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Chrysodeixis includens (Lepidoptera: Noctuidae) on soybean treated with resistance inducers

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Plant resistance levels can be boosted to enable better pest management. The objective of this study was to evaluate resistance interaction between four soybean genotypes and three inducers resistant to integrated management of *Chrysodeixis includens*. The assays were performed in the entomology laboratory. The soybean genotypes were seeded and plants were treated with calcium and magnesium silicate on soil and sodium silicate and Acibenzolar-S-methyl (ASM) spray on leaves. The weight, longevity and viability of the larvae, the duration and viability of the pre-pupal stage; the weight, longevity and viability of the pupae and the duration and viability of the egg to adult stage of *C. includens* were observed using plants leaves. The resistant cultivars IAC 100 and IAC 17 associated with the calcium and magnesium silicate inducers and sodium silicate lengthened larval stage and induced high mortality in the *C. includens* adults.

Key words: Insect, pest management, plant resistance to insects.

INTRODUCTION

Anticarsia gemmatalis Hübner and *Chrysodeixis includens* Walker (Lepidoptera: Noctuidae) are major pests in soybean in Brazil (Bueno et al., 2012; Mourão et al., 2014). The last species has been found to occur more frequently and in high populations in this crop (Bueno et al., 2012; Silva et al., 2012). These larvae feed on the leaves, leaving behind only the veins, which drastically reduces the leaf area, transpiration and photosynthesis of soybean plants and, consequently, the yield of this crop (Fortunato et al., 2004; Owen et al., 2013).

Chemical insecticides cause undesirable effects (Sosa-Gómez and Silva, 2010) which increases the reason to find effective alternative methods for pest control (Zanuncio et al., 2010; Fagundes et al., 2013; Souza et al., 2014). The alternative methods includes induction of plant resistance using silicon (Almeida et al., 2008, 2009; Lemes et al., 2011), plant resistance to insect, biological control or interaction between the various control methods (McPherson and Buss, 2007; Souza et al., 2012; Zanuncio et al., 2012; Jesus et al., 2014).

Inducers like silicon and Acibenzolar-S-methyl (ASM) can increase the resistance levels without in any way altering the plant genome (Lemes et al., 2011; Peixoto et al., 2011). The silicon increases the plant resistance stimulating growth and protecting plant against biotic and abiotic's stresses; rising silica mechanical barrier on the

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leaf tissues and trichomes as well producing phenolic defensive compounds (Lemes et al., 2011; Cruz et al., 2012). The ASM may activate the genes that encode resistance against biotic's agents (Goussain et al., 2002; Costa et al., 2007).

Resistance inducers have revealed promising results to other insect species on different crops. Silicon application reduce the number of nymphs on peanut plants (Dalastra et al., 2011) and reproduction and biological parameters of *Schizaphis graminum* Rondani (Hemiptera: Aphididae) in sorghum and wheat (Costa et al., 2007; Pereira et al., 2012).

Rhopalosiphum maidis Fitch (Hemiptera: Aphididae) showed a lower degree of preference for the corn plants treated with silicon (Moraes et al., 2005) and this treatment induced the non-preference mechanism to *Aphis gossypii* Glover (Hemiptera: Aphididae) in cotton plants (Alcantra et al., 2010). The calcium and magnesium silicate added, increased the number of trichomes in the resistant cultivars IAC 100 and IAC 17 and induced non-preference by *Euschistus heros* Fabricius (Hemiptera: Pentatomidae) to these cultivars (Souza et al., 2014).

Thus the objective of this work was to evaluate the interactions effects between resistance inducers and soybean cultivars on the biology of the Soybean Looper *Chrysodeixis includens*.

MATERIALS AND METHODS

The experiment was conducted in the Entomology Laboratory from Instituto Federal Goiano – Campus Urutaí, Goiás, Brasil with four soybean cultivars and three resistant inducers from September 2012 to July 2013. *C. includens* adults were collected and maintained in plastic cages (21.5 cm high and 14.5 cm diameter) with the BRS valiosa soybean cultivar for oviposition and the eggs of this insect were collected.

