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Screening test of Diatomaceous Earth as an alternative viability method for *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) eggs

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Resumo: The sugarcane borer *Diatraea saccharalis,* (Fabricius, 1794) (Lepidoptera: Crambidae), is a pest that causes damage to sugarcane fields. The female lays imbricated egg masses on the leave. They cause the greatest damage as larvae, and thus, control before egg hatching is desired to prevent the development of this pest and avoid damage to the plant. Diatomaceous Earth, or diatomite, is inert dust with insecticidal properties. In this work, we aimed to test the activity of Diatomaceous Earth on viable eggs of *D. saccharalis* as a potential alternative control method for sugarcane borer. *D. saccharalis* eggs 1 day after oviposition were immersed in a Diatomaceous Earth solution (10% - weight/volume) for 5, 10, and 15 minutes and observed every 24 hours for 216 hours of analysis. During the first 72 hours, there were no changes observed in egg morphology, which maintained an oval shape, pale yellow in color. After 96 hours, chorion striations were observed in the treatments with 10 and 15 minutes of exposure and were not present in the chorion controls eggs. Differences between embryo's number it was observed after 96 hours in the 15 minutes treatment when compared with the untreated eggs control. Besides that, control larvae completely hatched in 192 hours and the treatment with 10 minutes and 15 minutes not shown completely hatching suggesting a delay in the embryonic development of the *D. saccharalis* larvae. Overall, our study is the first report of the application of *D. saccharalis* eggs, and we believe that Diatomaceous Earth could be a potential tool for the control of *Diatraea* eggs, however, thorough investigations are required to elucidate the action of this powder on *Diatraea* and would be an alternative method to interrupt the biological cycle of the pest.
Palavras-chave: Sugarcane borer; Pest control; Diatomite.

Teste de triagem da Terra de Diatomáceas como método alternativo para viabilidade de ovos da *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) eggs

Resumo: A broca da cana *Diatraea saccharalis*, (Fabricius, 1794) (Lepidoptera: Crambidae), é uma praga que causa danos a cultura canavieira. A fêmea deposita os ovos em massas imbricadas nas folhas. Na fase larval causam os maiores danos, e assim, o controle antes da eclosão dos ovos pode interromper o desenvolvimento desta praga diminuindo os danos à planta. A Terra diatomácea, ou diatomita, é um

pó inerte com propriedades bioinseticidas. Neste trabalho buscamos realizar uma triagem da Terra Diatomácea em ovos viáveis de *D. saccharalis* como potencial método alternativo no controle da broca da cana. Ovos de *D. saccharalis* 1 dia após a oviposição foram imersos em suspensão de Terra de Diatomácea (10% - peso / volume) por 5, 10 e 15 minutos e observados em microscópio a cada 24 horas até 216 horas após o tratamento. Durante as primeiras 72 horas, não foram observadas alterações na morfologia dos ovos, que mantiveram uma forma oval, de cor amarelo pálido. Após 96 horas, estrias no córion foram observadas nos tratamentos com 10 e 15 minutos de exposição e não estavam presentes no córion dos ovos do controle. As diferenças entre o número de embriões foram observadas após 96 horas no tratamento de 15 minutos, quando comparado com o controle não tratado. Além disso, as larvas do controle eclodiram completamente em 192 horas e o tratamento com 10 minutos e 15 minutos não eclodiram completamente, sugerindo um atraso no desenvolvimento embrionário das larvas de *D. saccharalis*. Este trabalho é o primeiro relato da aplicação da Terra de Diatomáceas em ovos de *D. saccharalis*, o qual pode ser uma potencial ferramenta para o controle de ovos de *Diatraea*.
Palavras-chave: Broca da cana; Controle de pragas; Diatomita.

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INTRODUÇÃO

Diatraea have been considered to be pests since their introduction to the Americas. *Diatraea saccharalis* Fabricius, 1794 (Lepidoptera: Crambidae) is considered to be the most aggressive and has a wide distribution causing great losses for agricultural industries (MENDONÇA, 1996; MACEDO, 2000; RUVOLO-TAKASUSUKI et al., 2002; VARGAS et al., 2015; VARGAS et al., 2018). Its polyphagic habit affects several cultures of agronomic importance (PINTO et al., 2006; CRUZ et al., 2011; DINARDO-MIRANDA et al., 2012; SVEDESE et al., 2013). Females oviposit on both the abaxial and adaxial sides of leaves, depositing from 5–50 eggs in imbricated masses. Newly-ovulated eggs are yellow, turning pink to brown when the cephalic capsules of the embryos are visible (TERAN, 1987; LIMA FILHO, 2001).

