

# Kaolin spraying protects cotton plants against damages by boll weevil *Anthonomus grandis* Boheman (Coleoptera: Curculionidae)

C. A. D. Silva · F. S. Ramalho

Received: 24 September 2012 / Accepted: 8 February 2013 / Published online: 19 February 2013  
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**Abstract** The high cost of insecticides, the emergence of insecticide resistance in populations of a number of insect species and other problems arising from their continuous use, such as biological imbalance, cotton fiber waste, and environmental pollution, have prompted the development of new technologies aiming the control of *Anthonomus grandis* Boheman in cotton crops. This study evaluated the level of protection conferred by kaolin clay foliar spraying to cotton plants against boll weevil damage. Treatment-tested spraying kaolin or endosulfan on cotton plants. The highest percentage of oviposition-punctured squares were observed in the control, and the lowest percentages in the treatments sprayed with endosulfan and kaolin in a systematic manner and where the boll weevil reached the economic threshold at all assessments. The greatest numbers of non-attacked bolls by weevils and cotton-seed yield were observed under the endosulfan treatments, followed by the treatments of kaolin spraying. The smallest number of bolls and lowest cotton-seed yield were observed for the control plots. These finding are of practical significance because they may reduce the cotton production cost and environmental impacts of chemical pesticides and make possible the production of organic cotton with the presence of boll weevils.

**Keywords** *Gossypium hirsutum* L · Insect pest · Alternative control · Pesticide reduction

## Introduction

The boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), is originally from the tropics and subtropics of Mesoamerica (Burke et al. 1986), but its modern-day distribution extends from temperate parts of the U.S. Cotton Belt to Brazil and Argentina (Ramalho and Jesus 1988; Ramalho and Wanderley 1996; Rummel and Summy 1997). This insect has a high capacity for survival, reproduction, and dispersal. These physiologic and behavioral aspects explain why the boll weevil has been responsible for severe economic losses to the Brazilian cotton industry, and why it is considered a major pest of this crop (Ramalho and Silva 1993). The losses caused by the boll weevil in the northern Brazil are both direct and indirect, and extend throughout the entire social, financial, and economic structure of the region (Ramalho and Wanderley 1996). An estimate of the magnitude of yield loss can be estimated from field studies in which boll weevil injury has been eliminated (Ramalho and Santos 1994). In the states of the Paraíba and Pernambuco, Brazil, where the boll weevil originally caused yield losses of 54–87 %, increases in yield of seed-cotton over control (untreated plots) varied from 116 to 657 % (Ramalho and Santos 1994). Estimates indicate that one hectare of cotton (*Gossypium hirsutum* L.) can produce more than 1.5 million (exclude of) boll weevil adults, and in high pest population levels, the (exclude current) control techniques are not economically viable (Showler 2003, El-Sayed et al. 2006). Therefore, during some periods of cotton cultivation, (excluded commercial) chemical insecticides are (excluded directly) applied to the cotton square to eliminate and protect the crop against losses caused by this pest (Ramalho 1994; Ramalho and Wanderley 1996; Page et al. 1999; Ramalho et al. 2003). However, the high price of these products, increase of resistant insect populations, and other

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Communicated by T. Zaviezo.

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C. A. D. Silva · F. S. Ramalho (✉)  
Biological Control Unit/Embrapa Algodão, Av. Osvaldo Cruz,  
1143, Campina Grande, Paraíba 58107-720, Brazil  
e-mail: ramalhohvv@globo.com

problems arising from their use, such as biological imbalance, cotton fiber waste and environmental pollution, have prompted the development of new technologies to control boll weevils (Ramalho and Wanderley 1996; Ramalho et al. 2003).

