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Potential of antranilic diamides applied in seeds of soybeans with and without cry1ac protein for *Helicoverpa armigera* caterpillar control

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In Brazil, the caterpillar *Helicoverpa armigera* (Hübner) is a key economic pest of soybean, from seedling emergence to the reproductive stage. Control of this pest has relied on foliar insecticide sprays, and the use of insect-resistant cultivars. Characterization of the efficacy of insecticides that can be used for seed treatment, as a complement or alternative control for this pest, is needed for the production sector. The study objective was to evaluate the insecticidal potential of diamides for control of *H. armigera* larvae, when applied to soybean seeds. The effects of seed treatment with cyantraniliprole or chlorantraniliprole on 1st- and 3rd-instar *H. armigera* larvae, in both conventional and in "Bt soybean" (Cry1Ac), were evaluated under laboratory conditions. Two infestations were carried out at 8, 13 and 21 days after plant emergence, to determine the leaf area consumed, and mortality of larvae. The insecticides cyantraniliprole and chlorantraniliprole, used at rate of 60 and 62.5 g a.i./100 kg seeds, respectively, have potential for the control of 1st-instar *H. armigera* larvae up until at 13 days after the emergence of soybeans plants. The Cry1Ac protein controlled both 1st- and 3rd-instar *H. armigera* larvae at least until 21 days after plants emergence.

Key words: *Glycine max,* pest management, insecticides.

INTRODUCTION

The caterpillar *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae: Heliothinae) was recorded in the American continent in 2013, first in agricultural regions of Brazil (Czepak et al., 2013; Ávila et al., 2013), later in Paraguay (Senave, 2013), Argentina (Murúa et al., 2014) and, more recently, in the USA (North American Plant Protection Organization, 2015).

In Brazil, *H. armigera* is widely disseminated as a

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soybean pest and several other economically important crops, and feeding on weeds (Ávila et al., 2013; Czepak et al., 2013; Arnemann et al., 2014; Thomazoni et al., 2013). The management of this pest is hampered by its wide range of host plants, which includes non-monitored and fallow areas that serve as a refuge and source of multiplication of the pest (Tay et al., 2013).

In the soybean crop, this pest occurs from seedling emergence to the reproductive stage, when it feeds on flower buds and seed pods (Landim Filho et al., 2014). The occurrence as a pest at the beginning of the crop cycle can be due to two situations (Salvadori et al., 2013): caterpillars that are already present in the area, from the crop that precedes soybean, or infestations from eggs laid on the soybean plants, just after emergence.

The main tactic for control of *H. armigera* used by Brazilian soybean farmers has been foliar spraying of insecticides. The use of transgenic cultivars expressing the entomotoxic protein Cry1Ac from *Bacillus thuringiensis* (Fitt and Wilson, 2000), and seed treatment with insecticides are presented as alternatives to control *H. armigera*. Seed treatment products based on cyantraniliprole and chlorantraniliprole, from the diamide group, were recently introduced to the Brazilian market, and their efficacy in conventional and Bt soybean cultivars needs to be properly evaluated.

The objective of this study was to evaluate the insecticidal potential of cyantraniliprole and chlorantraniliprole for management of small *H. armigera* larvae, applied to soybean as seed treatments, with and without the presence of Cry1Ac protein.

MATERIALS AND METHODS

Experiments were conducted in the Entomology Laboratory of the Faculty of Agronomy and Veterinary Medicine (FAMV), University of Passo Fundo (UPF), Passo Fundo (RS), Brazil, in a controlled environment (25±2°C, 60±10% RH and 12-h photoperiod).

The effect of seed treatments with the insecticides on 1st- and 3rd-instar *H. armigera* larvae, in a conventional soybean cultivar (cv. BMX Potencia RR) and in a transgenic “Bt soybean” (cv. AS 3570 IPRO RR2, protein Cry1Ac) was recorded. Four chemical insecticides were applied in the treatment of seeds (Table 1), all are registered for this purpose in soybeans in Brazil (Agrofit, 2016).

The insecticides fipronil and imidacloprid+thiodicarb were used as positive controls, due to their established use in seed treatment of soybean. To obtain a uniform distribution, each insecticide was applied to 1.0 kg of seeds in a polyethylene bag with a small amount of water (0.3 L /100 kg seed). All the seeds were also treated with the carbendazim + thiram fungicides.

