Australian Journal of Crop Science AJCS

AJCS 13(10):1723-1730 (2019) ISSN:1835-2707
doi: 10.21475/ajcs.19.13.10.p2052

Challenges in chemical management of soybean looper (*Chrysodeixis includes*) using several insecticides

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Abstract

Soybean looper, *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: Noctuidae), is the most important caterpillar pest in Brazil due to its high tolerance to insecticides and control failures that have been reported in fields. Based on this, we assessed the performance of several insecticides against *C. includens* on soybean over three seasons (2014, 2015, and 2016), performing four experiments in southern Brazil. Experiments I / 2014, II / 2015, III / 2016 were carried in completely randomized block designs with eight insecticides. Experiment IV / 2016 was arranged in completely randomized block design in an 8 x 2 factorial arrangement, eight insecticides x two spraying time. Mortality of small (< 1.5 cm) and large (> 1.5 cm) soybean looper larvae was assessed with a vertical beat cloth, sampling in each plot 1.0 m^2 at 3, 7 and 10 days after spray. Percentage of defoliation injury was assessed visually comparing to a scale of soybean leaf injury in experiment IV / 2016. Data were subjected to one-way (type I SS) analysis of variance (ANOVA) using the general linear models. The insecticides indoxacarb and chlorfenapyr had consistently high mortality upon small and large larvae of *C. includens*. Spinosyn, spinosad and spinetoram insecticides showed higher effect over small larvae (< 1.5 cm) than large (> 1.5 cm). Number of spraying was significant for spinetoram and indoxacarb that increased mortality of large larvae (from 4.50 to 2.75 and from 3.75 to 0.87 larvae m^{-2}, respectively) and reduced defoliation injury significantly (from 22.5% to 9.3% and from 14.5% to 3.3%, respectively) with two applications. The treatment chlorfluazuron+acephate showed low defoliation injury in 2016 but did not affect larval density. Chlorfenapyr showed reduced defoliation (3.0%) and low larvae density (1.37 larvae m^{-2}) with only one application. Spinosyns have satisfactory control of small larvae and indoxacarb and chlorfenapyr show high mortality of both sizes, small and large larvae. Therefore, we recommend that 7 days from the first one, a second application should be considered for indoxacarb, and spinetoram to achieve higher mortality. Considering our results, we demonstrate that the satisfactory control of soybean looper larvae is difficult to achieve with most chemical insecticides. It needs a more accurate management strategy of *C. includens* in soybeans.

Keywords: soybean looper, caterpillar, defoliation, insecticide application, pest management.
Abbreviations: Bt_*Bacillus thuringiensis*; IRM_ Insect Resistance Management; DAS_days after spray; IRAC_Insecticide Resistance Action Committee; SCBI_Sodium channel blocker insecticide; nAChR_Allosteric modulators of the nicotinic acetylcholine receptor; NPK_nitrogen−phosphorus−potassium; 1S_one spray; 2S_two sprays.

Introduction

Chrysodeixis includens (Walker, [1858]) (Lepidoptera: Noctuidae) has significantly increased its predominance over the last decade in soybean due to successive changes in management practices and soybean cultivation (Guedes et al., 2010). Those factors contributed to proportional increase of *C. includens* on soybean in Brazil among other species, from 20% in the 80s and 90s (Moraes et al., 1991), to more than 70% nowadays (Guedes et al., 2015). This growth can also be related to areas where soybean and cotton crops are cultivated nearby (Burleigh, 1972), as it happens in the Midwestern Brazil. The area of soybean cultivation in Brazil has reached over 35 million hectares from north to south (Conab, 2018) distributed along seven months, indicating the immense food availability to be colonized.

Currently, management tactics against soybean looper in Brazil have mainly relied on the use of chemical insecticides, transgenic soybean expressing the Cry1Ac insecticidal protein from *Bacillus thuringiensis* (Bt) and baculovirus. There are currently no reports of *C. includens* resistance to *Bt* soybean (Bernardi et al., 2012; Yano et al., 2016) or chemical insecticides. Frequency of insecticide applications is often the most important issue for increasing the frequency of resistant insects if Insect Resistance Management (IRM) plans are not used correctly (Onstad and

Gassmann, 2014). Thus, as the number of insecticide application to control arthropod pests in Brazil range from four to six per soybean season (Bortolotto et al., 2015), the selection pressure on *C. includens* is very high.

The highest *C. includens* population density occurs generally during the reproductive stage of soybean, when the canopy is closed (Czepak and Albernaz, 2014) and the larvae are predominately located in the lower half of the soybean canopy (Papa and Celoto, 2007). The positioning of *C. includens* in the lower half of the soybean canopy hinders control and requires an improved pesticide sprayer system or an insecticide with greater toxicity. Larvae of *C. includens* are more tolerant to a range of doses usually being used to control other caterpillars and most insecticides recommended to control soybean caterpillars. They do not show satisfactory efficacy to control the soybean looper (Guedes et al., 2015). The tolerance of *C. includens* to insecticides was reported to be related to its capacity of enzymatic detoxification (Dowd and Sparks, 1986).