The technology of mineral particle films is a potential alternative to some insecticides in the control of different pests (Alavo and Abagli 2011). Kaolin is mineral composed of aluminum silicate ( $\text{Al}_4\text{Si}_4\text{O}_{10}(\text{OH})_8$ ) of fine grain, white color, flat, porous, and non-expanding, dissolving in water and chemically inert at a wide range of pH (Harben 1995). Preliminary studies have shown that kaolin has a deterrent effect on the feeding and oviposition behaviors of the boll weevil (Showler 2002), although the effectiveness of this product may be specific to some species of insects and must be studied for each pest in their own environment (Glenn and Puterka 2005). Kaolin has been shown to be effective against aphids (Cottrell et al. 2002; Wiss and Daniel 2004, Alavo and Abagli 2011); beetles (Showler 2002); fruit flies (Mazor and Erez 2004; Lo Verde et al. 2011), psyllids, leafhoppers and mites in various cropping systems (Puterka et al. 2000; Peng et al. 2011). The mechanisms of action of kaolin against (excluded targeted) insect pests include repellency, tactile or visual cue interference, impairment or disruption of oviposition and feeding activity, and decreased longevity and survivorship (Glenn and Puterka 2005). The abrasive mineral particles promote insect dissection due the disruption of their cuticle, obstruction of their digestive system, and also change the host plants color, affecting the recognition and attractiveness of the plant (Showler 2002).

Therefore, the use of kaolin might reduce the level of damage caused by boll weevils in the semiarid and Cerrado Savannah regions of Brazil where cotton is grown without much negative effects on the environment, because it is non-toxic to humans and relatively safe to natural enemies (Friedrich et al. 2003, Glenn and Puterka 2005, Marko et al. 2008).

This study aimed at assessing the level of protection conferred by kaolin to cotton plants against boll weevil.

## Materials and methods

### Experimental site and cotton cultivar

The study was conducted between January 31 and June 6, 2009, at an experimental farm of the “Embrapa Algodão”, in Barbalha municipality, State of Ceará, Brazil (latitude  $7^\circ 18' 40''$ , longitude  $39^\circ 18' 15''$ , elevation 414 m) in an area historically infested with boll weevils. An area of approximately one hectare was used to cultivate cotton (cultivar BRS Safira) under dry land conditions with two

plants per hill after thinning. This cotton cultivar has tender leaves with little fiber and was produced by crossing upland cotton introduced from the United States, which exhibits a dark brown colored fiber, with the CNPA 87–33 cultivar, which has good quality white lint. The rows were spaced 0.80 m apart with 0.20 m between plants in rows with two plants per hill. The number of cotton plants per plot was 1,000 plants.

The temperature ( $^\circ\text{C}$ ), relative humidity (%), and weekly rainfall (mm) during the assessment experiment were recorded by the National Institute of Meteorology (INMET) located in the Municipality of Barbalha, in the State of Ceará, Brazil. During this research the mean weekly temperature was  $24.9^\circ\text{C}$ , ranging from  $23$  to  $26.3^\circ\text{C}$ ; the mean weekly relative humidity was  $88.7\%$ , ranging from  $76$  to  $97\%$ ; and the mean weekly rainfall was  $11.1$  mm, ranging from  $0.6$  to  $19$  mm.

### Experimental design

The experimental design consisted of randomized blocks with five treatments and four replications. Treatment-tested spraying kaolin or endosulfan on cotton plants, as follows:  $T_1$ ) (excluded a) weekly systematic spraying with kaolin ( $60\text{ g l}^{-1}$  of water) after the emergence of the cotton plants at 7-day intervals;  $T_2$ ) spraying with kaolin ( $60\text{ g l}^{-1}$  of water) when the number of plants with oviposition-punctured squares reached a level of  $5\%$ ;  $T_3$ ) a weekly systematic spraying with endosulfan (Thiodan 35 EC,  $1.5\text{ g a.i. ha}^{-1}$ ) after the emergence of the cotton plants at 7-day intervals;  $T_4$ ) spraying with endosulfan (Thiodan 35 EC,  $1.5\text{ g a.i. ha}^{-1}$ ) when the number of plants with oviposition-punctured squares reached a level of  $10\%$  (Ramalho and Silva 1993), and  $T_5$ ) control (no spray). We utilize an economic threshold of  $5\%$  damaged squares for kaolin due to the fact that with this product applications need to occur weekly, and continuous good coverage is important for maintaining its effect (Showler 2002). Because this is not necessary for endosulfan, a  $10\%$  damaged squares threshold was used in this treatment (Ramalho and Silva 1993). Each experimental unit comprised 10 rows of cotton of  $10\text{ m}$  long, and  $5\text{ m}$  separating each experimental unit. The kaolin-based particle film used in this study was Potiguar wettable powder (Equador, RN, Brazil), here referred to as kaolin. A knapsack sprayer, with a capacity of  $20\text{ L}$  of spray and nozzle cone empty, was used. The spray nozzle was positioned laterally to the row and maintained at approximately  $20\text{ cm}$  from the plants (Ramalho and Jesus 1988). The flow rate was adjusted depending on the cotton crop growth stage, and varied between  $150$  and  $300\text{ L ha}^{-1}$ .