The insects used came from the rearing kept in artificial diet. Soybeans were grown in a greenhouse, in pots (5 plants per pot) with a capacity of 8 liters of soil and a surface area of 0.53 m^2. The pots were placed in trays, in which water was applied to irrigate the plants.

From plants in the V2 stage (second node on the main stem), leaf discs (1 or 4 cm², as required) were removed for bioassay with larvae placed individually in Petri dishes (9 cm diameter). Two infestations were performed for each larval stage (1st- and 3rd-instar), at 8, 13 and 21 days after plant emergence. Observations were performed daily, but it is considered for analysis the sum consumption, and the number of dead caterpillars five days after infestation. The caterpillars that did not show movement when touched with a brush was considered dead.

The experimental design was completely randomized, with 5 treatments (4 insecticides + negative control with only water), and 25 larvae/treatment. Data were subjected to analysis of variance, and means compared by Tukey's test (p≤0.05). For mortality analysis, the caterpillars were gotten together in 5 replicates of 5 individuals each.

RESULTS AND DISCUSSION

Seed treatment in conventional soybean

In 1st-instar caterpillars, at 8 days after plant emergence, all insecticides significantly suppressed foliar consumption by 1st instar larvae (Table 2).

However, the lowest foliar consumption was observed for cyantraniliprole and chlorantraniliprole, which also produced the greatest larval mortality (96.0 and 84.0%, respectively), demonstrating faster action than the other insecticides. Imidacloprid+thiodicarb caused less but still significant mortality, possibly explaining why it produced intermediate foliar consumption.

At 13 days after emergence, cyantraniliprole and chlorantraniliprole continued as the best treatments, both in terms of consumption and mortality, showing a persistent effect with comparable efficacy (Table 2). The other insecticides failed to significantly reduce consumption or kill larvae. At 21 days after the emergence, there was no residual effect (Table 2).

The rapid interruption of consumption in insects poisoned by cyantraniliprole or chlorantraniliprole applied to the seeds appears to provide protection to soybean against attack by small (1st and 3rd-instar) *H. armigera* larvae. Antranilic diamides, even before paralyzing the body of the insect leading to death, paralyzes the mandibles of larvae (Cordova et al., 2006; Lahm et al., 2009; Hannig et al., 2009; Álvarez and Abbate, 2013), so that only a small amount needs to be ingested for its effect to be observed (Table 2).

Regarding 3rd-instar larvae, the superiority of both diamides over the other insecticides was confirmed at 8 days after plant emergence, but only in terms of leaf consumption (Table 3). At 13 days after emergence, only ciantraniliprole reduced significantly leaf consumption, but offering only 59.6% protection. Due to this result, no evaluation was performed at 21 days after plants emergence, even though for the 1 st-instar larvae there was no residual effect after this time.

For the doses evaluated, the antranilic diamides proved to be a viable control option for neonate *H. armigera* larvae during the first week after emergence of soybean plants. The use of cyantraniliprole or chlorantraniliprole as seed treatments may be an important option for Brazilian producers, as the occurrence of infestations of this pest in the first days of the soybean cycle from eggs laid directly on the emergent plants is common (Salvadori

Table 1. Insecticides evaluated: active ingredient and respective trademark (name and rate).

Active ingredient	Trademark	Rate (ml/100 kg seeds)
Cyantraniliprole	Fortenza 600 FS®	100
Chlorantraniliprole	Dermacor®	100
Fipronil	Belure®	200
Imidacloprid + thiodicarb	Cropstar®	500

Table 2. Mean foliar consumption and mortality of 1st-instar *H. armigera* larvae at 8, 13 and 21 days after emergence of soybean plants, whose seeds were treated with insecticides (25 ± 2 ºC, 60 ± 10% RH, 12-h photoperiod).

Treatment (g a.i./100 kg seeds)	8 days				13 days				21 days	
	Consumption (cm²)		Nº dead		Consumption (cm²)		Nº dead		Consumption (cm²)	Nº dead
Cyantraniliprole (60)	0.20	c	4.80	ab	0.18	b	4.60	a	0.34ns	3.60ns
Chlorantraniliprole (62.5)	0.22	c	4.20	ab	0.22	b	4.20	a	0.28	4.00
Fipronil (50)	0.72	b	1.40	cd	0.70	a	2.80	ab	0.46	2.60
Imidacloprid+thiodicarb (75+225)	0.56	b	2.00	bc	0.71	a	1.20	b	0.46	3.40
Control (water)	0.97	a	0.20	d	0.87	a	1.60	b	0.46	2.00
C.V. (%)	8.14	-	18.19		14.31	-	14.86	-	35.05	17.80

Means followed by the same letter do not differ statistically (Tukey, p≤0.05).