Studies on insecticide efficacy against *C. includens* in Brazil were performed a long time ago. In 2004, insecticide growth regulators were tested and had control efficacy >85% from 7 to 30 days after spraying (Pinto Junior et al., 2011). In 2006 and 2007, the insecticides fenitrothion + esfenvalerate, methomyl, thiodicarb, and chlorpyrifos were considered efficient (Martins and Tomquelski, 2015). However, these results are outdated and no longer match the current reality of control of soybean looper, as the majority of those insecticides are out of market nowadays. In order to address this critical knowledge gap, we performed experiments for three years to assess the effects of insecticides that are currently widely used to manage *C. includens* in soybean in Brazil.

Results

There was a significant effect of insecticides, DAS, size of larvae and number of spraying on all experiments, with exception in 2014 for DAS (Supplementary Table 1). For these reasons, the data of small and large larvae were analyzed and are presented separately at different DAS for each insecticide. It shows early and late effect of on small and large larvae of *C. includens* by some insecticides. The number of spray (1x and 2x) also have affected significantly the larvae mortality.

Mortality assessment of soybean looper over three seasons

In the experiment I / 2014, insecticides IND, CLF and SPD reduced the number of small larvae significantly already at 3 DAS, with 3.50, 1.75 and 3.00 larvae m^{-2}, respectively (Figure 1). A similar result was observed on IND only at 3 DAS for large larvae with 0.25 larvae m^{-2}. At 7 and 10 DAS, IND and CLF presented the lowest numbers of large larvae, 2.25 larvae m^{-2} at 7 DAS - 2.75 larvae m^{-2} at 10 DAS and 2.75 larvae m^{-2} at 7 DAS - 0.75 larvae m^{-2} at 10 DAS, respectively, representing to be highly effective compared to the other insecticides. Looking at the mean value of small and large larvae among the evaluation dates on Table 1, indoxacarb, chlorfenapyr, and spinosad had high mortality against small larvae with the lowest mean value of 1.8, 1.5, and 1.4 larvae m^{-2}, respectively. These insecticides were not differed from one another (Supplementary Table 2.1). But, for large larvae only indoxacarb and chlorfenapyr decreased the population density among the evaluation period significantly, presenting 1.7 and 2.2 larvae m^{-2}, respectively (Table 1), not differing significantly (*Pr* = 0.6832).

In 2015, some treatments were modified based on results of 2014 and company's recommendation to experiment insecticides for higher mortality. Similar to I / 2014, SPI caused the highest reduction of small larvae of *C. includens* at 3 DAS, with a mean of 1.00 larvae m^{-2} (Figure 1). Insecticides SPI, CLZ+ACF, CLF, and IND had the lowest density of small larvae, comparing to UNT and other insecticides, at 7 and 10 DAS. Analyzing the effect of insecticides on large larvae of *C. includens*, only CLF achieved high mortality at 3, 7, and 10 DAS, with a mean of 2.75, 2.50, and 1.25 larvae m^{-2}, respectively. In addition, IND reduced the population density at 7 DAS (2.00 larvae m^{-2}), but at 10 DAS its late residual effect was absent and the soybean looper density was increased. Similar to the most effective insecticides in the experiment in 2014, low variation between minimum and maximum data values of small larvae was observed for SPI at 3, 7 and 10 DAS, for CLZ+ACF, CLF, and IND at 7 and 10 DAS, and of large larvae for CLF and IND at 3, 7, and 10 DAS. Other insecticides showed higher variation but not consistent on mortality of *C. includens*. Spinetoram resulted in the lowest mean number of small larvae among evaluation dates with 0.8 larvae m^{-2}, followed by chlorfenapyr (1.0 larvae m^{-2}, indoxacarb (1.2 larvae m^{-2}), diflubenzuron + chlorpyrifos (1.3 larvae m^{-2}), methoxyfenozide (1.5 larvae m^{-2}), and chlorfluazuron + acephate (1.6 larvae m^{-2}) (Table 1 and Supplementary Table 2.2). In contrast, only chlorfenapyr showed a significant reduction (2.0 larvae m^{-2}) on large larvae population during the evaluation period, followed by indoxacarb with 3.8 larvae m^{-2} (*Pr* = 0.0063).

