wing scales to facilitate the emamectin benzoate penetration. Filter paper strips containing the egg batches were cut into small circles (approximately 15 mm diameter) and dipped for 3 seconds into one of three concentrations of the insecticide (0.5, 1 and 1.5 mg/L a.i.) as well as into control solutions (Pineda *et al.* 2004). Two different egg age classes were evaluated: young (<24 h old) and old (>48 h old, when the neonate's head could be observed through the chorion). Once they had dried at room temperature, the circles of filter paper containing the treated egg batches were transferred into plastic cages (9.5 cm in diameter by 3 cm in height with a 5.5-cm in diameter ventilation hole covered with mesh on the top of the cage for aeration) with filter paper on the floor and containing an artificial diet cube (1.5 cm^3). Each treatment was replicated four times,

and each replicate consisted of a single filter paper circle containing the egg batch. The number of neonates that hatched from each treatment was recorded daily for four days after the start of hatching. The development of the neonates was followed for three days.

Larval toxicity induced by residual contact

To evaluate the residual contact activity of the insecticide, glass plates (12 × 12 × 0.5 cm thick) were treated using a Potter precision spray tower (Burkard Manufacturing Co., Hertfordshire, UK) with a standard deposit of 1.63 ± 0.01 mg/cm^2 (1 mL; 55 kPa), which followed the IOBC recommendations (Sterk *et al.* 1999). Concentrations of 0.25, 0.50, 1, 1.5 and 2 mg/L a.i. of emamectin benzoate were tested alongside distilled water controls. The test units consisted of a round methacrylate frame (10 cm in diameter, 3 cm high) and the two square glass plates described above. The plastic frame had seven holes (1 cm in diameter): four covered by a brass mesh for ventilation; two used for insect manipulation and closed with plasticine; and one that held a hypodermic needle connected to a rubber tube that provided a continuous flow of air produced by an aquarium pump to ensure forced ventilation. As soon as the plates were dry, they were mounted with two crossed rubbers (test units slightly modified from Giolo *et al.* 2009) (Figure 1). Four replicates and ten L2 or L4 larvae per replicate were used for each treatment. The mortality was scored at 24-hour intervals during the three days following the application. If no movement was observed, the larvae were recorded as dead. Surviving larvae were later moved to untreated test units consisting of round plastic cages (12 cm in diameter by 5 cm in height with a 5.5-cm in diameter ventilation hole covered with mesh on the top of the cage for ventilation) with filter paper in the bottom to absorb humidity. Each cage contained 1.5 or 3 cm^3 diet cubes (for L2 and L4 larvae, respectively). The development of surviving individuals was tracked until their pupation. The filter paper and the artificial diet were changed three times per week.

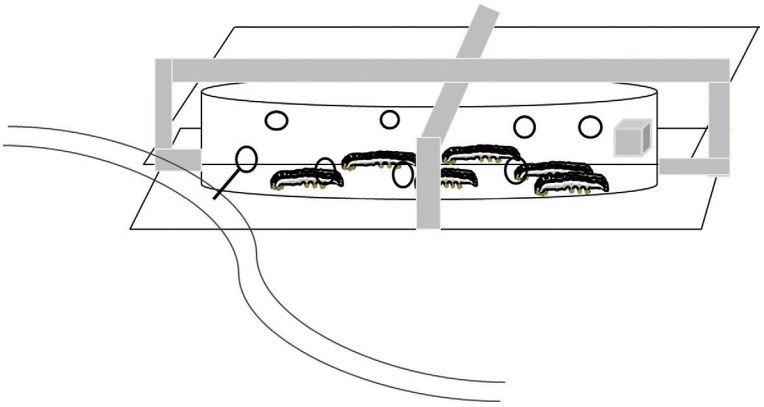

Figure 1. Experimental units used to evaluate the residual contact activity of the insecticide on a glass surface: the round methacrylate frame (with the seven holes) and the two square glass plates hold together with two crossed rubber bands. One of the holes held a hypodermic needle connected to a rubber tube that provided a continuous flow of air produced by an aquarium pump to assure forced ventilation.