All larval phase causes the greatest amount of damage in sucarcane plantations (RUVOLO-TAKASUSUKI et al., 2002; DOSSI et al., 2006), however, conventional methods of pest control based on chemical insecticide (ENDO et al., 2018; DAQUILA et al., 2019) are not efficient against larvae. The most works have been mentioned biological control of *Diatraea* involving the use of parasitoids (DINARDO-MIRANDA, 2010; SANDOVAL; SENÔ, 2010; MARTINS et al., 2011; AYA et al., 2017; AYA et al., 2019) or plants extracts (JUSTINIANO et al., 2012). Therefore, it is important to control the egg or adult phases, preventing pest development and avoiding plant damage (CRUZ, 2008). It is also important to develop products that attack the pest without damaging the plants or other insects (BARROS et al., 2006; MOURA et al., 2009; MOLNÁR et al., 2016).

Alternative control methods, especially those that use natural products, represent an important pest control tool (SCUDELER et al., 2017). These methods are important because they advocate the development of sustainable agriculture by replacing the use of chemical pesticides that often trigger adverse effects on the environment (MARTÍNEZ-EIXARCH et al., 2017; YING, 2018; SHAO et al., 2019).

Diatomaceous Earth, or diatomite, is an inert powder extracted from fossilized diatomaceous rocks formed from accumulated algal diatoms, which contain silica dioxide. It usually has a density of 1.98–2.5 g.cm^{-3} and can be white, brown, or pink (WYPYCH, 2016; EROGLU et al., 2017). It has been found in abundance and has a wide range of uses in engineering (OLIVEIRA et al., 2013; POLIZOS et al., 2014; PURETSKIY et al., 2015; PERERA et al., 2016). Silica, one of the most important components of diatomite, can dehydrate insects leading to death in a variable period, depending on the species biological cycle (LORINI, 1999). Diatomaceous Earth or its derivatives have been proposed as an alternative method for controlling insects that act as pests of stored grain products (ALDRYHIM, 1990; ARTHUR, 2001; MEWIS, 2000; ULRICHS, 2001; BALDASSARI et al., 2002; PINTO JUNIOR, 2008; NWAUBANI et al., 2014; ANTUNES et al., 2014; KAVALLIERATOS et al., 2015; JAIROCE et al., 2016; RUMBOS et al., 2016; BOHINC et al., 2018; PERISIC et al., 2018).

Sugarcane production was approximately 626 million tons in 2018/2019, which was about 1.2% lower than the previous year's harvest according to the Companhia Nacional de Abastecimento (CONAB) (CONAB, 2018). A potential reason for lower agricultural production could be the pests that attack sugarcane plantations and affect the development of the plants. This issue of reduced production is not only for Brazilian agriculture but is also of global concern (BARBOSA et al., 2011). By the way, the objective of this work was to evaluate the application of the Diatomaceous Earth as an alternative method of the viability of *D. saccharalis* eggs.

MATERIALS AND METHODS

Insects

D. saccharalis eggs were obtained from the Laboratory of Morphology and Cytogenetics of Insects in the Department of Biotechnology, Cell Biology and Genetics of the State University of Maringá, Paraná, Brazil. Five pairs of moths were kept in PVC tubes internally wrapped with an A4 sheet of sulfite paper coated with plastic to a thickness of 15 mm and protected from light. We selected eggs within 1 day of oviposition, and masses containing on average 25–40 eggs were separated and transferred to plastic pots of 147 x 100 x 40.5 mm. The eggs did not undergo any surface disinfection.

Diatomaceous Earth

The product was obtained commercially from Diatomite CI/325 CX, certified by analysis n. 105/15, supplied by CIEMIL, Com. Indústria e Exportação de Minérios Ltda, Vitória da Conquista, BA, Brasil.