On experiment III / 2016, the treatments CLF and IND had an early effect on large larvae mortality at 3 DAS, with 1.50 and 2.00 larvae m^{-2}, respectively, compared to UNT that showed 7.00 larvae m^{-2} (Figure 1). After this evaluation, CLF and IND maintained the large larvae density at low levels compared to other insecticides (CLF: 4.25 larvae m^{-2} at 7 DAS - 2.25 larvae m^{-2} at 10 DAS; IND: 3.25 larvae m^{-2} at 7 DAS - 3.00 larvae m^{-2} at 10 DAS). Moreover, small larvae were affected by a greater number of insecticides, as previous results. At 3 DAS, no insecticide showed significant reduction on population of insect. But, at 7 and 10 DAS, CLF, IND and SPI had higher larvae mortality, compared to other insecticides (Figure 1). Chlorfenapyr and indoxacarb were the most effective insecticides against small and large larvae, as shown in Table 1, which did not differ among one another (*Pr* of 0.3306 and 0.9022, respectively - Supplementary Table 2.3). For small larvae, chlorfenapyr and indoxacarb were mostly effective followed by diflubenzuron + chlorpyrifos (1.5 larvae m^{-2}) and spinetoram (1.8 larvae m^{-2}). For large larvae, chlorfenapyr and indoxacarb were significantly different from the other insecticides (*Pr* < 0.0001).

Number of insecticide sprays comparison

The number of sprays had significant differences among insecticides only for large larvae (Figure 2). The insecticide CLF was the only one that significantly reduced small and large larvae with one spray, having 0.37 and 1.37 larvae m^{-2}, respectively (Figure 3). It represents a late residual effect of

CLF, contrasting from the other insecticides. SPI, CLF, IND, DIF+CLF, and FLU+TIO showed a positive response in reducing the population density of large larvae of *C. includens* with the second application, presenting 2.75, 0.12, 0.87, 1.37, and 1.50 larvae m^{-2}, respectively. Chlorfenapyr was the most effective against small and large larvae, with 0.5 and 0.7 larvae m^{-2}, respectively (Table 1). For small larvae, chlorfenapyr was followed by diflubenzuron + chlorpyrifos, spinetoram, and indoxacarb, with mean values ranging from 1.2 to 1.7 larvae m^{-2}. For large larvae, chlorfenapyr was followed by diflubenzuron + chlorpyrifos (1.7 larvae m^{-2}) and indoxacarb (2.3 larvae m^{-2}).
Damage on soybean leaves was reduced by the treatments with 2 applications, including methoxyfenozide (from 19.8% to 12.0%), spinetoram (from 22.5% to 9.3%) and indoxacarb (from 14.5% to 3.3%) (Figure 4). Considering that defoliation injury is caused mainly by large larvae of *C. includens*, it suggests that larvae mortality by indoxacarb and chlorfenapyr exhibited the lowest defoliation injury ranging from 1.3% to 3.3% (Supplementary Table 2.5). In contrast, even though the treatment CLZ+ACF was not effective in reducing the number of small and large larvae with one or two sprays (Figure 3), the mean values of defoliation percentage on this treatment was low - 6.0% and 7.3% - even with one or two applications, respectively.

