Susceptibility and residual effect of indoxacarb formulations on major soybean lepidopteran pests

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Abstract *Chrysodeixis includens* and *Helicoverpa armigera* can negatively impact soybean yield due to defoliation and direct injury on the pods, respectively. Insecticides, such as indoxacarb, are still an important controlling tool to manage these insects. To support Integrated Pest Management (IPM) and Insect Resistance Management (IRM) as well as to reduce product decantation during storage and nozzle clogging in field application, a new indoxacarb formulation (emulsifiable concentrate - EC) was developed to replace the suspension concentrate formulation (SC). The objective of this study was to evaluate the susceptibility and compare the residual effects of two indoxacarb formulations on *C. includens* and *H. armigera* on soybean. A dose-response curve and the residual effect from in-field application were obtained using a laboratory strain of both insect pests. Soybean leaflets were collected and dipped into aqueous indoxacarb solutions. The evaluations were performed 96 h after the infestation to calculate the insect mortality percentage and foliar damage.

Also, plants were sprayed in the field and leaflets from mid and upper regions of the plants were collected and used to feed third-instar larvae of both species. Results demonstrated that *C. includens* and *H. armigera* are similarly sensitive to indoxacarb. The residual activity was up to 1 h after application for *H. armigera* and up to 3 days for *C. includens*. No difference was observed on this activity between mid and upper regions of the plant. Overall, the enhanced EC formulation is as effective as the SC formulation for the control of both species on soybean.

Keywords *Chrysodeixis includens* · *Helicoverpa armigera* · Dose-response curve · Residual activity

Introduction

The soybean looper *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae) is known as an economically important pest to a wide range of host plants such as cotton, beans, tomatoes, potatoes, peanuts (Herzog 1980, Bueno et al. 2007) and soybean (Moscardi et al. 2012). In Brazil, *C. includens* was considered a secondary soybean pest for many years and was mainly controlled by naturally occurring parasitoids and entomopathogenic fungi (Sosa-Gómez et al. 2003). However, the occurrence of Asian rust in Brazil increased considerably the use of fungicides in soybean (Garcés-Fiallos 2011). These fungicides affect negatively beneficial fungi (Sosa-Gómez 2005) and were most likely responsible for *C. includens* outbreaks as well as for its status

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change to a major soybean insect pest (Bueno et al. 2007, Santos et al. 2017).

In addition, *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae), an invasive species identified in Brazil during the 2012/2013 growing season (Czepak et al. 2013), is also a polyphagous insect pest and feeds on vegetative (leaves) and reproductive (flowers and pods) parts of host plants (Czepak et al. 2013, Specht et al. 2013). Injuries caused by this insect leads to yield losses in various agricultural crops such as tomato, cotton, corn, beans and soybean (Fitt 1989, Romeis and Shanower 1996, Pogue 2004, Moral García 2006). Its control is challenging and resistance of this insect pest to several conventional insecticides including synthetic pyrethroids (Forrester et al. 1993), carbamates (Gunning et al. 1996), organophosphates (Gunning et al. 1997) and spinosad (Wang et al. 2009) have been reported (Fitt and Wilson 2000, Yang et al. 2013).

These two species can cause great impact on soybean yields. *Helicoverpa armigera* alone was responsible for approximately US\$ 800 million in Brazil and *C. includens* has become a recurrent problem and is considered a key pest in soybean (Specht et al. 2015). Genetically modified (GM) soybean expressing *Bacillus thuringiensis* toxic protein is the main pest control strategy and was adopted in ca. 58% of the soybean area cultivated in Brazil (ISAAA 2017). Despite of the great adoption of this strategy, Integrated Pest Management (IPM) tools need to be enhanced to cope with pests that are not targets of GM plants as well as those present in non-Bt soybean.