Bioassay

The commercial product was diluted to 10% (weight/volume) in distilled water (10 g Diatomaceous Earth: 100 mL water) and shaking for 5 min. The egg masses were immersed in the solution for 5, 10, and 15 minutes. The whole experiment was performed in triplicate, and the controls for each treatment were performed with distilled water only. After the treatments, the egg masses were transferred to plastic pots (147 x 100 x 40.5 mm) and maintained at 25 ± 2 °C, a relative humidity of 70 ± 10% and photophase of 12 h (N/D).

Microscopic analysis

Observations were conducted every 24 h for 9 days, totaling 216 h. We used an Olympus microscope coupled to a Canon 7 megapixel camera. Low light intensity views were used to avoid the interference of light as a mortality factor, avoiding heating of the egg surface. The number of eggs with embryos and the presence or absence of larval emergence were recorded.

Statistical analyses

Data from all experiments were analyzed by ANOVA (analysis of variance) and the means were compared by the Scott-Knott test ($p < 0.05$). Tests were carried out in the statistical program SISVAR 5.6 (FERREIRA, 2011).

RESULTS

During the first 72 h, there were no changes observed in egg morphology, which maintained an oval shape, pale yellow in color. In the control group (Figure 1 A1), it is possible to observe the vitellogenesis process, represented in this case by these yellowish edges in the periphery of the eggs. During embryogenesis the calf will be used to construct a new individual, the first instar larva. After 96 hours, chorion striations were observed in the treatments with 10 and 15 minutes of exposure and were not present in the chorion controls eggs (Figure 1. A3 and A4).

In Figure 1 in B2 to B4, which are the treated eggs and observed at 144 hours, we noticed delayed development of the larvae compared to B1 (control eggs) where the larvae are well developed and with very evident cephalic capsules. In C2 and C4, in the 168-hour treatment, it is observed that inside the eggs there are more evident development larvae compared to the previous treatment (B2 and B4), but still anomalous when compared to control (B1 and C1).Besides that, all control larvae hatched in 192 h (Figure 1 D1 and E1) and the treatment with 10 minutes (Figure 1 D3 and E3) and 15 minutes (Figure 1 D4 and E4) not shown completely hatching suggesting a delay in the embryonic development of the *D. saccharalis* larvae. In E2 and E4 treatment corresponding to 216 hours, we observed spotted eggs and some larvae remaining inside the eggs.

Analyzing the number of embryos in relation to the treatment time (Table 1), statistical differences were found for the treatment of 15 minutes after 96 hours (20 embryos), when compared to untreated eggs control (36 embryos). After 120 hours, differences in the number of embryos for the 15-minute treatment (18 embryos) in relation to eggs control (36 embryos) were also observed. All treatments differed from eggs control after 144 hours (Table 1).

DISCUSSION

Due to the fact that the larval stages of *D. saccharalis* are the ones that cause the most damage to the sugarcane fields and are located within the stem of the plant, studies that aim alternative methods of control not only for these larval stages but also for stages prior to them, like egg, are extremely important (BORTOLI, 2013).

Table 1. *Diatraea saccharalis* eggs treated with Diatomaceous Earth 10% (w/v). Analysis carried out up to 216 hours.

	Time/ Embryon number		
Treatments	5 minutes	10 minutes	15 minutes
Untreated eggs control*	36a	36a	36a
24 hours	30a	26a	21a
48 hours	30a	26a	21a
72 hours	30a	26a	21a
96 hours	29a	26a	20b
120 hours	29a	26a	18b
144 hours	28b	26b	18c
168 hours	28b	26b	18c
192 hours	26b	26b	17c
216 hours	26b	26b	17c

*Untreated eggs control: eggs not treated with diatomaceous earth, the egg masses were immersed only in distilled water for 5, 10 and 15 minutes. The number of embryos is represented by the average of the triplicate eggs of each treatment. Means of the triplicate followed by different letters are significantly different according to the Scott-Knott test ($p < 0.05$).