Discussion

In these experiments, the insecticides chlorfenapyr and indoxacarb exhibited reliable results among the three years of experiments, with high mortality of *C. includens* larvae. Chlorfenapyr, an inhibitor (uncoupler) of oxidative phosphorylation disrupting the proton gradient (IRAC MoA group 13), kept the number of large and small larvae at low levels during all evaluation dates. Moreover, chlorfenapyr had low defoliation percentage with only one spray in 2016. The insecticide indoxacarb, a sodium channel blocker insecticide (SCBI) (IRAC MoA sub-group 22A), had high mortality of small and large larvae of *C. includens* in 2014, 2015 and 2016 (Table 1). Also, indoxacarb decreased in the residual effect over time, especially in 2014 and 2016.
Early stages of *C. includens* appeared to be more susceptible to insecticides, since its population density was significantly reduced by a major number of insecticides than large larvae. The spinosyns has shown its major effect against early stages of *C. includens* larvae among all experiments, representing a suitable option to control soybean looper in soybeans at early infestations to control greater number of small larvae. Spinosyns are allosteric modulators of the nicotinic acetylcholine receptor (nAChR) (IRAC MoA group 5) and has high effectiveness to Lepidopteran pests (Thompson et al., 1995).
A satisfactory control of *C. includens* in Brazil is demanding and we have shown failure of most tested insecticides to control the insect. The number of insecticide applications to control arthropod pests in soybeans ranged from four to six per soybean season (Bortolotto et al., 2015), from which at least one or two are used to control soybean looper in non Bt soybean.
The low susceptibility of *C. includens* to some insecticides may be related to its capacity to detoxify insecticides as previously found (Dowd and Sparks, 1986; Rose et al., 1990). Recently, the high metabolic process of cytochrome P450, glutathione S-transferase, and esterase in a resistant population for pyrethroids has been detected in Brazil (Perini et al., 2018). This metabolic advantage might be related to the higher efficacy of chlorfenapyr and indoxacarb during the larvae stages. Chlorfenapyr is a broad-spectrum pro-insecticide activated by cytochrome P450, glutathione S-transferase, carboxylesterase, which activate the pro-insecticide with oxidative removal of the N-ethoxymethyl group of chlorfenapyr to form a toxic compound that uncouples oxidative phosphorylation at the mitochondria (Hunt and Treacy, 1998; Feyereisen, 2012). Indoxacarb also is a pro-insecticide bioactivated by enzymes that convert this compound to N-decarbomethoxyllated active metabolites, which are highly potent to block the voltage-gated sodium channel in the inactivated state (Wing et al., 1998).
Chlorfenapyr and indoxacarb were also reported to have high efficacy controlling *Helicoverpa armigera* on soybean in Brazil, where indoxacarb showed a low residual effect (Perini et al., 2016). Indeed, indoxacarb has a short period of residue due to its high photodegradation ratio (DT50 = 4.5 days; FAO), compared to chlorfenapyr, which is less soluble in water and has less photolysis along five to eight days (DT50 = 5-8 days; FAO). Thus, based on our results, a monitoring of *C. includens* should be taken after application of indoxacarb in soybean field to make a decision whether it is required to spray the second time or not.
The issue that make *C. includens* difficult to control by several chemical insecticides is related to its biology. The period from egg to 3rd instar larvae of *C. includes* takes about seven days (Moscardi et al., 2012) and these stages only scrape on the underside of leaves with little consumption (Bueno et al., 2007). At this point, we suggest that spinetoram had its major effect on causing mortality on early stages of *C. includens* larvae, because its translaminar activity is able to penetrate the leaf cuticle and to move into the leaf tissue, as previous reported (Shimokawatoko et al., 2012).
The major consumption activity of *C. includens* (97%) is from 4º to 6º instar and its development takes about 8 days (Reid and Greene, 1973), and consequently in this period it has the highest probability to get contaminated by insecticides. Thus, the insecticide needs a longer residual period (more than seven days) or additional applications after this interval to release the amount of chemicals available on soybean leaves, when the greatest consumption of large larvae begins (after 3rd instar). In 2014, 2015 and especially in 2016, we observed long residual effect on large larvae of *C. includens*, after application of chlorfenapyr treatment even with just one application (Figures 1 and 2).
The low efficacy of insecticides can lead to leaf injury and yield loss on soybean, as reported when leaf injury occurs during the reproductive stages (Reichert and Costa, 2003). Defoliation injury was reduced by treatments with one and two application including chlorfluazuron+acephate and chlorfenapyr; and with two applications, including spinetoram and indoxacarb (Figure 4). Interestingly, chlorfluazuron+acephate had low efficacy (Figures 1 and 2). but presented low defoliation percentage by *C. includens*. We suggest that the larvae did not die in this treatment because the insecticide probably caused feeding inhibition. The type of injury in looper larvae on soybean leaves, consuming only between veins, can result in water loss and reduction in photosynthetic efficacy, in addition of reduction

Table 1. Assessment of mean number of small and large larvae of *C. includens* among the experiments in 2014, 2015, and 2016 and corrected mortality, considering only treatments.