The *C. includens* ovipositions were daily removed and placed in plastic cups (16.5 × 5 cm) until caterpillar eclosion occurred and these were reared on an artificial diet (Greene et al., 1976). The *C. includens* adults which emerged were placed in plastic cages (21.5 cm hight and 14.5 cm to diameter) with 10% honey solution soaked in a cotton wad placed in PET type soft drink cover. *C. includens* rearing was maintained in an acclimatized room (25±2°C, 70±10% RH and 14 h photoperiod).

IAC 100 and IAC 17 (resistant), BRS Conquista (moderate resistant) and BRS Jatai (susceptible) seeds were treated with Captan fungicide and seeded in plastics pots (5 L) on an organic substrate (29) to obtain the leaves used in the experiments.

The treatments included spraying the plants with 0.3% ASM solution (T1) until draining; application of calcium and magnesium silicate on the soil (T2); 1% sodium silicate (T3); and distilled water (T4) 15 days after plant emergence. The leaves were collected after 30 days and transported to the laboratory to study the biology of insect pest.

Chrysodeixis includens on soybean cultivars treated with resistant inducers

This experiment was conducted using a completely randomized design in a factorial scheme (4 cultivars × 3 inducers + untreated) with 16 treatments and 10 replications. One Petri dish (6 cm diameter lined with moistened filter paper) constituted a replication. The apical leaves of each soybean cultivar per treatment were placed with one newly hatched caterpillar and the biological cycle of *C. includens* was followed. The leaves of each of the cultivars were daily changed.

The biological parameters evaluated included, a) larva stage: larval period and weight of the ten-day-old caterpillars; b) pupa stage: pupal period and weight of the 24-hour-old pupa and c) egg-to-adult period, longevity and the total cycle of *C. includens*.

Statistical analyses

Values from biological parameters were submitted to variance analysis (ANOVA) as well as to the F-test. Means values were compared with the Tukey test at 5% probability employing the Sisvar software (Ferreira, 2011).

RESULTS AND DISCUSSION

The larval, pupal periods, weights, adults longevity and development period to egg and to adult of *C. includens* differed among the soybean cultivars tested. The resistance inducers were observed to affect the pupal period, weight and development period of this insect (Table 1).

C. includens larval and pupal weights were lower (98.10 and 55.70 mg, respectively) when feed to IAC 100 cultivar, and also larval period was longer (15 days). This genotype is considered highly resistant and less damaged by *Spodoptera eridania* Smith (Lepidoptera: Noctuidae) and the stink bug *E. heros* (Souza et al., 2012; Souza et al., 2014).

The negative impact of the IAC 100 cultivar on *C. includens* shows the indirect defense which may be due to increase production of the secondary compounds induced by the herbivory performed by the lepidopteran larvae (Piubelli et al., 2003; Li et al., 2004; Piubelli et al., 2005). These inducer compounds include the flavonoids, mainly rutin and the genistein, which can reduce the degree of feeding by the *C. includens* (Hoffmann-Campo et al., 2001).

The development period of *C. includens* was longer with the resistance inducer ASM (26.94 days), than with the other treatments, Ca+Mg silicate (19.75) and Na silicate (20.19 days). These results in the lengthened duration of the life cycle of *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) on the cucumber plants treated with the ASM (Correa et al., 2005).

The larval period (14.87 days) of *C. includens* was longer on the BRS Jatai (susceptible) with the resistance inducer ASM than that observed in the IAC 17 and BRS Conquista (Table 2). This demonstrates the resistance induction by the ASM in plants (Goussain et al., 2002; Costa et al., 2007).

Significant interaction was observed between inducers and IAC 17 cultivar. The calcium and magnesium silicates lengthened the larval stage of *C. includes*; the phase was completed in 15.50 days and 2.6 days

Table 1. Larval period (days), larval weight (mg), pupal period (days), pupal weight (mg), adult longevity (days) and egg-to-adult period (days) of *Chrysodeixis includens* (Lepidoptera: Noctuidae) in soybean cultivars treated with the resistance inducers ASM, Ca+Mg silicate, Na silicate and untreated (25°C, 70% RH and 14h photoperiod).