Table 3. Mean foliar consumption and mortality of 3rd-instar *H. armigera* larvae at 8 and 13 days after emergence of soybean plants, whose seeds were treated with insecticides (25 ± 2 ºC, 60 ± 10% RH, 12-h photoperiod).

Treatment (g a.i./100 kg seeds)	8 days				13 days		
	Consumption (cm²)		Nº dead		Consumption (cm²)		Nº dead
Cyantraniliprole (60)	5.46	b	1.40	ab	3.26	b	2.40^{ns}
Chlorantraniliprole (62.5)	3.93	b	2.60	a	4.93	ab	1.80
Fipronil (50)	9.79	a	0.40	b	7.01	a	1.20
Imidacloprid+thiodicarb (75+225)	12.79	a	1.40	ab	7.65	a	1.40
Control (water)	12.76	a	0.60	ab	8.06	a	1.20
C.V. (%)	19.34	-	22.95		30.22	-	23.06

Means followed by the same letter do not differ statistically (Tukey, p≤0.05), ns = non-significant diferences.

et al., 2013).

The study results show that the effect of cyantraniliprole and chlorantraniliprole applied as soybean seed treatments depends on the age of the larvae, as it has been observed in cotton (Barbosa et al., 2014) and in soybean (Landim Filho et al., 2014), with chlorantraniliprole. On the other hand, confirm the efficiency of the active ingredient for the control of this pest, as already seen in spraying of plant leaves (Wakil et al., 2012; Carneiro et al., 2014; Misra, 2015).

H. armigera sensitivity tests for cyantraniliprole show that oral toxicity is on average 400 times greater than that for contact toxicity (Bird, 2016), demonstrating that ingestion is important for control of *H. armigera* by this active ingredient, supporting its importance as a seed treatment (Table 3).

The residual effect of chlorantraniliprole applied as a soybean seed treatment against *Anticarsia gemmatalis* larvae has been previously reported (Rodrigues et al., 2014). In the present study, in 1st- and 3rd-instar *H. armigera* larvae, the effect was observed beyond 7 days after emergence, consistent with the results of Filho et al. (2014).

The performance of fipronil and imidacloprid+thiodicarb was lower than that of the cyantraniliprole and chlorantraniliprole. In the case of the mixture imidacloprid+thiodicarb, the small effect shown on 1st-instar *H. armigera* larvae at 8 days after plant emergence

Table 4. Mean foliar consumption and mortality of 1st-instar *H. armigera* larvae at 8, 13 and 21 days after emergence of Bt soybean (Cry1Ac) plants, whose seeds were treated with insecticides (25 ± 2 ºC, 60 ± 10% RH, 12-h photoperiod).

Treatment (g a.i./100 kg seeds)	8 days		13 days		21 days	
	Consumption (cm²)	Nº dead	Consumption (cm²)	Nº dead	Consumption (cm²)	Nº dead
Cyantraniliprole (60)	0.19[ns]	4.80[ns]	0.14[ns]	5.00[ns]	0.20	5.00
Chlorantraniliprole (62.5)	0.20	5.00	0.16	4.80	0.20	5.00
Fipronil (50)	0.20	5.00	0.18	4.80	0.20	5.00
Imidacloprid+thiodicarb (75+225)	0.20	5.00	0.14	5.00	0.20	5.00
Control (water)	0.20	4.60	0.21	4.60	0.20	5.00
C.V. (%)	0.78	2.78	4.29	3.31	-	-

Ns = non-significant differences (p≤0.05).

may have been due to the presence of thiodicarb (Bueno et al., 2010), but in an insufficient dose.

The inability of fipronil in prevent foliar consumption by *H. armigera* larvae indicates a limitation of this active ingredient, at the dose tested, when applied to soybean seeds. Its toxic effect on caterpillars (Colliot et al., 1992), including *H. armigera* (Carneiro et al., 2014) has been observed when sprayed in soybean foliage.