Experiment I / 2014	Small	Large	Corrected mortality	
			Small	Large
1. Untreated control	6.3 a†	10.9 a	-	-
2. Chlorantraniliprole	2.6 cd	8.9 ab	58.7	18.3
3. Flubendiamide	3.6 bc	8.9 ab	42.9	18.3
4. Indoxacarb	1.8 d	1.7 d	71.4	84.4
5. Chlorfenapyr	1.5 d	2.2 d	76.2	79.8
6. Spinosad	1.4 d	5.1 c	77.8	53.2
7. Chlorfluazuron + methomyl	3.9 bc	6.0 c	38.1	45.0
8. Methoxyfenozide	4.9 ab	9.7 a	22.2	11.0
9. λ-cyhalothrin + chlorantraniliprole	5.2 ab	11.2 a	17.5	-2.8
10. Chlorpyrifos	3.0 cd	4.9 c	52.4	55.0
Coefficient of variation (%)	62.2	44.1		
Experiment II / 2015	**Small**	**Large**	**Corrected mortality**	
			Small	Large
1. Untreated control	3.7 a	7.2 a	-	-
2. λ-cyhalothrin + chlorantraniliprole + diafenthiuron	1.9 bc	6.6 ab	48.6	8.3
3. Methoxyfenozide	1.5 bcd	3.6 d	59.5	50.0
4. Spinetoram	0.8 d	4.4 cd	78.4	38.9
5. Chlorfluazuron + acephate	1.6 bcd	6.1 ab	56.8	15.3
6. Chlorfenapyr	1.0 cd	2.0 e	73.0	72.2
7. Indoxacarb	1.2 bcd	3.8 d	67.6	47.2
8. Diflubenzuron + chlorpyrifos	1.3 bcd	5.6 bc	64.9	22.2
9. Flubendiamide + thiodicarb	2.1 b	5.8 b	43.2	19.4
Coefficient of variation (%)	69.5	31.9		
Experiment III / 2016	**Small**	**Large**	**Corrected mortality**	
			Small	Large
1. Untreated control	3.1 a	7.5 ab	-	-
2. λ-cyhalothrin + chlorantraniliprole + diafenthiuron	2.4 abc	8.4 a	22.6	-12.0
3. Methoxyfenozide	2.6 ab	7.6 ab	16.1	-1.3
4. Spinetoram	1.8 bcd	6.7 bc	41.9	10.7
5. Chlorfluazuron+acephate	2.2 abc	7.2 ab	29.0	4.0
6. Chlorfenapyr	1.2 bcd	2.6 d	61.3	65.3
7. Indoxacarb	1.1 d	2.7 d	64.5	64.0
8. Diflubenzuron+chlorpyrifos	1.5 cd	5.6 c	51.6	25.3
9. Flubendiamide+thiodicarb	2.9 a	6.3 cb	6.5	16.0
Coefficient of variation (%)	58.6	27.2		
Experiment IV / 2016	**Small**	**Large**	**Corrected mortality**	
			Small	Large
1. Untreated control	2.6 ²	4.4 ²	-	-
2. λ-cyhalothrin + chlorantraniliprole + diafenthiuron	3.2 a	5.6 a	-23.1	-27.3
3. Methoxyfenozide	2.6 ab	4.1 b	0.0	6.8
4. Spinetoram	1.5 bcd	3.6 bc	42.3	18.2
5. Chlorfluazuron+acephate	2.4 ab	3.4 bcd	7.7	22.7
6. Chlorfenapyr	0.5 d	0.7 f	80.8	84.1
7. Indoxacarb	1.7 bc	2.3 de	34.6	47.7
8. Diflubenzuron+chlorpyrifos	1.2 cd	1.7 ef	53.8	61.4
9. Flubendiamide+thiodicarb	1.9 bc	2.7 cde	26.9	38.6
Coefficient of variation (%)	81.8	60.5		

† Values followed by the same letter are not significantly different at *Pr* ≤ 0.05 according to Tukey's test.

Table 2. Cost of each insecticide treatment to control *C. includens* in soybeans based on its rates per hectare.

Chemical name	Trade name	g a.i. ha^{-1}	Cost (U\$ ha^{-1}) †
Chlorantraniliprole	Premio 200 SC	10	$7.69
Flubendiamide	Belt 480 SC	33.6	$11.00
Indoxacarb	Avatar 150 CE	60	$20.89
Chlorfenapyr	Pirate 240 SC	240	$31.85
Spinetoram	Exalt 120 SC	12	$19.58
Chlorfluazuron + methomyl	Atabron 50 CE + Lannate 215 SL	25+215	$12.79
Chlorfluazuron + acephate	Atabron 50 CE + Orthene 750 PS	25+750	$17.62
Methoxyfenozide	Intrepid 240 SC	96	$9.92
λ-cyhalothrin + chlorantraniliprole	Ampligo 50+100 SC	3.75+7.5	$8.22
λ-cyhalothrin + chlorantraniliprole + diafenthiuron	Ampligo 50+100 SC + Polo 500 SC	6+12+75	$13.16
Chlorpyrifos	Lorsban 480 EC	480	$15.14
Diflubenzuron + chlorpyrifos	Dimax 480 SC + Klorpan 480 EC	72+720	$23.97
Flubendiamide + thiodicarb	Belt 480 SC + Larvin 800 WG	38.4+200	$22.37

† Price survey of insecticides during 2018/2019 soybean season.