Cultivars (C)[1]	Lar. Per.[2]	Lar. Wei.	Pup. Per.	Pup. Wei.	Long.	eg-Ad.
BRS Jataí	13.62^{ab}	144.00^{b}	6.33^{a}	126.90^{b}	3.75a^{b}	27.12^{a}
BRS Conquista	13.47^{b}	166.60^{a}	6.62^{a}	149.20^{a}	4.06^{a}	27.00^{b}
IAC 17	13.84^{ab}	154.30^{ab}	5.96^{a}	141.90^{a}	3.00^{b}	26.19^{a}
IAC 100	15.00^{a}	98.10^{c}	3.92^{b}	55.70^{c}	0.44^{c}	6.69^{b}
F (C)	3.28*	24.41**	17.12**	157.25**	52.61**	107.45**
Inducers (I)						
ASM	13.37^{a}	151.60^{a}	6.67^{a}	139.60^{a}	3.12^{a}	26.93^{a}
Ca+Mg silicate	14.50^{a}	132.80^{a}	6.21^{a}	127.30^{a}	2.50^{a}	19.75^{b}
Na silicate	14.00^{a}	133.40^{a}	4.87^{b}	99.70^{b}	2.50^{a}	20.19^{b}
Untreated	14.06^{a}	145.20^{a}	5.08^{b}	107.10^{b}	3.12^{a}	20.12^{b}
F (I)	1.46^{ns}	2.32^{ns}	8.57**	28.72**	2.53^{ns}	119.66**
F (C x I)	2.14*	2.96**	6.62**	26.00**	5.15**	115.31**
C.V. (%)	15.50	24.41	25.41	11.55	32.25	5.82

[1]Means followed by the same lower-case letter do not differ significantly according to Tukey's test at 0.05 probability. **Significant at 1% probability. *Significant at 5% probability. [NS]Not significant. [2]Lar. Per, Larval period; Lar. Wei, larval weight; Pup. Per, pupal period; Pup. Wei, pupal weight; Long, adult longevity; eg-Ad, egg-to-adult period.

Table 2. Larval period (days) and larval weight (mg) of *Chrysodeixis includens* (Lepidoptera: Noctuidae) in soybean cultivars treated with the resistance inducers ASM, Ca+Mg silicate, Na silicate and untreated (25ºC, 70% RH and 14 h photoperiod).

Cultivars (C)[1]	Inducers (I)				F (C)
	ASM	Ca + Mg	Na	Untreated.	
Larval period (days)					
BRS Jataí	14.87^{aA}	13.00^{aB}	13.12^{aA}	13.50^{aAB}	1.26^{ns}
BRS Conquista	12.12^{aB}	13.50^{aAB}	14.12^{aA}	14.12^{aAB}	1.51^{ns}
IAC 17	12.50^{bB}	15.50^{aAB}	14.50^{abA}	12.87^{abB}	3.36*
IAC 100	14.00^{aAB}	16.00^{aA}	14.25^{aA}	15.75^{aA}	1.77^{ns}
F (I)	2.82*	3.69*	0.62^{ns}	2.60*	–
Larval weight (mg)					
BRS Jataí	119.80 b C	169.00 a A	147.50 ab A	139.80 ab AB	2.82*
BRS Conquista	181.00 a A	152.70 a A	165.30 a A	167.60 a A	0.91^{ns}
IAC 17	172.90 a AB	134.40 a A	145.10 a A	164.80 a A	2.13^{ns}
IAC 100	132.90 a BC	75.20 b B	75.70 b B	108.80 ab B	5.35**
F (I)	6.09**	11.44**	10.66**	5.11**	–

[1]Means followed by the same lower-case letter do not differ significantly according to Tukey's test at 0.05 probability. **Significant at 1% probability. *Significant at 5% probability. [NS]Not significant.

longer than that seen in the control, a fact that could be explained by the higher lignin content of the soybean genotype (Moraes et al., 2009) or by the lower degree of feeding by the *S. frugiperda* caterpillars on corn due to the mechanical barrier caused by the silicon accumulation (Goussain et al., 2002).