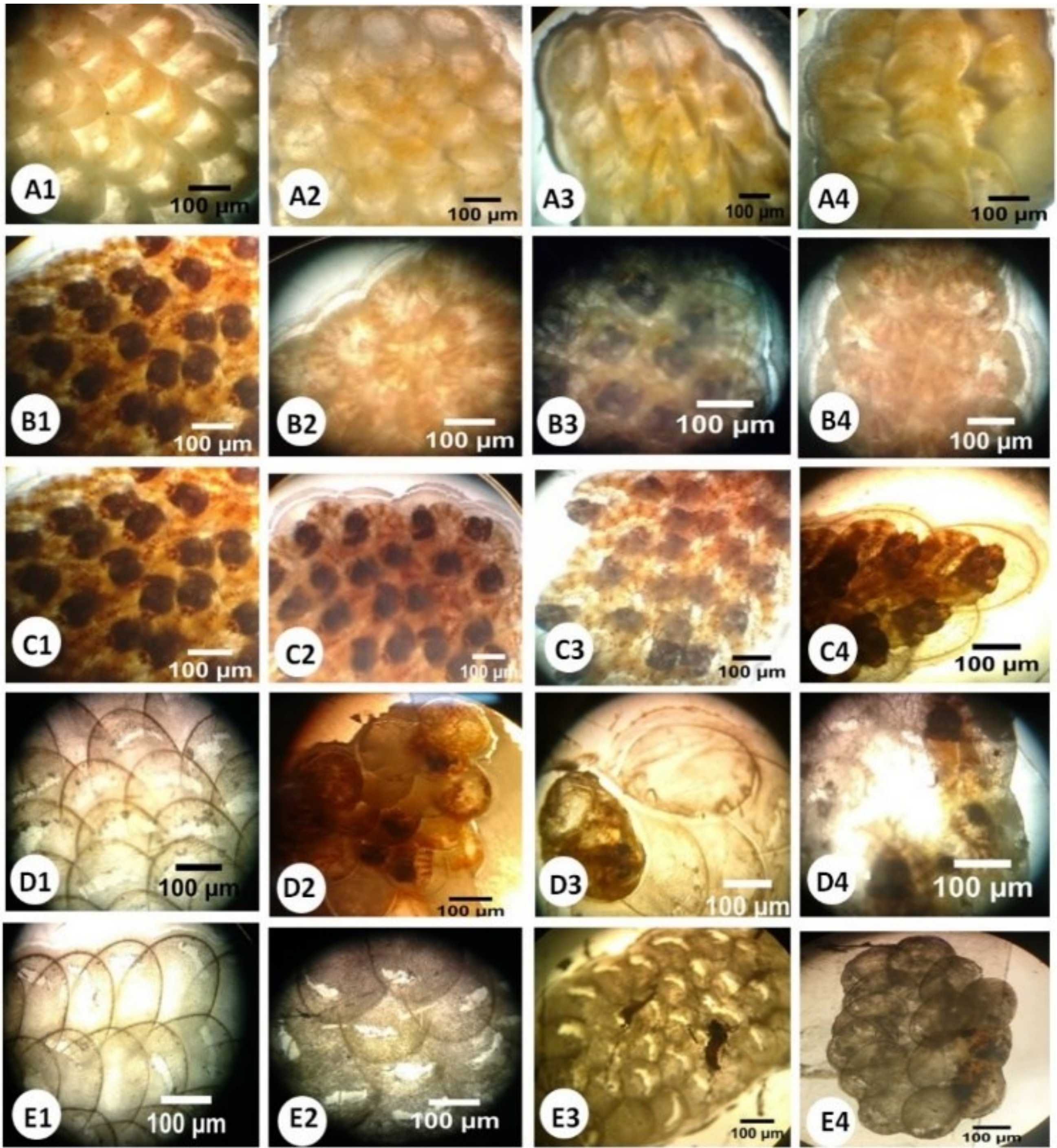

Figure 1. *Diatraea saccharalis* eggs treated with Diatomaceous Earth. Eggs after: **(A)** 96 hours (A1 eggs control, A2 5 minutes, A3 10 minutes, and A4 15 minutes). **(B)** 144 hours (B1 eggs control, B2 5 minutes, B3 10 minutes, and B4 15 minutes). **(C)** 168 hours (C1 eggs control, C2 5 minutes, C3 10 minutes, and C4 15 minutes). **(D)** 192 hours (D1 eggs control, D2 5 minutes, D3 10 minutes, and D4 15 minutes). **(E)** 216 hours (E1 eggs control, E2 5 minutes, E3 10 minutes, and E4 15

DISCUSSION

Due to the fact that the larval stages of *D. saccharalis* are the ones that cause the most damage to the sugarcane fields and are located within the stem of the plant, studies that aim alternative methods of control not only for these larval stages but also for stages prior to them, like egg, are extremely important (BORTOLI, 2013).