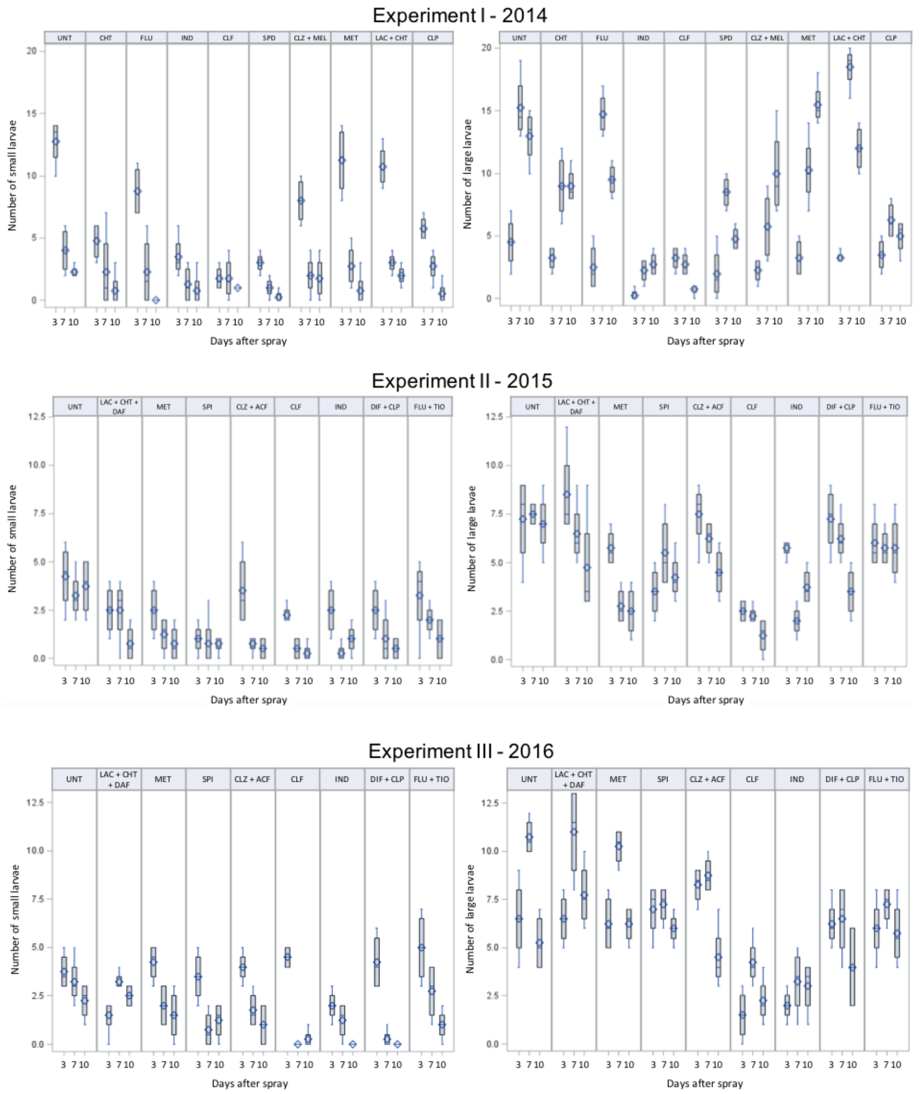

Fig 1. Assessment of small and large larvae of *C. includens* that survived after insecticide spray in 2014, 2015, and 2016.

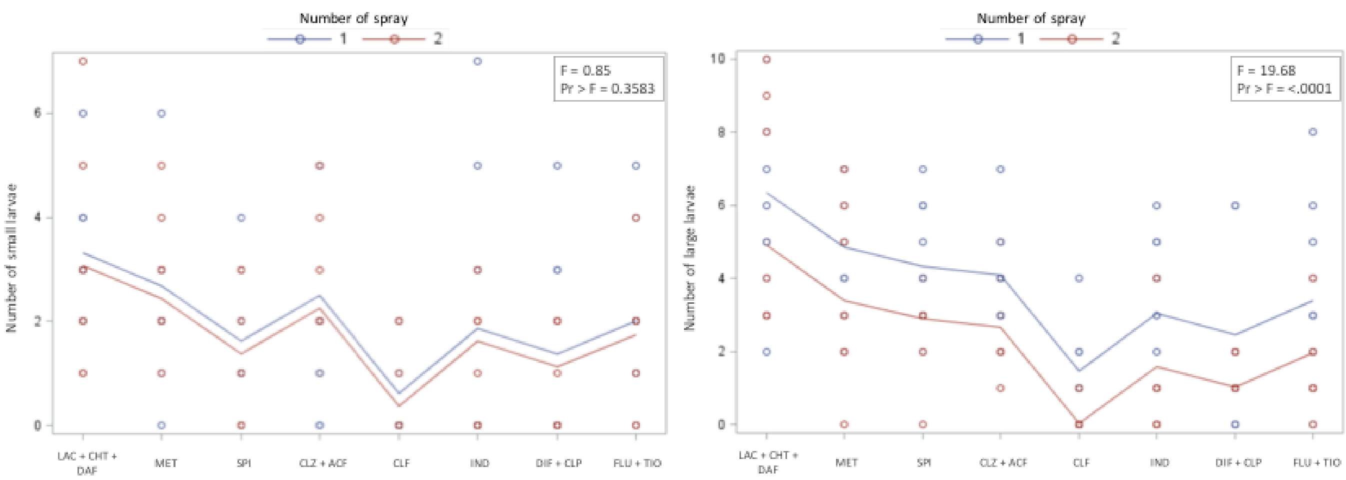

Fig 2. Significance comparison between one and two sprays for small and large larvae of *C. includens* in 2016.