The effect of the ASM inducer ranged amongst the cultivars. *C. includens* larvae had less weight when feed on the BRS Jatai (119.8 mg), showing the resistance being induced in the susceptible cultivar (Table 2). On the other hand, the larvae weight was higher on the BRS Conquista (moderate resistance) (181.0 mg). This difference regarding larvae weight shows that the ASM can induce resistance against the biotic agents, as

Table 3. Pupal period (days) and pupal weight (mg) of *Chrysodeixis includens* (Lepidoptera: Noctuidae) in soybean cultivars treated with the resistance inducers ASM, Ca+Mg silicate, Na silicate and untreated (25ºC, 70% RH and 14h photoperiod).

Cultivars (C)[1]	Inducers (I)				F (C)
	ASM	Ca + Mg	Na	Untreated	
Pupal period (days)					
BRS Jataí	6.00^{aA}	6.50^{aA}	6.50^{aAB}	6.33^{aA}	0.16^{ns}
BRS Conquista	6.67^{aA}	6.00^{aA}	7.00^{aA}	6.83^{aA}	0.55^{ns}
IAC 17	6.67^{abA}	7.00^{aA}	4.50^{bB}	5.67^{abA}	3.61*
IAC 100	7.33^{aA}	5.33^{aA}	1.50^{bC}	1.50^{bB}	24.11**
F (I)	0.85^{ns}	1.44^{ns}	17.76**	16.92**	–
Pupal weight (mg)					
BRS Jataí	115.80^{aC}	136.30^{aA}	123.40^{aA}	132.10^{aB}	1.79^{ns}
BRS Conquista	143.80^{aAB}	140.80^{aA}	146.80^{aA}	165.40^{aB}	2.63^{ns}
IAC 17	163.20^{aA}	145.10^{abA}	128.60^{bA}	130.80^{bB}	5.44**
IAC 100	135.70^{aBC}	86.90^{bB}	0.00^{cB}	0.00^{cC}	96.84**
F (I)	8.26**	15.79**	96.67**	114.51**	–

[1]Means followed by the same lower-case letter do not differ significantly according to Tukey's test at 0.05 probability. **Significant at 1% probability. *Significant at 5% probability. ^{NS}Not significant.

reported for *S. frugiperda* on corn and *S. graminum* on wheat (Goussain et al., 2002; Costa et al., 2007).

The weight of the *C. includens* larvae was lower on the IAC 100 cultivar with the use of the calcium, magnesium and sodium resistance inducers (75.2 and 75.7 mg). The lower weight of this caterpillar with the silicon inducers can be explained by the Si deposition in the leaf cell walls, forming a mechanical barrier, thus increasing the hardness of the leaf tissues and the wearing out of the caterpillar mandibles, thereby reducing the degree of feeding.

The cultivars showed significant interactions without the use of the resistance inducers (untreated). The *C. includens* caterpillars were heavier (167.6 mg) on the BRS Conquista cultivar with the use of the IAC 100 (108.8 mg). This is similar to the resistance recorded in the IAC 100 cultivar to insects such as *C. includens* and stink bugs (McPherson and Buss, 2007; Souza et al., 2014).

The pupal period of *C. includens* was shorter on the IAC 17 treated with the calcium and magnesium silicate resistance inducer (7.0 days) than with the treatment with sodium silicate (4.50 days) (Table 3). This may be related to the length of the larval stage because each one depends on the earlier one to obtain, synthesize and accumulate the nutritional substances (Fugi et al., 2005). The resistance inducer, sodium silicate, increased the pupal period of *C. includens* on the BRS Conquista (7.0 days) compared with those on the IAC 100 and IAC 17 (4.5 and 1.5 days), respectively. The shorter pupal period of *C. includens* on this latter cultivar shows the antibiosis type of resistance, as observed with the cultivars with all the inducers. Sodium silicate and the treatment without using the inducer (control) showed lower values, with 1.50 days for the pupal stage of this insect compared with the 7.33 days with the inducer ASM, prolonging the *C. includens* pupal stage.