Larval toxicity induced by contaminated artificial diets

Newly molted (0-8 h) L2 and L4 *S. exigua* instars were continuously fed on diets containing different concentrations of emamectin benzoate (0.25, 0.5, 1, 1.5 and 2 mg/L a.i.). Dilutions of the insecticide were prepared in 10 mL of distilled water and mixed with the artificial diet during its preparation (Budia, Marco and Viñuela 1994). Prior to the experiment, the larvae were starved for three to six hours. Test units consisted of the round plastic cages described above. Each cage contained 1.5 or 3 cm^3 diet cubes (for L2 and L4 larvae, respectively). Four replicates and forty L2 or L4 larvae per concentration were assayed. Untreated diet was offered to the controls. Larval mortality was scored daily until the last larva of the treatments was dead or pupated. If no movement was observed, the larvae were recorded as dead.

Larval toxicity induced by treated pepper leaves

Sweet pepper plants (*Capsicum annuum* L. (cv. Dulce Italiano) (20 cm high) were sprayed with various concentrations of emamectin benzoate (0.25, 0.5, 1, 1.5 and 2 mg/L a.i.) using hand sprayers until the liquid ran off the leaves. Untreated leaves were offered to the controls.

One treated leaf per replicate was offered to ten L4 larvae, and four replicates per concentration were performed. Untreated leaves were offered to the controls. The larvae were starved for three hours prior to the assay, and the leaves were replaced daily to prevent cannibalism. This experiment was carried out again with a new *S. exigua* generation, using a range of ten different concentrations from 0 to 1 mg/L a.i. to determine LC_{10}, LC_{50} and LC_{90}. The mortality was determined as explained above.

Adult toxicity induced by emamectin benzoate ingestion

Serial dilutions of 0, 0.5 and 1 mg/L a.i. of emamectin benzoate were prepared by dilution into a 15% honey solution in distilled water. Insecticide solutions were offered continuously to newly emerged adults (<24 h old) in glass troughs (1.5 cm in diameter by 2 cm high) covered with Parafilm® pierced by a piece of Spontex wiper. The solutions offered to the moths were replaced every two days to prevent fungal growth. The test solutions were stored in a refrigerator until use and were warmed to room temperature. The experimental arenas were the round plastic cages described for the experiments with larvae. The floor and the walls of each cage were covered with filter paper, and a layer of kitchen paper (Collogar®) was placed on top of each cage to provide an oviposition substrate to females. Two pupae from the rearing stock (one male and one female) that were close to emergence were placed into each cage. Six experimental arenas were used per concentration alongside controls. A total of twelve adults per concentration were used. Once the adults emerged, the mortality was recorded daily. An adult was considered dead when it could not right itself after being placed upside down. Male and female lifespans were recorded separately. The cumulative number of eggs per female that were laid during the first eight days after the onset of oviposition and the percentage of eggs hatched from those collected on the third oviposition day were recorded. Fertility was not scored because the number of eggs laid was so low that it could not be statistically analysed.

Statistical analysis

As a general procedure, data (mean ± standard error (S.E.)) were subjected to general linear model analysis. The specific

model employed depended on the number and type of factors considered in each experiment. Insecticide dose and egg age were considered as fixed factors and time of evaluation of larval mortality as a repeated measures factor. Means of fixed factors were separated using the Fisher's least significant difference (LSD) test. When a repeated measure ANOVA was employed, the sphericity of the variance-covariance matrix was first tested by Mauchly's W statistic. If the assumption of sphericity of the data was not confirmed, the effect of the repeated measures factor (time) was tested using the F value generated by Pillai's trace. When needed, the data were previously transformed to meet the assumptions of parametric statistics. The transformation was chosen according to their ability to change the distribution of residuals to satisfy the requirements for parametric tests. Thus, percentages were best transformed by arcsin $\sqrt{(x/100)}$ and the remaining parameters by log (x+1). Only the non-transformed data are shown in the tables. If any of the assumptions of the analysis of variance were violated after appropriate transformations, the effect of insecticide dose was analysed by means of a non-parametric Kruskal-Wallis test. The median values were considered significantly different if the 95% median confidence intervals did not overlap. When significant differences among variances were found in the case of repeated measure ANOVA, dose effects were compared using Tamhane test. The significance level of $P < 0.05$ was considered for all tests. All analyses were performed using SPSS 13.0 statistical software (SPSS 2004).