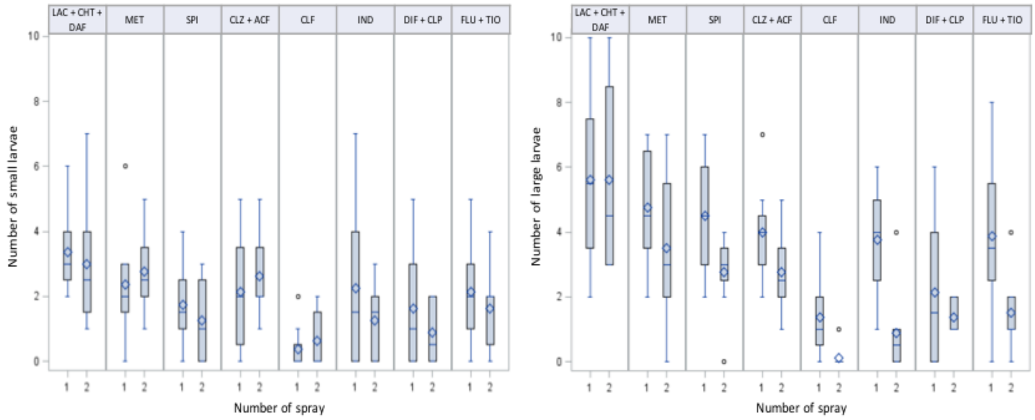

Fig 3. Comparison between one and two sprays of each insecticide over small and large larvae of *C. includens* in 2016.

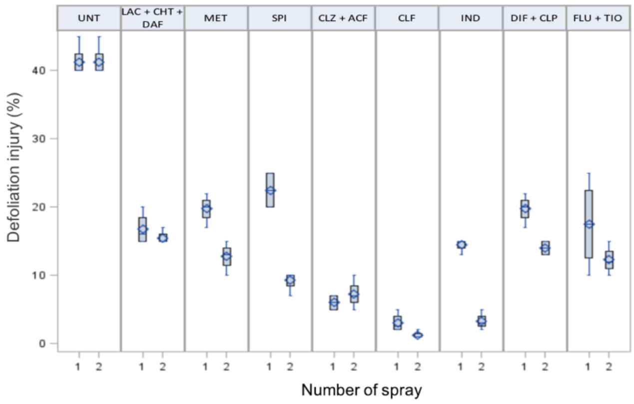

Fig 4. Defoliation injury among treatments between one and two sprays in 2016.

Fig 5. Location where experiments were performed in Santa Maria city, Rio Grande do Sul, Brazil.

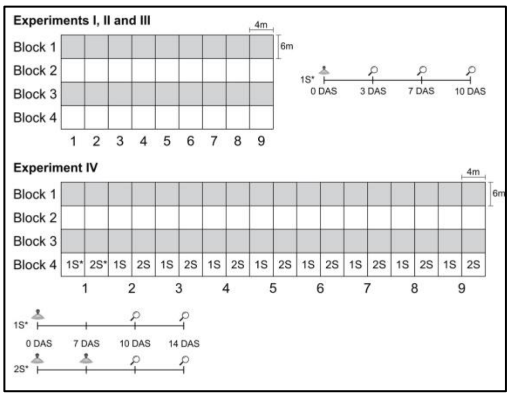

Fig 6. Experimental design how the trials were configured and evaluated in Santa Maria city, Rio Grande do Sul, Brazil. (1S: one spray; 2S: two sprays).

reduction of light interception and carbon assimilation as reported for cabbage looper with the same type of injury (Tang et al., 2006). Thus, low defoliation percentage helps to maintain the potential of soybean yield.

A relevant issue of looper management in soybean is the cost of insecticides, wherein the most effective insecticides were also the most expensive (Table 2). Thus, the usual decision of growers is to spray a cheaper insecticide first, leading to use those effective and expensive products just when the larvae density is very high. It is problematic because it might lead to fail controlling insect. However, in regards of our results, soybean growers should consider the tactics to control *C. includens* in an integrated pest management, regardless of costs. The consecutive use of only one strategy has a high risk to generate resistant caterpillars and the tactics to control this pest on soybean should consider not only the efficient chemical insecticides (chlorfenapyr and indoxacarb), but also biological insecticides and varieties of transgenic soybean that express Bt toxins.

Materials and methods

Plant genotype and soybean cultivation

The soybean variety cultivated in the experiments in Santa Maria, Rio Grande do Sul State, was BMX Potencia RR, which is widely used in Southern Brazil. Soybean was sown at a population density of 15 seeds linear m^{-1} with a spacing of 0.5 m between rows. We applied 250kg of nitrogen−phosphorus−potassium (NPK; 2-20-30) during the sowing. Seeds were treated with fipronil + pyraclostrobin + thiophanate-methyl (Standak®Top, FS, 250+25+225 g a.i. L^{-1}) at 50+5+45 g a.i. per 100 kg of seed. Glyphosate (Crucial, SL, 540 g L^{-1} of acid equivalent) was applied (1,35 g ha^{-1} of acid equivalent) at pre-sowing to aid as a desiccant and at post-emergence of soybean at V_3 stage of growth (third node on the main steam with fully developed leaves) for weed management. Further details of location (Figure 5), spraying dates on soybean growth stages and larvae density are presented (Supplementary Table 3).