Silva et al. (2013) investigated the action of neem oil on *D. saccharalis* eggs. These authors evaluated the effect of treatments carried out with neem oil for six days (144 hours) and their results indicated that neem oil when sprayed on *D. saccharalis* eggs, demonstrated reduced hatching, increasing the mortality of hatched caterpillars, compromising embryonic development and the production of new individuals. Silva et al. (2013) findings corroborate the idea that alternative methods that affect the viability of eggs, such as the Diatomaceous Earth, can be positive control strategies.

Although investigations using Diatomaceous Earth on *D. saccharalis* eggs and other stages are few found, works with larval and adult phases of other classes of insects can be used as models to test and set up experiments. Many previous studies have been described as Diatomaceous Earth as an alternative bioinsecticide, especially for adult beetles. Pinto (2008), reported satisfactory mortality rates applying Diatomaceous Earth on three beetle species. Antunes et al. (2013) performed another study with beetles and found positive results. Nwaubani et al. (2014) and Kavallieratos et al. (2015) corroborate the potential of Diatomaceous Earth for pest control proposed by the previous authors.

Hosseini et al. (2014) and Jairoce et al. (2016) suggested that the mortality rate of tested pests was proportionally related to the applied dosage and the time of exposure. The findings of these authors are consistent with our results, in that the emergence rate and the number of viable embryos of *D. saccharalis* were significant at the time of major treatment. It is considered that the effect of Diatomaceous Earth on adult individuals is linked to their gregarious habits, whereby many individuals form groups facilitating the transfer of the control agent from insects exposed to the product to those not directly exposed (AKHTAR; ISMAN, 2013).

A viable explanation for the susceptibility of adult insects to Diatomaceous Earth, might be related to the anatomy, physiology, and behavior of these insects, which differs greatly between the egg and larval stages of development. The inert powder may enter the insect through small holes preventing gas exchange or even directly or indirectly influencing metabolic activity. Insects with large surface areas are usually more sensitive (ALKAN et al., 2019). In *D. saccharalis* eggs, the powder might come through the aeropyle making possible the ovicidal action that could be happens by the permeability of the egg chorion affecting this way the embryonic development of the larvae (MUÑOZ et al., 1991).

Inert dust is marketed as insecticides; however, high doses are usually required for them to be effective (PIERANTTINI et al., 2019). Silica dioxide (SiO_2), one of the main components of Diatomaceous Earth, is used as an alternative method of pest control in agriculture by adding to the soil and increasing plant resistance to insects. Resistance occurs through accumulation in the epidermal cells of the leaves and trichomes, increasing thickening and stiffness, and thus constituting a mechanical barrier. Alternatively, the formation of defense substances with low digestibility or that decrease attraction to the target insect (GOUSSAIN et al., 2002; KORNDORFER, 2010; OLIVEIRA et al., 2012).

There are few previous reports of bioassays on the use of Diatomaceous Earth for biological control of larvae and eggs of insect pests, especially *D. saccharalis*. Nevertheless, there have been studies on the use of Diatomaceous Earth focusing on other taxa and stages of insect development. An example is the tests performed by Constanski et al. (2016) with second instar larvae of *Spodoptera eridania* and *S. frugiperda,* and the results indicated significant indices for the mortality for both insects.

CONCLUSION

Diatomaceous Earth is generally used to control the later stages of insect development, which contain chitin since one of the mechanisms of action might occur through dehydration when the powder comes into contact with it. To our knowledge, our study is the first report of Diatomaceous Earth application on *D. saccharalis* eggs.

Thorough investigations are required to elucidate the action of this powder on lepidopteran insects by analyzing more stages of this powder on lepidopteran insects by analyzing more stages of development. In addition, as proposed by previous authors, we advocate the implementation of new methodologies that use different concentrations and exposure times of Diatomaceous Earth solutions to determine its potential as a control agent. Our findings contribute to the development of sustainable agriculture by reducing the use of pesticides.

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