The insecticide indoxacarb presents low toxicity to the environment (McCann et al. 2001) and to non-target organisms when compared to pyrethroids or carbamates (Michaud and Grant 2003). Belonging to the group of oxidiazines (McCann et al. 2001), indoxacarb is composed of a chiral carbon with two enantiomers (–)-R-indoxacarb and (+)-S-indoxacarb. It is a pyrazoline-type sodium channel blocker (IRAC MoA subgroup 22A) with activity on lepidopteran, coleopteran, and hemipteran insect pests (Wing et al. 1998, 2004). Indoxacarb is not systemic (McKinley et al. 2002) and is active by contact and ingestion resulting in paralysis and death (Jones and Bryant 2012). One of the main indoxacarb products used in Brazil is Avaunt® SC (DPX-KN128- suspension concentrate). However, product decantation occurred under storage conditions and nozzle clogging observed during field application could be a limiting

factor of its effectiveness and use as a control tool. Trying to improve the indoxacarb efficacy, a new formulation was developed: Avatar® EC (emulsifiable concentrate). This formulation contains an oil solvent and a surfactant to enhance wetting of leaves. The EC formulation blooms into a white cloudy emulsion when poured into water and disperses with minimal agitation in spray tank containing either hard or soft water. The indoxacarb is completely dissolved with no particles to resuspend (DuPont 2013). To support IPM and Insect Resistance Management (IRM), the objective of this study was to evaluate the susceptibility and compare the residual effects of the new indoxacarb formulations EC in comparison to the SC formulation for the control of *C. includens* and *H. armigera* on soybean.

Material and methods

Insects

Indoxacarb-susceptible laboratory mass reared strains of *C. includens* and *H. armigera* were used in all studies. Insect colonies were founded after collection of insects in soybean fields in 2008 and 2013, respectively. The insects were reared under controlled conditions (25 ± 2 °C, 46–47% RH, 12:12 h [L: D]) at FMC Research and Technology Center (formerly DuPont do Brasil Research Station), located in Paulínia, SP, Brazil.

Susceptibility of laboratory insect strains to indoxacarb

Commercial insecticide formulations of indoxacarb were used: Avaunt SC® (15% active ingredient) and Avatar EC® (15% active ingredient). The two indoxacarb formulations (SC and EC) were compared using dose-response curves to evaluate the susceptibility.

Soybean variety BMX Potencia® (Brasmax Genética Ltd., Passo Fundo, RS, Brazil), insecticide free, was grown under greenhouse conditions. Leaflets were randomly collected from soybean plants and taken to the laboratory to be treated when plants were at V4, i.e., 4th trifoliate stage (Fehr and Caviness 1977). They were dipped into different indoxacarb concentrations (0.001; 0.01; 0.1; 1; 10; 100; and 250 µg a.i./ml) for 10s. The untreated control was dipped into water. The leaflets were left to

Fig. 1 Defoliation scale used to evaluate injury caused by *Chrysodeixis includens* and *Helicoverpa armigera* on soybeans leaflets

air dry at room temperature for approximately one hour. Leaflets were individualized into 16-cell plastic trays (Advento do Brasil, Diadema, SP, Brazil) and a third-instar larvae of *C. includens* was individually introduced (one larva per well and 16 larvae per dose). The same methodology was used for *H. armigera*. The bioassay was carried out under controlled conditions (25 ± 2 °C, 46–47% RH, 12:12 h [L:D]) and the evaluations were performed

96 h after the infestation. The number of dead, moribund, and alive larvae were registered. A larva was considered dead when there was no movement when softly prodded by a brush or when presented paralysis of the prolegs (Bird 2015). The data were expressed as percentage of mortality. We performed three bioassays for each insect species and product formulation. Each bioassay was considered one replication.