An outbreak of cotton leafworm, *Alabama argillacea* (Hübner) (Lepidoptera: Noctuidae) occurred at the seedling

stage, and was controlled by sprayings of *Bacillus thuringiensis* Berliner at the commercial dosage (16 g a.i. ha<sup>-1</sup>).

**Boll weevil damage**

Evaluations were performed weekly by the randomly surveying 30 cotton plants per plot. For each plant, one cotton square of median size, taken at random in the upper half of the plant (Ramalho and Jesus 1988), was examined for the presence or absence of oviposition puncture damage. The plant height, number of cotton bolls and cotton-seed yield were determined by observing 5, 6, and 12 plants at random from different rows per experimental unit, respectively.

**Analysis of data**

The effects of kaolin and insecticide sprays on (1) plant height, (2) the mean percentage of oviposited squares (i.e., squares that had oviposition marks), and (3) number of non-attacked cotton bolls were subjected to two-way analysis of variance (ANOVA) [treatment and plant age], using PROC GLM of SAS (SAS Institute 2006). We use the mean of the measurements for each experimental unit in the analyses. The proportions of oviposition-punctured squares made by boll weevil were transformed in arcsine square root before repeated measures analyses; however, untransformed means are presented. The number of non-attacked bolls per cotton plant and cotton-seed yield (kg ha<sup>-1</sup>) were regressed against percentage of oviposition-punctured squares by boll weevil using PROC REG of SAS (SAS Institute 2006).

**Results**

The emergence of the cotton plants, the appearances of the first square, and boll opening occurred at 8, 31, and 73 days after planting, respectively.

The two-way ANOVA revealed a significant effect of treatment, plant age, and interaction between treatment and plant age ( $P < 0.0001$  for all; Table 1) for percentages of oviposition-punctured squares by boll weevil.

The percentages of oviposition-punctured squares by boll weevil differed significantly between the treatments at the sixth, seventh, and eighth assessments (Table 2). The highest percentage of oviposition-punctured squares were observed in the control, and the lowest percentages in the treatments sprayed with endosulfan and kaolin in a systematic manner and where the boll weevil reached the economic threshold at all assessments (Table 2). When sprayed consistently, kaolin showed a higher percentage of oviposition-punctured squares (27.5 %) than the obtained after treatment with endosulfan sprayed systematically (5.8 %) at the plants 80 days old, but the percentages did not differ from those obtained at the plants 66 and 73 days old (Table 2).

The number of non-attacked bolls per plant 94 days old ( $F_{(4, 12)} = 83.26, P < 0.0001$ ) (Fig. 1a), plant height at 94 days old ( $F_{(4, 12)} = 2.26, P < 0.0590$ ) (Fig. 1b), and cotton-seed yield per hectare ( $F_{(4, 12)} = 18.92; P < 0.0001$ ) (Fig. 2) differed among the tested treatments. The tallest cotton plants were in the control, which differed significantly from those weekly sprayed with endosulfan. Both kaolin treatments and endosulfan at 10 % threshold had intermediate plant heights (Fig. 1b). In contrast, the shortest plants were observed under the systematic spraying with endosulfan (Fig. 1b). The greatest number of non-attacked bolls by weevils (Fig. 1a) and cotton-seed yield (Fig. 2) were observed in the endosulfan treatments, followed by the kaolin treatments. The smallest number of bolls and the lowest cotton-seed yield were observed for the control plots. In the treated plots with kaolin and endosulfan (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>), the cotton-seed yields were 3.88, 3.03, 2.37, and 2.49 larger than the control cotton plot.