In the L4 larval ingestion assay in which sweet pepper leaves were treated with 0 to 1 mg/L a.i. emamectin benzoate, the mortality data were subjected to probit regression using the POLO-PC program (LeOra Software 1994). Failure of 95% to overlap was used as criterion for significant difference at LC_{10}, LC_{50} and LC_{90}.

Results and Discussion

Treatment of the eggs and neonate mortality

Emamectin benzoate diluted in water did not exhibit any ovicidal activity against young (<24 h-old) or old (>48 h-old) *S. exigua* eggs. Hatching percentages four days after treatment ranged from 78.8 ± 5.1 to 95.6 ± 4.4% and were not significantly different from those of the controls (Table 1).

In contrast, neonates from both treated egg classes exhibited significant mortality due to treatment with emamectin benzoate. The mortality rates one day after emergence for neonates from young and old eggs treated with 0.5 mg/L a.i. emamectin benzoate were 62.9 ± 9.5 and 70.2 ± 13.8, respectively. The three doses tested provoked a significant mortality compared to the control. Furthermore, the percentage mortality increased during the three-day evaluation period (Table 2). Nevertheless, it cannot be concluded that the larval mortality is a purely larvicidal effect of emamectin benzoate stemming

Table 1. Effect of emamectin benzoate on the eclosion of *Spodoptera exigua* eggs treated at two different ages (mean data ± standard errors)

Concentration (mg/L a.i.)	Egg hatching[1] (%)	
	<24 h old eggs	>48 h old eggs
Control	80.6 ± 5.8	93.6 ± 4.4
0.5	87.2 ± 6.0	78.8 ± 5.1
1	79.8 ± 5.7	85.1 ± 7.5
1.5	95.6 ± 4.4	84.3 ± 3.6

[1]Eggs hatched in the 4-day period after treatment. No statistical difference in percentage of egg hatching was observed due to concentration of insecticide or to age of the eggs ($P = 0.633$ and 0.711, respectively, two-way ANOVA).

Table 2. Effect of emamectin benzoate on neonate mortality of *Spodoptera exigua* after treating eggs of two different ages (mean data ± standard errors)

Concentration (mg/L a.i.)	Mortality[1] (%)					
	<24 h old eggs			>48 h old eggs		
	24 h	48 h	72 h	24 h	48 h	72 h
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 3.0	4.6 ± 2.9
0.5	62.9 ± 9.5	88.1 ± 6.9	95.6 ± 2.6	70.2 ± 13.8	94.7 ± 1.8	100.0 ± 0.0
1	90.2 ± 4.8	93.3 ± 6.7	95.0 ± 5.0	83.8 ± 10.0	95.5 ± 0.5	100.0 ± 0.0
1.5	79.5 ± 17.8	92.1 ± 7.9	100.0 ± 0.0	74.4 ± 13.4	94.1 ± 3.8	99.0 ± 1.0

[1]Mortality of neonates in the three days following hatching.
The three doses tested significantly increased mortality with regard to control ($P < 0.001$, repeated measures ANOVA); a significant effect on mortality was observed through time ($P = 0.001$, repeated measures ANOVA); no statistical difference on mortality was observed between eggs of different ages ($P = 0.721$, repeated measures ANOVA).

solely from ingestion or contact with the product after hatching. It is likely that the mortality is explained by a combined ovolarvicidal effect. In this kind of system, the mortality of neonates is related to the treated eggs because low amounts of the insecticide might penetrate the chorion and affect the embryos. Nevertheless, our experiment cannot demonstrate this phenomenon. However, once the larvae hatch, they feed on the chorion and thus ingest the remains of the compound (Breuer and Gillham 2008).