Table 1 Susceptibility parameters for third-instar larvae of *Chrysodeixis includens* and *Helicoverpa armigera* (Lepidoptera: Noctuidae) laboratory susceptible strain tested on soybean leaflets

Species	Insecticide	N	LC ₅₀ (95% FL)	LC ₉₉ (95% FL)	Slope $\pm$ SE	Chi-Square	P
<i>C. includens</i>	indoxacarb (15% EC)	32	0.065 (0.028–0.147)	12.969 (3.054–186.259)	1.011 $\pm$ 0.157	6.850	0.0089
	indoxacarb (15% SC)	32	0.127 (0.046–0.340)	28.055 (5.437–762.121)	0.992 $\pm$ 0.174		
<i>H. armigera</i>	indoxacarb (15% EC)	32	0.113 (0.039–0.314)	46.768 (8.234–1352.086)	0.890 $\pm$ 0.148	1.995	0.1578
	indoxacarb (15% SC)	32	0.111 (0.045–0.277)	31.568 (6.430–631.207)	0.948 $\pm$ 0.152		

N: number of insects exposed; LC₅₀ and LC₉₉ are the lethal concentration values with 95% fiducial limits (FL) to kill 50 and 99% of the larvae exposed; Lethal concentrations are expressed as active ingredient ($\mu\text{g ml}^{-1}$)

Table 2 Mortality (% $\pm$ SEM) of third-instar *Chrysodeixis includens* larvae in the upper and middle soybean canopy layers treated with two formulations of indoxacarb (EC - emulsifiable suspension and SC - suspension concentrate)

Canopy Layer	Days after treatment	indoxacarb (15% EC)		indoxacarb (15% SC)	
		30 g a.i./ ha	60 g a.i./ ha	30 g a.i./ ha	60 g a.i./ ha
Upper	0	97.50 $\pm$ 2.50 Aa	98.41 $\pm$ 0.92 Aa	92.92 $\pm$ 5.16 Aa	86.62 $\pm$ 11.27 Aa
	3	79.29 $\pm$ 6.67 Bab	93.32 $\pm$ 2.20 Aa	67.08 $\pm$ 8.59 Bb	91.16 $\pm$ 4.89 Aa
	7	17.07 $\pm$ 7.25 Cc	44.81 $\pm$ 7.60 Bb	33.33 $\pm$ 4.99 Cb	72.31 $\pm$ 3.23 Ba
	10	29.62 $\pm$ 8.17 Cb	55.90 $\pm$ 12.69 Bab	39.41 $\pm$ 11.20 Cb	72.53 $\pm$ 7.94 Aba
	16	9.04 $\pm$ 5.52 Cb	15.73 $\pm$ 7.26 Cb	6.68 $\pm$ 4.00 Db	39.82 $\pm$ 10.42 Ca
Middle	0	98.17 $\pm$ 1.07 Aa	97.21 $\pm$ 0.94 Aa	79.03 $\pm$ 3.11 Ab	87.39 $\pm$ 9.07 Aab
	3	68.06 $\pm$ 7.46 Bb	89.97 $\pm$ 3.49 Aa	60.94 $\pm$ 5.06 Ab	80.09 $\pm$ 5.31 ABab
	7	15.22 $\pm$ 3.82 Cc	45.21 $\pm$ 8.39 Bb	29.84 $\pm$ 5.28 Bbc	70.78 $\pm$ 5.20 Ba
	10	32.27 $\pm$ 9.97 Cb	54.77 $\pm$ 3.94 Bab	30.86 $\pm$ 4.25 Bb	74.62 $\pm$ 2.88 Ba
	16	3.70 $\pm$ 3.70 Db	6.48 $\pm$ 4.38 Cab	0.00 $\pm$ 0.00 Cb	24.41 $\pm$ 15.33 Ca

Uppercase letters indicate differences between days after application within treatment and layer (column within a layer) whereas lowercase letters indicate differences between treatments on the same day after application (row)