Linear models were the best fits to represent the relationship between cotton-seed yield (kg ha<sup>-1</sup>) ( $y = 3079.32 - 67.77x, F_{(1,3)} = 50.16, R^2 = 0.94, P < 0.0058$ ) (Fig. 3a) or number of non-attacked bolls ( $n \text{ plant}^{-1}$ ) ( $y = 9.99 - 0.22x, F_{(1, 3)} = 45.82, R^2 = 0.95, P < 0.0066$ ) (Fig. 3b) as a function of the percentage of oviposition-punctured squares by boll weevil. This mean that 94 and 95 % of the variation in the cotton-seed yield and number of non-attacked bolls, respectively, was due to the percentage of oviposition-punctured squares by boll weevil.

**Table 1** Summarized model of two-way analysis of variance (ANOVA) for the effects of treatment and plant age on the percentage of cotton plants with oviposition-punctured squares by boll weevil in cotton plots

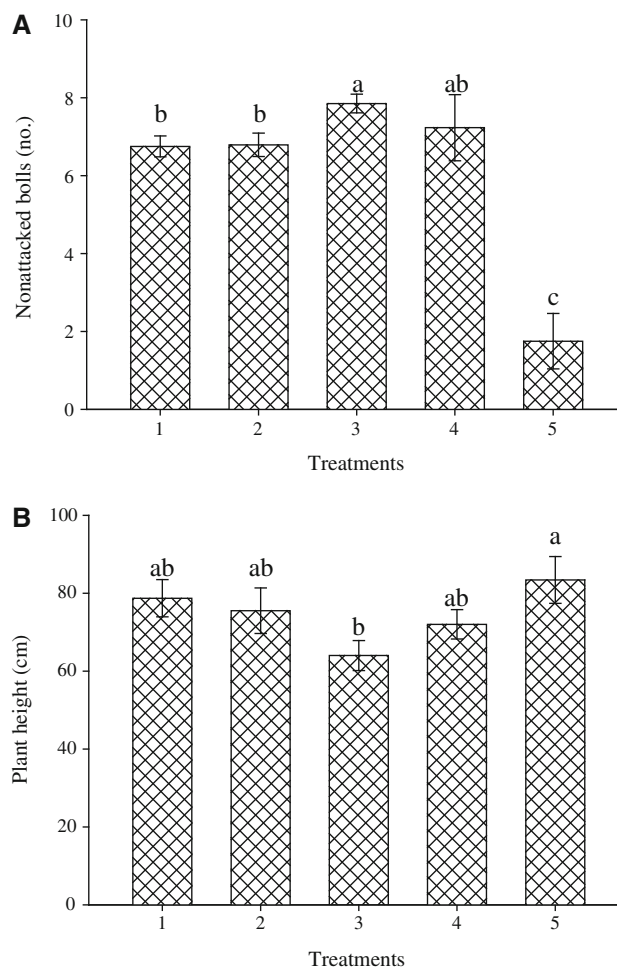
Source	Models	DF	F ratio	Prob > F
Oviposition-punctured squares plant <sup>-1</sup>	Model	42	12.92	0.0001
	Treatment (T)	4	15.34	0.0001
	Plant age (Pa)	7	52.27	0.0001
	T × Pa	28	3.74	0.0001

Treatments: T<sub>1</sub>, kaolin weekly after cotton emergence; T<sub>2</sub>, kaolin weekly from 5 % oviposition-punctured squares; T<sub>3</sub>, endosulfan weekly after cotton emergence; T<sub>4</sub>, endosulfan weekly from 10 % oviposition-punctured squares, and T<sub>5</sub>, control (no spray)

**Table 2** Mean ( $\pm$ SE) percentage of oviposition-punctured squares by boll weevil in treated and non-treated cotton plots through time