Venkateswari *et al.* (2008) carried out a similar bioassay, dipping groups of 30 eggs (24h-old) of *S. litura* (F.) for 30 seconds into different concentrations of emamectin benzoate. These researchers reported an LC_{50} of 0.1 mg/L a.i. for neonates 24 hours after hatching, indicating that the emamectin benzoate may be slightly more toxic for *S. litura* than for *S. exigua*. The authors did not observe any effect on hatchability, which is in agreement with our data. In contrast, Charmillot *et al.* (2007) observed a strictly ovicidal effect, described as a reduction in neonate hatching, when females of *G. lobarzewskii* oviposited on apples previously treated with the compound at different concentrations. Emamectin treatment resulted in significant mortality with LC_{50} values of approximately 2 mg/kg a.i.. The LC_{90}, however, was 256 mg/kg a.i.. Our results on neonates showed that early treatments can achieve good control, and this result has also demonstrated by Argentine *et al.* (2002). These authors found that the LC_{90} values for emamectin benzoate ranged from 0.005 to 0.0218 mg/L for six species of Lepidoptera, including *S. exigua*, after neonates ingested a treated artificial diet. These findings show that emamectin benzoate possesses excellent insecticidal potency against neonates.

In summary, the ovicidal efficacy of emamectin benzoate is not comparable to its larvicidal activity and depends on the lepidopteran larvae species. The differences observed could be due to the physical properties of the chorion (ease of penetration), the abundance of wing scales, the layers of eggs treated or even how the physical characteristics of the batches were managed in the experiments.

Larval toxicity

L2 and L4 *S. exigua* instars were not susceptible to emamectin benzoate when exposed to treated inert surfaces (Table 3). No significant differences in L2 mortality at either 3 or 15 days after the

Table 3. Mortality of *Spodoptera exigua* second and fourth instars after exposure for three consecutive days to emamectin benzoate applied on an inert surface (mean data ± standard errors)

Concentration (mg/L a.i.)	L2		L4	
	% Mortality 72 h	% Mortality 15 d	% Mortality 72 h	% Mortality 7 d
Control	0.0 ± 0.0	2.8 ± 2.8	0.0 ± 0.0	2.5 ± 2.5
0.25	8.3 ± 8.3	12.5 ± 12.5	0.0 ± 0.0	7.8 ± 4.8
0.5	3.6 ± 3.6	6.7 ± 3.9	0.0 ± 0.0	2.8 ± 2.8
1	2.5 ± 2.5	18.6 ± 7.9	0.0 ± 0.0	0.0 ± 0.0
1.5	8.7 ± 5.4	18.2 ± 11.0	0.0 ± 0.0	2.5 ± 2.5
2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.5 ± 2.5

No statistical difference was observed among the percentages of mortality registered at each dose ($P = 0.535$, repeated measures ANOVA for the percentage mortality of L2 and $P = 0.737$, one-way ANOVA for the percentage mortality at 7d of L4).

treatment were found between the different concentrations and the controls ($P > 0.05$). Eighteen days after the treatment, 100% of surviving larvae in all concentrations and controls were able to pupate. Little to no mortality was recorded for L4 instars within 7 days of their exposure to residual contact with the insecticide ($P > 0.05$). Eight days after the exposure, 100% of surviving larvae in both the different insecticide concentrations and controls reached the pupal stage. In contrast, L2 larvae suffered 100% mortality within 24 hours after ingesting the diet contaminated with emamectin benzoate at concentrations of 0.25, 0.5, 1, 1.5 and 2 mg a.i/L (Table 4). For the lower dose of 0.25 mg a.i/L, the mortality after 24 hours was 60.0 ± 8.2% and reached 100% after 72 hours. Fourth instar larvae consuming an emamectin benzoate-treated diet were less susceptible to the compound in comparison to the second instar: 24 h after the beginning of the treatment statistical differences were found for the concentrations 1, 1.5 and 2 mg/L a.i., compared to the controls. All treated L4 larvae died within 48 hours of consuming the contaminated diet at the highest concentration (2 mg/L a.i.). The rest of the larvae died within 72 hours at the 0.5, 1 and 1.5 mg/L a.i. doses and by day 4 at the lowest dose (0.25 mg/L a.i.).