Indoxacarb residual effect

Soybean variety BMX Potencia® (Brasmax Genética Ltd., Passo Fundo, RS, Brazil) was planted in the field in June, 2015 using recommended cultural practices (EMBRAPA 2013), but without insecticide applications. Indoxacarb (30 and 60 g a.i./ha for both SC and EC formulations – product label rate) was applied when plants reached R3-R4 growth stage, i.e., pods presented 30% of final length (Fehr and Caviness 1977). Water was applied on the untreated control. Application of the treatments was performed through a manual costal sprayer coupled to a three-meter bar with six nozzles type XR 11001 VS (TeeJet Technologies, Spraying Systems Co., Glendale Heights, IL, USA). The spraying system was operated with a constant pressure (2.1 bar) and application volume of 200 l ha⁻¹. The experimental design adopted was CRBD (Completely randomized block design) with four replications per treatment. Each plot (replication) measured 3.5 m $\times$ 6 m.

After application, soybean leaflets were collected 1 h (0 d), 3 d, 7 d, and 10 d (days after application). Leaflets were also collected at 16 d, but just for *C. includens*. For each collection, 128 leaflets were randomly selected from each replication being 64 from the upper third and 64 from the middle layer of the plants, because the insects usually feed on these regions on soybean plants (Moscardi et al.

2012, Stacke et al. 2018). The leaflets were individually transferred into the cells of plastic trays (Advento do Brasil, Diadema, SP, Brazil). For each region of the plant, 32 cells were infested with third-instar larvae of *H. armigera* and 32 cells with *C. includens*. The assay was maintained in laboratory conditions (25 $\pm$ 2 °C, 46–47% RH, 12:12 h [L:D]). For both species, larval mortality (expressed in percentage) and defoliation were accessed. Defoliation was visually evaluated 96 h after infestation using a 0% (no injury) to 100% (all the leaflet fed with some preserved nervures) scale (Fig. 1).

Data analysis

Mortality obtained from the dose-response bioassays was adjusted to the Probit curve (dose-mortality) to obtain LC₅₀ and LC₉₉ (lethal concentration to kill 50% and 99% of the population). The residual effect data (mortality and injury) were submitted to factorial analysis of variance and the means were compared using Tukey test ($P = 0.05$) using PROC GLM. Every mortality datum was transformed into arcsine $\sqrt{x/100}$. Percent mortality was corrected based on mortality in the untreated control using Abbott's (1925) formula and response curves were compared using Chi-Square (Robertson et al. 2007). The statistical analyses were performed using JMP 12.2.0 or SAS studio (SAS Institute Inc., Cary, NC, USA).

Fig. 2 Defoliation (+ SEM) caused by *Chrysodeixis includens* in the upper (A) and middle (B) soybean regions at 0 (1 h), 3, 7, 10, and 16 days after application of treatments (Uppercase letters

indicate differences between times after application within treatment and lowercase letters indicate differences between treatments on the same time after application)

Table 3 Mortality (% $\pm$ SEM) of third-instar *Helicoverpa armigera* larvae in the upper and middle soybean canopy layers treated with two formulations of indoxacarb (EC - emulsifiable suspension and SC - suspension concentrate)