Treatments <sup>a</sup>	Plant age (day since emergence)										80	87	94	101	
	31	38	45	52	59	66	73	80	87	94					
2	0.00 $\pm$ 0.00aB	0.00 $\pm$ 0.00aB	0.00 $\pm$ 0.00aB	2.50 $\pm$ 1.60aB	15.83 $\pm$ 4.98bA	15.84 $\pm$ 5.16bcdA	16.67 $\pm$ 3.04bA	27.50 $\pm$ 4.59bA	–	–	–	–	–	–	–
3	0.00 $\pm$ 0.00aB	0.00 $\pm$ 0.00aB	0.00 $\pm$ 0.00aB	14.16 $\pm$ 8.43aA	16.67 $\pm$ 5.77bA	26.67 $\pm$ 13.40bcA	15.83 $\pm$ 6.85bA	20.82 $\pm$ 9.27bA	–	–	–	–	–	–	–
4	0.00 $\pm$ 0.00aC	0.00 $\pm$ 0.00aC	0.00 $\pm$ 0.00aC	0.84 $\pm$ 0.08aBC	15.00 $\pm$ 3.19bABC	3.33 $\pm$ 1.36dABC	8.33 $\pm$ 3.19bABC	5.83 $\pm$ 3.44cABC	–	–	–	–	–	–	–
5	0.00 $\pm$ 0.00aC	0.00 $\pm$ 0.00aC	0.00 $\pm$ 0.00aC	5.83 $\pm$ 5.22aBC	35.00 $\pm$ 7.33abA	5.83 $\pm$ 3.54cdBC	13.33 $\pm$ 3.33bBC	5.84 $\pm$ 2.97cBC	–	–	–	–	–	–	–
5	0.00 $\pm$ 0.00aD	0.00 $\pm$ 0.00aD	0.00 $\pm$ 0.00aD	7.50 $\pm$ 3.44aCD	23.33 $\pm$ 4.91abB	38.33 $\pm$ 5.53aAB	50.83 $\pm$ 7.62aAB	60.00 $\pm$ 9.43aAB	–	–	–	–	–	–	–
									1 <sup>st</sup> squares					Less than five	First open bolls
															Non-attacked Squares/plant

<sup>a</sup> Treatments: T<sub>1</sub>, kaolin weekly after cotton emergence; T<sub>2</sub>, kaolin weekly after cotton emergence; T<sub>3</sub>, endosulfan weekly after cotton emergence; T<sub>4</sub>, endosulfan weekly from 10 % oviposition-punctured squares, and T<sub>5</sub>, control (no spray). Means with the same upper case letter within rows and means with a common lower case letter within columns do not differ significantly using the student–Newman–Keuls test ( $P = 0.05$ )

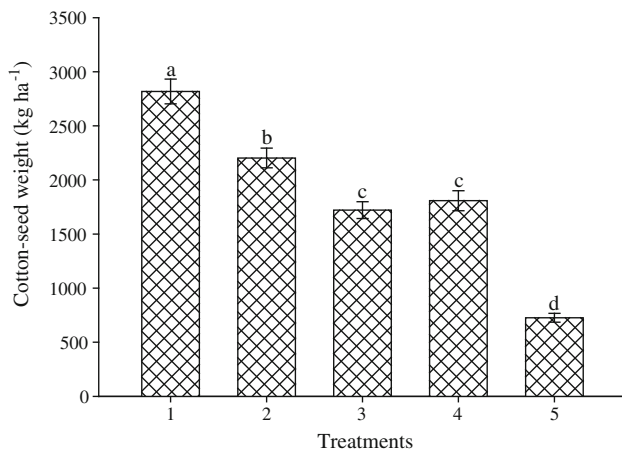


**Fig. 1** **a** non-attacked bolls by boll weevil (mean no. per plant ( $\pm$ SE) and **b** mean ( $\pm$ SE) plant height (cm) of cotton plants and in the following treatments: T<sub>1</sub>, kaolin weekly after cotton emergence; T<sub>2</sub>, kaolin weekly from 5 % oviposition-punctured squares; T<sub>3</sub>, endosulfan weekly after cotton emergence; T<sub>4</sub>, endosulfan weekly from 10 % oviposition-punctured squares, and T<sub>5</sub>, control (no spray). Means with the same upper case letter within rows and means with a common lower case letter within columns do not differ significantly using the student–Newman–Keuls test ( $P = 0.05$ )