When L4 larvae were fed with treated sweet pepper leaves, significant mortality occurred within 24 hours of the beginning of the experiment, even for the lowest dose (0.25 mg/L a.i.). After 48 hours of continuously ingesting treated leaves, 100% of the larvae subjected to all emamectin benzoate concentrations died (Table 4).

The LC_{50} values were 0.8 mg/L a.i. (confidence limits = 0.75-0.91) 24 hours after feeding on treated sweet pepper leaves, 0.4 mg/L a.i. (0.39-0.48) 48 hours after feeding and 0.2 mg/L a.i. (0.15-0.21) 72 hours after feeding (Table 5).

Our results showed that although emamectin benzoate intoxicates arthropods via both contact and ingestion, the latter is the primary route by which insects accumulate a lethal dose (Jansson and Dybas 1997). Venkateswari *et al.* (2008) reported an LC_{50} = 19.1 mg/L a.i. when the third instar of *S. litura* were topically treated, a concentration 38.2 times greater than that obtained after these instars actually consumed leaf discs (LC_{50} = 0.5 mg/L a.i.). The LC_{50} emamectin benzoate values for larval mortality after leaf disc ingestion by final instar larvae were 1.1 mg/L a.i., much lower than the 359.5 mg/L

Table 4. Mortality of *Spodoptera exigua* second and fourth larval instars after consuming an artificial diet of pepper leaves treated with different concentrations of emamectin benzoate (mean data ± standard errors)

Concentration mg/L a.i.	Ingestion of contaminated artificial diet						Ingestion of treated sweet pepper leaves	
	L2 larvae			L4 larvae			L4 larvae	
	% Mortality 24 h	% Mortality 48 h	% Mortality 72 h	% Mortality 24 h	% Mortality 48 h	% Mortality 72 h	% Mortality 24 h	% Mortality 48 h
Control	$0.0^{a} \pm 0.0$	$5.0^{a} \pm 2.9$	$5.0^{a} \pm 2.9$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	0.0 ± 0.0
0.25	$60.0^{b} \pm 8.2$	$97.5^{b} \pm 2.5$	$100.0^{b} \pm 0.0$	$0.0^{a} \pm 0.0$	$67.5^{b} \pm 10.3$	$95.0^{b} \pm 5.0$	$87.5^{b} \pm 4.8$	100.0 ± 0.0
0.5	$100.0^{c} \pm 0.0$	–	–	$5.0^{a} \pm 2.9$	$72.5^{b} \pm 4.8$	$100.0^{b} \pm 0.0$	$82.5^{b} \pm 6.3$	100.0 ± 0.0
1	$100.0^{c} \pm 0.0$	–	–	$45.0^{b} \pm 10.4$	$82.5^{bc} \pm 8.5$	$100.0^{b} \pm 0.0$	$87.5^{b} \pm 9.5$	100.0 ± 0.0
1.5	$100.0^{c} \pm 0.0$	–	–	$57.5^{bc} \pm 11.1$	$92.5^{c} \pm 2.5$	$100.0^{b} \pm 0.0$	$85.0^{b} \pm 6.5$	100.0 ± 0.0
2	$100.0^{c} \pm 0.0$	–	–	$72.5^{c} \pm 8.5$	$100.0^{c} \pm 0.0$	–	$90.0^{b} \pm 4.1$	100.0 ± 0.0
	$H^{1} = 22.87$	$F = 586.71$	$H^{1} = 6.22$	$F_{5,18} = 20.02$	$F_{5,18} = 37.27$	$H^{1} = 16.6$	$F_{5,18} = 35.74$	$H^{1} = 23.0$
	$P = 0.0003$	$P < 0.0001$	$P = 0.0126$	$P < 0.0001$	$P < 0.0001$	$P = 0.0023$	$P < 0.0001$	$P = 0.0003$