Seed treatment in Cry1Ac soybean

With 1st-instar larvae, there was no difference in leaf consumption or larval mortality between Bt soybean (Cry1Ac) treated or untreated with insecticides, either at 8, 13 or 21 days after plant emergence (Table 4). Consumption was not significantly affected in either evaluation and mortality was high in all treatments (including the negative control) reaching 100% or near (Table 4).

Although consumption was modest (limited to a few test bites), it was sufficient to kill 1st-instar larvae, regardless of the treatment and the time between plant emergence and infestation. The rapid and high mortality caused by the Cry1Ac protein was also observed for neonate larvae of *H. virescens* (Bortolotto et al., 2014).

In 3rd-instar larvae, all insecticides significantly reduced leaf consumption at 8 and 13 days after plant emergence, relative to the control (Table 5). This result indicates that for larger larvae, the use of seed treatments in Bt soybean can minimize caterpillar damage to foliage. The average reduction in consumption in insecticide treatments was 30.1% and 55.3%, at 8 and 13 days after plant emergence, respectively.

However, the number of dead larvae did not differ between treatments. At 8, 13 and 21 days, the mortality of 3rd-instar larvae fed discs from soybean leaves whose seeds were treated with insecticides did not exceed that observed in the cultivar expressing the entomotoxic protein Cry1Ac, which alone caused complete larval mortality (100%). These data suggests that there is no advantage to seed treatment with the tested insecticides in Bt soybeans for control of *H. armigera* small caterpillars, when the infestation comes from oviposition after plants emergence.

The toxic effect of the Cry1Ac protein on *H. armigera* larvae of different stages in soybean, showing a high mortality (Bortolotto et al., 2014), is confirmed. This insecticidal protein has also been shown to be efficacious in controlling *H. virescens* larvae in soybean and cotton (Terán-Vargas, 2005; Bortolotto et al., 2014). In soybean, even in more developed plants (withseed pods), the concentration of the insecticidal protein is sufficient to control *H. virescens* larvae (Bernardi et al., 2013; Bortolotto et al., 2014) (Table 5).

Foliar consumption by 1st- and 3rd-instar larvae was lower in Bt soybean leaves than in the conventional cultivar (Tables 2 to 5). Aside from the fact that they are different genotypes, this is still indicative of the action of Cry1Ac protein on *H. armigera*. This protein causes a reduction in feeding and, consequently, starvation of larvae by specific binding in the ciliated membranes of the midgut, leading to death (Yu et al., 2011).

The results of both experiments demonstrated the potential of cyanthraniliprole and chlorantraniliprole (in seed treatment), and entomotoxin Cry1Ac in the control of infestations of *H. armigera* caterpillars hatched in newly emerged soybean plants. However, these results require confirmation under field conditions and natural pest infestation.

CONCLUSIONS

(1) The insecticides cyantraniliprole and chlorantraniliprole, used at rate of 60 and 62.5 g a.i./100 kg seeds, respectively, have potential for the control of 1st-instar *H. armigera* larvae up until at 13 days after the emergence of soybeans plants.

(2) Constitutively, expressed Cry1Ac protein also controls 1st- and 3rd-instar *H. armigera* larvae at least until 21 days after the emergence of plants.

Table 5. Mean foliar consumption and mortality of 3rd-instar *H. armigera* larvae at 8, 13 and 21days after emergence of Bt soybean (Cry1Ac) plants, whose seeds were treated with insecticides (25 ± 2 ºC, 60 ± 10% RH, 12-h photoperiod).

Treatment (g a.i./100 kg seeds)	8 days			13 days			21 days	
	Consumption (cm²)		Nº dead	Consumption (cm²)		Nº dead	Consumption (cm²)	Nº dead
Cyantraniliprole (60)	0.80	b	5.00	0.86	b	5.00ns	1.056ns	5.00
Chlorantraniliprole (62.5)	0.80	b	5.00	0.83	b	5.00	0.832	5.00
Fipronil (50)	0.90	b	5.00	1.17	b	4.71	0.928	5.00
Imidacloprid+thiodicarb (75+225)	0.80	b	5.00	0.80	b	5.00	0.992	5.00
Control (water)	1.18	a	5.00	2.05	a	5.00	0.896	5.00
C.V. (%)	5.36	-	-	16.05	-	1.65	15.85	-

Means followed by the same letter did not differ statistically (Tukey, p≤0.05). ns: non-significant differences.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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