Canopy Layer	Days after treatment	indoxacarb (15% EC)		indoxacarb (15% SC)	
		30 g a.i./ ha	60 g a.i./ ha	30 g a.i./ ha	60 g a.i./ ha
Upper	0	98.26 $\pm$ 1.02 Aa	100.00 $\pm$ 0.00 Aa	96.52 $\pm$ 2.03 Aa	91.94 $\pm$ 8.07 Aa
	3	38.70 $\pm$ 13.98 Bbc	69.47 $\pm$ 9.01 Ba	17.02 $\pm$ 7.96 BCc	52.11 $\pm$ 4.97 Bab
	7	15.66 $\pm$ 7.22 BCb	19.47 $\pm$ 9.49 Cb	23.39 $\pm$ 5.43 Bab	48.35 $\pm$ 6.86 Ba
	10	1.57 $\pm$ 0.90 Ca	3.91 $\pm$ 1.97 Ca	2.35 $\pm$ 0.78 Ca	3.13 $\pm$ 1.80 Ca
Middle	0	75.38 $\pm$ 5.61 Ab	100.00 $\pm$ 0.00 Aa	93.58 $\pm$ 4.12 Aa	95.63 $\pm$ 3.26 Aa
	3	27.88 $\pm$ 9.62 Bb	72.01 $\pm$ 6.07 Ba	26.68 $\pm$ 7.25 Bb	50.30 $\pm$ 15.45 Bab
	7	11.07 $\pm$ 6.77 Cb	37.16 $\pm$ 10.76 Ca	2.59 $\pm$ 2.59 Cb	40.74 $\pm$ 11.55 Ba
	10	0.81 $\pm$ 0.81 Ca	0.81 $\pm$ 0.81 Da	0.81 $\pm$ 0.81 Ca	0.81 $\pm$ 0.81 Ca

Uppercase letters indicate differences between days after application within treatment and layer (column within layer) whereas lowercase letters indicate differences between treatments on the same day after application (row)

Results

Susceptibility to indoxacarb

The dose response curves of both indoxacarb formulations were similar for *H. armigera* ($X^2 = 1.995$; $P = 0.1578$) but not for *C. includens* ($X^2 = 6.850$; $P = 0.0089$) (Table 1). The lethal concentrations (LC_{50}) of EC and SC indoxacarb formulations were 0.065 (0.028–0.147) $\mu\text{g a.i. ml}^{-1}$ and 0.127 (0.046–0.340) $\mu\text{g a.i. ml}^{-1}$ for *C. includens* and 0.113 (0.039–0.314) $\mu\text{g a.i. ml}^{-1}$ and 0.111 (0.045–0.277) $\mu\text{g a.i. ml}^{-1}$ for *H. armigera*, respectively. Therefore, considering the fiducial limits, both formulations of indoxacarb are similarly toxic to *C. includens* and *H. armigera* (Table 1).

Indoxacarb residual effect

The mortality caused by indoxacarb to *C. includens* decreased significantly over time ($F_{4,132} = 164.52$; $P < 0.0001$) and was the highest just after the application (1 h) for Avaunt® and up to 3 days after application for Avatar® (Table 2). Consequently, defoliation increased with time and was the lowest just after the application ($F_{4,166} = 131.22$; $P < 0.0001$) when compared to the untreated control (Fig. 2). Mortality of *C. includens* fed on treated leaflets collected from the upper layer was higher ($F_{1,132} = 7.58$; $P = 0.0067$; Table 2). Higher doses of both formulations caused higher mortality of *C. includens* ($F_{1,132} = 20.00$; $P < 0.0001$ for EC formulation and $F_{1,132} = 61.51$; $P < 0.0001$ for SC formulation) and reduced defoliation ($F_{1,166} = 14.01$; $P = 0.0003$

for EC formulation and $F_{1,166} = 40.82$; $P < 0.0001$ for SC formulation).

Both formulations of indoxacarb (SC - Avaunt® and EC - Avatar®) were similarly toxic to *H. armigera* ($F_{1,105} = 0.21$; $P = 0.6494$) leading to likewise reduced injury ($F_{1,132} = 2.15$; $P = 0.1448$) caused by this insect pest (Table 3; Fig. 3). There was no difference between the upper and middle soybean layers to insect mortality ($F_{1,105} = 3.73$; $P = 0.0561$) and to foliar injury ($F_{1,132} = 1.89$; $P = 0.1719$) in each evaluating time. On the other hand, mortality decreased ($F_{3,105} = 242.11$; $P < 0.0001$; Table 3) while injury increased ($F_{3,132} = 86.92$; $P < 0.0001$; Fig. 3) over time. Similar to the results observed for *C. includens*, higher doses of both formulations caused higher mortality of *H. armigera* ($F_{1,105} = 24.64$; $P < 0.0001$ for SC formulation and $F_{1,105} = 15.07$; $P = 0.0002$ for EC formulation) and lower defoliation by *H. armigera* ($F_{1,132} = 12.11$; $P = 0.0007$ for SC formulation and $F_{1,132} = 4.26$; $P < 0.0410$ for EC formulation).