The ASM inducer increased the pupal weight (163.5 mg) of the *C. includens* on the IAC 17 to a higher degree than in the other treatments, more than the IAC 100 (86.90 mg) treated with the calcium and magnesium silicate and the BRS Jatai (115.8mg) (Table 3). This may be related to the increased resistance of the susceptible genotype with the resistance inducer (Costa et al., 2007; Souza et al., 2014).

The *C. includens* larval mortality reached 100% with the use of the inducer sodium silicate; however, in the control it prevented the pupal weight from being recorded with the IAC 100 genotype. Such mortality revealed by the resistant cultivar IAC 100 is explained by the flavonoid rutin and the genistein isoflavones linked to the anti-nutritional effect and injury to cells of the digestive tract as shown for *A. gemmatalis* fed on a diet containing these metabolites (Hoffmann-Campo et al., 2001; Salvador et al., 2010).

The increased longevity of *C. includens* adults on the BRS Conquista without the use of the resistance inducers shows a lower impact on the IAC 17 treated with the sodium silicate on the adults of this insect (Table 4). The mortality of the insects on the IAC 100 cultivar with the use of the calcium and magnesium silicate and sodium silicate inducers and in the control disallowed the evaluation of the longevity of this insect and thus corroborates the beneficial effect of the interaction of the resistant plants with the inducers to control the spread of *E. heros* on soybean (Souza et al., 2014).

Table 4. Adult longevity (days) and egg-to-adult period (days) of *Chrysodeixis includens* (Lepidoptera: Noctuidae) in soybean cultivars treated with the resistance inducers ASM, Ca+Mg silicate, Na silicate and untreated (25ºC, 70% RH and 14h photoperiod).

Cultivars (C)[1]	Inducers (I)				F (C)
	ASM	Ca + Mg	Na	Test.	
Adult longevity (days)					
BRS Jataí	2.50 c BC	3.25 bc A	5.00 a A	4.25 ab AB	5.87**
BRS Conquista	4.50 ab A	3.25 b A	3.50 ab A	5.00 a A	3.32*
IAC 17	3.75 a AB	3.50 a A	1.50 b B	3.25 a B	5.06**
IAC 100	1.75 a C	0.00 b B	0.00 b B	0.00 b C	3.72*
F (I)	7.39**	13.57**	23.49**	23.59**	–
Cycle egg-to-adult period (days)					
BRS Jataí	28.00 a A	26.00 a B	28.00 a A	26.50 a AB	2.65^{ns}
BRS Conquista	26.50 ab A	24.50 b B	28.35 a A	28.75 a A	9.25**
IAC 17	26.50 ab A	28.50 a A	24.50 b B	25.25 b B	7.62**
IAC 100	26.75 a A	0.00 b C	0.00 b C	0.00 b C	46.06**
F (I)	1.29^{ns}	439.06**	458.94**	454.08**	–

[1]Means followed by the same lower-case letter do not differ significantly according to Tukey's test at 0.05 probability. **Significant at 1% probability. *Significant at 5% probability. ^{NS}Not significant.

The lengthened time for the total cycle of *C. includens* on the IAC 17 cultivar with the calcium and magnesium silicate (28.50 days) than in the control (25.25 days) indicates an adverse effect on the biology of this insect, thus characterizing the antibiosis (Paixão et al., 2013). This may be related to the protective layer formed by the accumulation of silicon on the epidermal cells, which inhibits feeding, thus impacting the life cycle of this insect (Savant et al., 1997; Datnoff et al., 1991).

The IAC 100 cultivar treated with the resistance inducers calcium and magnesium silicate and sodium silicate as well as without them (untreated) increased the length of the larval stage of *C. includens*. This indicates that this effect is caused by the anti-nutritional compounds and induction of the secondary defense metabolites in these plants. The ASM did not prevent this insect from completing its cycle on this genotype.

Conclusion

The resistant cultivars IAC 100 and IAC 17 associated with the calcium and magnesium silicate inducers and sodium silicate lengthened the larval stage and induced high mortality in the *C. includens* adults.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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