Data with different superscript letters are significantly different (ANOVA, LSD; $P \leq 0.05$).
[1]Data analysed using Kruskal-Wallis test.

a.i. obtained using the topical method. Mortality rates differed between instar stages, a finding that could be explained by the effects of different larval weights in our experiments and in Venkateswari *et al.* (2008) assays. Differences observed between the two routes of uptake clearly indicate that emamectin has higher toxicity when administered via consumption than through contact. The polar nature of emamectin benzoate may explain its poor contact toxicity, as polarity is a limiting factor in the penetration of insect cuticle. Hydrophilic compounds, however, have better solubility in digestive enzymes and in haemolymph (Stanley *et al.* 2006).

The results were also dependent on the type of diet. L4 larvae were killed faster when they fed on treated pepper leaves than when they fed on an artificial diet, which is a trend that could be due to the translaminar effect of emamectin benzoate. The quick degradation, absorption and translocation of emamectin benzoate may diminish the amount of insecticide deposited on the plant surface, which, while reducing the possibility of natural enemies contacting the insecticides, would ensure a sufficiently large amount of the product inside the leaf tissues so as to effectively control pest (Prabhu et al. 1991; Lopez et al. 2011). Nevertheless, it should be taken into account that our experiments did not measure the exact amount of insecticides that larvae ingested both for the artificial diet and the pepper leaves.

The efficacy of emamectin benzoate has already been tested on multiple different lepidopteran species. Several studies have shown that this product can effectively control Noctuidae pests. Argentine *et al.* (2002) demonstrated that when this insecticide was applied on the surface of artificial diets, it was able to control *S. exigua, Heliothis virescens* (F.), *Trichoplusia ni* (Hübner), *P. xylostella*, *Pseudoplusia*

Table 5. Emamectin benzoate toxicity (by Probit analysis) in *Spodoptera exigua* L4 instars that consumed treated pepper leaves (mean data ± standard errors)

Hours	N	Slope ± SE	LC^1_{10} (95% FL[2])	LC^1_{50} (95% FL[2])	LC^1_{90} (95% FL[2])	χ^2/df[3]
24	479	5.29 ± 0.75	0.5 (0.39–0.51)	0.8 (0.75–0.91)	1.4 (1.18–1.92)	2.80/9
48	478	3.83 ± 0.39	0.2 (0.16–0.24)	0.4 (0.39–0.48)	0.9 (0.82–1.14)	1.92/9
72	478	3.51 ± 0.35	0.1 (0.06–0.10)	0.2 (0.15–0.21)	0.4 (0.37–0.50)	5.67/9

[1]LC = Lethal concentration in milligrams of active ingredient per litre.
[2]FL = Fiducial limits.
[3]df = Degrees of freedom.

includens (Walker) and *Spodoptera frugiperda* (Smith). This product was also sprayed on different crops, and the treated leaves were offered to the larvae of those species. Utilised in this way, emamectin benzoate caused 100% mortality in all tested species, both in fresh residue and different aged-residues, and it was consistently more potent than all other tested insecticides, including chlorfenapyr, fipronil and tebufenozide, even at doses ten times lower than the projected field rate (8.4 g a.i./ha). These results are in agreement with those obtained in our experiments.