## Discussion

Climatic conditions can affect boll weevil populations and the persistence of kaolin particles on plants (Showler 2007). The survival of insects in cotton agro ecosystems is influenced by high temperature and low humidity (Ramalho and Silva, 1993), and the persistence of kaolin particles is also reduced in environments that experience high levels of precipitation (Lo Verde et al. 2011). In our study, the temperature, relative humidity and weekly rainfall were favorable to the pest population development and not hampered spraying with kaolin.

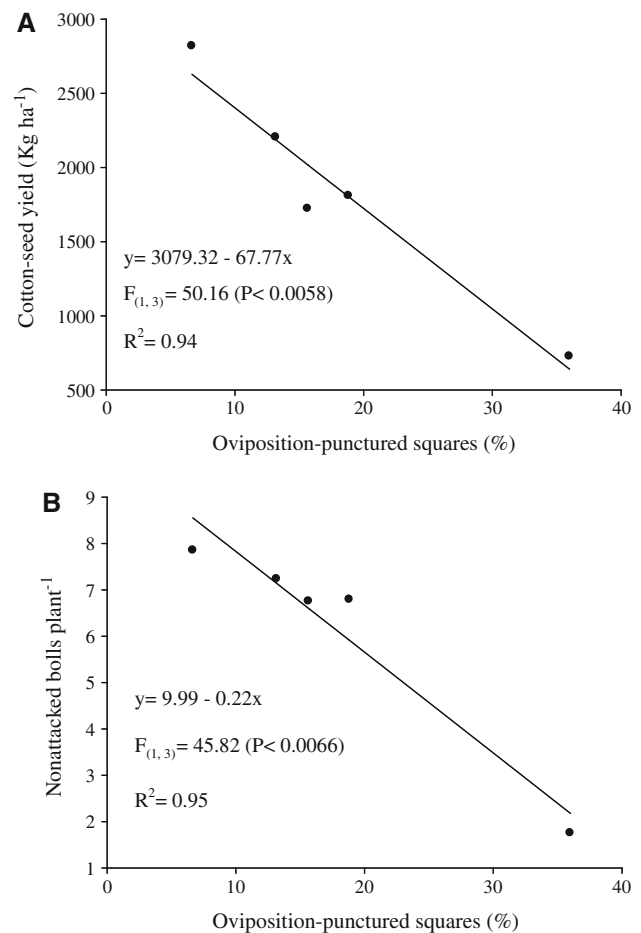
The tallest plants, lower number of bolls and lowest cotton-seed yield of the control plot may be attributed to the higher percentage of oviposition-punctured squares



**Fig. 2** Mean ( $\pm$ SE) cotton-seed yield ( $\text{kg ha}^{-1}$ ) in the followings treatments: T<sub>1</sub>, kaolin weekly after cotton emergence; T<sub>2</sub>, kaolin weekly from 5 % oviposition-punctured squares; T<sub>3</sub>, endosulfan weekly after cotton emergence; T<sub>4</sub>, endosulfan weekly from 10 % oviposition-punctured squares, and T<sub>5</sub>, control (no spray). Bars capped with different letters are significantly different ( $P = 0.05$ ) by Student–Newman–Keuls test

observed in this treatment. On the other hand, the smaller plant height, larger number of bolls, and higher cotton-seed yields of the cotton plots sprayed systematically with endosulfan, either weekly or from the 10 % economic threshold, can be attributed to the lower percentage of oviposition-punctured squares observed in these treatments. There has been found a negative relationship describing cotton-seed yield and non-attacked bolls as a function of oviposition-punctured squares by boll weevil (Bever and Slosser 1992, Slosser 1993, Showler et al. 2005).