Discussion

The two indoxacarb formulations were effective for the control of *H. armigera* and *C. includens* on soybean. The sensibility of the species to indoxacarb was similar. Therefore, the enhancement of indoxacarb formulation (SC - suspension concentrate to EC - emulsifiable concentrate) did not result in increased efficacy. Stacke et al. (2019) evaluated the Brazilian field populations of *C. includens* and noticed up to 6.7-fold resistance

Fig. 3 Defoliation (+ SEM) caused by *Helicoverpa armigera* in the upper (A) and middle (B) soybean regions at 0 (1 h), 3, 7, and 10 days after application of treatments (Uppercase letters indicate

differences between times after application within treatment and lowercase letters indicated differences between treatments on the same time after application)

ratio to indoxacarb in comparison to a lab susceptible population. The resistance ratio of other nerve action insecticides (e.g., spinetoram, thiodicarb, lambda-cyhalothrin) varied from 5.6 to 62.5. However, similar results for Brazilian field populations of *H. armigera* are not available yet.

In the study on indoxacarb residual effect, high mortality of *H. armigera* was shown on leaflets infested up to 1 h after indoxacarb application and up to 3 days for *C. includens*. Similar results were reported by Kuss et al. (2016) using indoxacarb EC formulation for the control of *H. armigera* on soybean. On the other hand, Vinaykumar et al. (2013) reported that indoxacarb controlled *H. armigera* up to 7 days after application on soybean. This difference could be explained by population sensibility to the insecticide indoxacarb (Yang et al. 2013).

Indoxacarb SC and EC formulations presented a good foliar coverage on soybean plants. No mortality difference was found on the leaflets located either on the top or the middle regions of soybean plants. This result is important mainly for *C. includens* that prefers to feed in leaves located in the middle region of plants (Conte et al. 2014). Failure on efficient foliar coverage should favor survivorship of insects. Consequently, more frequent or repeated applications of insecticides may be necessary for the control (Vivan and Degrande 2011). However, this decision may increase an ecosystem imbalance and costs.

The doses studied were in the range recommended for pest control in different crops. It was expected that higher doses would cause higher mortality and lower defoliation. However, the highest mortality was overall observed on the first day or, at most, 3 days after the application.

The enhanced insecticide indoxacarb EC formulation can be used as one of the strategies within an Integrated Pest Management program for the control of *H. armigera* and *C. includens* on soybean. However, spray cover is essential to assure mortality of these defoliators and particularly of *H. armigera* that feeds on pods within the canopy of plants. Moreover, this study brings information on the susceptibility of indoxacarb to both lepidopteran species. Such information is important as a reference to evaluate the progress and to support Insect Resistance Management (IRM) programs of population resistance development to indoxacarb.

Conclusions

The new indoxacarb emulsifiable concentrate formulation is as effective as the previous suspension concentrate formulation to control Lepidopterous defoliators. However, residual efficacy is no longer than 3 days after application.

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Availability of data and material Raw data were generated at Paulinia Experimental Station, Dupont do Brasil S. A. Derived data supporting the findings of this study are available from the corresponding author [MGSO] on request.

Authors' contributions MGSO conducted the research; MGSO, RHS, OAF analyzed the data; All authors designed the experiments, analyzed and discussed the results, worked and approved the final manuscript.

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Compliance with ethical standards

Conflicts of interest there is no conflict of interest.

Ethics approval the study was carried out in compliance with scientific ethical guidelines.

Consent to participate all authors were involved in the study.

Consent for publication all authors agreed to be part of this publication.

Code availability not applicable.

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