Adult experiments

Significant differences were observed in both the male and female life span when adults ingested solutions of emamectin benzoate via their drinking water (Table 6). In the females, lifespan reductions of 29.3% and 40.7% compared to the control were detected for the concentrations 0.5 and 1 mg/L a.i., respectively. In the males, these percentages were 55.3% and 63.8% for the concentrations 0.5 and 1 mg/L a.i., respectively (reductions were calculated using the Abbott formula: P (%) = [1- (Ptreated/ Pcontrol)] × 100) (Abbott 1925). This finding indicates that while emamectin benzoate does have an adulticidal effect on *S. exigua*, it is not as lethal as the larvicidal effect. López, Latheef and Hoddman (2010) reported a similar effect on adults of the noctuid *Helicoverpa zea* (Boddie). In this study, these researchers proposed a pest management strategy for

Table 6. Lifespan and fecundity of *Spodoptera exigua* adults when they were fed with emamectin benzoate at two different concentrations (mean data ± standard errors)

	Treatment of the adults		
Concentration (mg/L a.i.)	Life span (females) (No. days)	Life span (males) (No. days)	Fecundity[1] (No. eggs)
Control	$10.0^a \pm 0.8$	$9.4^a \pm 0.7$	$1{,}195.0^a \pm 93.8$
0.5	$7.0^b \pm 0.6$	$4.2^b \pm 0.6$	$8.0^b \pm 3.7$
1	$5.6^b \pm 0.6$	$3.4^b \pm 0.4$	$1.2^b \pm 1.2$
	$F_{2,12} = 12.03$	$F_{2,12} = 33.17$	$H^2 = 10.89$
	$P = 0.0014$	$P < 0.0001$	$P = 0.0043$

Data with different superscript letters are significantly different (ANOVA, LSD; $P \leq 0.05$).
[1]Cumulative number of eggs per female laid daily during the first eight days after the onset of oviposition.
[2]Data analysed using Kruskal-Wallis test.

the suppression of *H. zea* adults using a feeding attractant and stimulant in combination with a toxicant. Emamectin benzoate was highly toxic to feral *H. zea* males. The LC_{50} values were 0.7 (24 h), 0.5 (48 h) and 0.2 (72 h) mg/L a.i.. Moreover, these authors proved that sublethal concentrations did not significantly reduce proboscis extension or gustatory response, but that females did deposit fewer eggs and had a depressed larval hatching percentage (although this effect was not statistically examined).

The fecundity of the treated *S. exigua* adults was also negatively affected by the treatments (Table 6). Females that continuously ingested emamectin benzoate at 0.5 mg/L a.i. exhibited a 99% reduction in fecundity (also calculated using the Abbott formula). While some of this effect could be due to the lesser life span of treated females (seven days) than controls (ten days), the treated females were only able to oviposit eight eggs in comparison with the nearly 1,200 laid by untreated females. This decrease in fecundity rate could be due to the suppression of the movements of the muscles involved in egg laying (White *et al.* 1997). A large reduction or even a suppression of the reproduction ability of *S. exigua* could be exploited as a control strategy in Integrated Pest Management (IPM) programs as proposed by López, *et al.* (2010) for *H. zea*. This approach entails the use of a feeding attractant and stimulant in combination with a toxicant that when ingested by the adult will either reduce fecundity/fertility at sub-lethal dosages or kill the adult. *S. exigua* can breed 3 generations per year in the Spanish southeastern climate and up to 8 generations per year in protected crops (Cabello 2008). However, current chemical controls used against *S. exigua* adults have not been recently modernised because *S. exigua* adults have only nocturnal activity and also because of the potential negative impact on auxiliary fauna. The sole strategy currently developed for using against *S. exigua* adults is the use of pheromone-baited traps and light traps to capture males and both males and females, respectively. These tactics should also allow the monitoring and possible reduction of progeny size (Calvo and Belda 2008). Currently, this attract-and-kill strategy for *H. zea* seems to be in a conceptual stage, but further studies must be performed examining its potential and its possible application to other noctuids.

Overall, this work enhances the knowledge on insecticidal activity of emamectin benzoate at different developmental stages of the beet armyworm *S. exigua,* even when low doses are applied. Further research is needed to exploit the practical use of this insecticide at field level on developmental stages different from the larvae.

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