Irrespective of the tested treatment, the mean percentage of oviposition-punctured squares by *A. grandis* varied with age of the plants (Table 2). However, the mean percentage of oviposition-punctured squares found for the treatments sprayed with kaolin and endosulfan, both systematically or where the boll weevil reached the economic threshold, differed from the control plot at the last four assessments (Table 2). Plants were smaller, while the mean numbers of bolls and cotton-seed yield were higher with endosulfan treatments. We believe that these results can be attributed to the different modes of action of tested products. Cyclodiene, such as endosulfan, are antagonists of the neurotransmitter gamma-aminobutyric acid (GABA), inducing the absorption of chloride ions by neurons, promoting their blockage and uncontrolled excitement, and as consequence death of the insect (Klaassen and Watkins 1999). On the other hand, the kaolin particle film shows a repellent effect, reducing feeding and oviposition on insects, without causing their death (Showler 2002). Although the real modes of action of kaolin particles on insects are not fully understood,



**Fig. 3 a** Cotton-seed yield ( $\text{kg ha}^{-1}$ ) and **b** non-attacked bolls ( $n \text{ plant}^{-1}$ ) as a function of the oviposition-punctured squares (%) by boll weevil

the effects appear to be caused by the color (white), which repels the insects from landing (Liang and Liu 2002), and disruption of feeding and oviposition (Liang and Liu 2002, Liu and Trumble 2004).

The lowest percentage of oviposition-punctured squares, the largest number of bolls and cotton-seed yields in the plots treated with kaolin found in our study indicates that possibly plant color plays an important role in guiding the boll weevils for feeding and egg-laying in cotton. A similar effect was found in experiments with the cotton cultivar “Deltapine-50” sprayed with kaolin at the Kika de la Garza Subtropical Agricultural Center (Showler 2002). As kaolin has a repellent effect as the main mode of action (Glenn and Puterka 2005), the boll weevils appeared to prefer the cotton plants with green leaves (without kaolin) for their feeding and oviposition. Fecundated female weevils first move toward the buds without kaolin, and after contact feeding and oviposition (Showler 2002) stimulated by cotton plant volatiles (Grodowitz et al. 1992) and aggregation pheromone (Hardee et al. 1969). However, if the boll weevil contacts the cotton

square treated with kaolin, the feeding and oviposition behaviors will not be arrested (Showler 2002), indicating that the protection conferred by kaolin can be reduced in the absence of choice.

The percentages of oviposition-punctured squares, number of bolls and cotton-seed yield did not differ between the treatments sprayed with kaolin in a systematic manner and after the boll weevil reached the 5 % damaged squares economic threshold.

These results are of practical significance because they may reduce the cotton production cost and environmental impacts of chemical pesticides, making possible the production of organic cotton with the presence of boll weevils. However, there is the possibility that by spraying kaolin to entire field, at high boll weevil populations, the level of effectiveness of this approach may be reduced given the need of the female weevils to oviposit. The mechanism of action of this material is based on creating a kaolin-based particle barrier on the plant surface, so understanding how it affects the behavior and biology of the boll weevil is important in developing an effective deployment strategy for kaolin particle film against this pest.

The fact that yield was not only affected but also indicates that this product probably does not affect the photosynthetic capacity of the cotton plants. The kaolin particle film may contribute to the physiologic balance of the plant due to the reflective properties, which can reduce heat stress, protecting against sunburn (Glenn et al. 2003), and increase photosynthesis by reducing the plant temperature, resulting in increases in size and yield (Glenn et al. 1999; Thomas et al. 2004; Glenn and Puterka 2005; Lapointe et al. 2006).

Other advantages of kaolin particle film are the difficult of pests develop resistance to it (Liu and Trumble 2004); does not show phytotoxic effects, and lasts longer than most insecticides on the plants when it does not rain or there is no excessive dew formation (Sugar et al. 2005), being non-toxic to humans, and relatively safe to natural enemies (Delate and Friedrich 2004). Besides, it is washable and forms a suspension in water, being easily applied using conventional spray equipment, and it may eventually reduce the number of chemical insecticides applications (Peng et al. 2011).

**Acknowledgments** This research was supported by the following Brazilian Agencies: “Financiadora de Estudos e Projetos” (FINEP), and “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq).

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