Experimental design

Every field experiment (I / 2014, II / 2015, III / 2016, and IV / 2016) was accomplished with a natural infestation of *C. includens* on soybeans and scouting for population density prior to plots installation and insecticides application. When the population density of small (< 1.5 cm) and large (> 1.5 cm) larvae reached 10 larvae m^{-2} the experiments were performed (Table 1). We elected a location over soybeans area having the minimum of soil and insect heterogeneity. Experiments I / 2014, II / 2015, III / 2016 were carried in completely randomized block designs with four blocks and plot size of 8 rows of 6 meters (Figure 6). Insecticides and rate of each treatment for managing soybean looper was recommended by each company and are shown in Supplementary Table 4a-c. These insecticides are commonly used by growers to control larvae of *C. includens* and other caterpillar species on soybeans in Brazil. Commercial products were obtained from each company holder. As a consequence of some results in I / 2014 or insecticide disuse, some treatments were recommended to be changed by the companies for experiments in 2015 and 2016. Experiment IV / 2016 was arranged in completely randomized block design in an 8 x 2 factorial arrangement, with the same plot size, as previously described, and four blocks (Figure 6). Factor A was composed by eight insecticides (Supplementary Table 4d). Factor B was represented by one (1x) and two (2x) sprays. On all experiments, insecticides were applied with a pressurized-CO_2 backpack sprayer with a 4-m bar (the same plot width of 8 soybean rows) and 0.5-m nozzle spacing (XR 110.015 fan-type nozzle tips, Teejet Technologies Co., Glendale Heights, Illinois, USA). Application were performed during the morning period (9am-12pm) for better environmental conditions and a flow rate of 150 L ha^{-1} was used.

Evaluation of soybean looper on soybean

Larvae of *C. includens* was randomly collected during the experiment evaluations and identified at the Laboratory of Integrated Pest Management (LabMIP) at the Federal University of Santa Maria using the identification key of Eichlin (1975). The voucher specimens were deposited at LabMIP. Larvae mortality of soybean looper was assessed evaluating the number of alive larvae in each plot using a vertical beat cloth method (Drees and Rice, 1985; Guedes et al., 2006) in 2 meters of soybean row (area of sampling of 1.0 m^2). Larvae collected in the vertical beat cloth was separated in small (<1.5 cm) and large (>1.5 cm) in order to see the effect of insecticides in early and late stages of soybean looper. Evaluations were performed in order to understand the early and late effect of insecticides. For experiments I / 2014, II / 2015, and III / 2016 evaluations were done at 3, 7 and 10 days after spraying (DAS). For IV / 2016-experiment evaluations were accomplished at 3 and 7 days after second spray (DA2S). Damage on soybean leaves by natural infestation of soybean looper was evaluated visually and attributed damage rating to each plot in accordance with the Stewart scale (Stewart, 2014) at 7 days after the second spray on IV / 2016-experiment.

Statistical analyses

The number of small and large larvae of soybean looper of each experiment and the defoliation rate were subjected to one-way (type I SS) analysis of variance (ANOVA) using the General Linear Models (PROC GLM procedure) (SAS Institute, 2000). Insecticides, DAS, size of larvae, number of spray and its interaction were tested as variation sources prior to final analyses and representation of results. Because the significance of variation sources ($Pr \leq 0.05$): size of larvae, DAS, number of sprays, and treatments, these sources were used as categorical variables (Supplementary Table 1). Box plot for small and large larvae among evaluation dates and defoliation injury including the median, the 25th and 75th percentiles, and the minimum and maximum data values, in response to treatments, and number of sprays were generated. Abbott's formula was used to correct mortality of small and large larvae (Abbott, 1925).

Conclusion

Overall, few active ingredients have satisfactory efficacy to control small and large larvae of *C. includens* in soybeans, including indoxacarb and chlorfenapyr (both pro-insecticides), and spinosyns for small larvae. Moreover, insecticides with low residual effect increase larvae mortality and decrease damage by adding a second spray. However, the most effective treatments triggers high costs for control of soybean looper. Thus, the management of *C. includens* in soybean should consider the effectiveness and costs of these insecticides, the mode of action, and combination of tactics in an integrated pest management (IPM) and insecticide resistance management.

Acknowledgements

We thank all the people who helped during the experiments such as LabMIP-UFSM team and Prof Dr. Juan Luis Juarat-Fuentes for his helpful corrections and suggestions in the discussion of this manuscript. We also would like to thank the CAPES Foundation for the scholarship provided to the first author and Msc. Junior Mendes based on its assistance on statistical analysis.

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