PS7-18 – 6195

# Control of *Zabrotes subfasciatus* (Boheman) (Coleoptera, Chrysomelidae, Bruchinae) in *Phaseolus vulgaris* Linnaeus, using diatomaceous earth under different temperatures

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## Abstract

Diatomaceous earth (DE) is a nontoxic and efficient method to control many insect pests of stored grains, but there is no information about the viability of its use for controlling the Mexican bean weevil *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera, Chrysomelidae, Bruchinae). Laboratory assays were conducted under different temperature and DE dosage combinations in order to determine the effect of such interactions to control this pest species. Bean samples were treated with 0.0; 0.50; 0.75 and 1.00 g of DE/kg of grain. Couples of *Z. subfasciatus* were placed in the vials of each treatment and kept in rearing chambers at 15, 20, 27 and 30 °C, 70 ± 10 % r.h., and 24 h scotophase. Diatomaceous earth at any dosage tested caused significant *Z. subfasciatus* mortality, especially in temperature of 27 and 30 °C. After five days of exposure, death rates of *Z. subfasciatus* were about 100 % for all DE dosages and in temperature above 20 °C. However, at 27 °C the insects reproduced and laid a large number of eggs before dying. The use of 0.75 and 1.00 g of DE/kg dosages is recommended based on the tests. The interaction of DE and temperature leads to almost 100 % of mortality on the fifth day of exposure. It was concluded that *Z. subfasciatus* is very susceptible to DE treated beans.

*Key words:* Mexican bean weevil; physical control; inert dust; stored beans; grain protection.

## Introduction

The Mexican bean weevil, *Zabrotes subfasciatus* (Boheman), is one of the main pests of stored beans, causing expressive qualitative and quantitative losses in grains and seeds, mainly in the warmest regions of the world (Dendy and Credland, 1991; Mazzonetto and Vendramin, 2003; Sari et al., 2003). The presence of bean weevils affects the visual aspect of the beans, their odor, palatability and acceptability by consumers. Additionally, significant reduction of the nutritional quality, weight loss and commercial depreciation occur in the attacked grains, not only due to the presence of this weevil species, but also due to its fragments (Hohmann and Carvalho, 1989).

Insect control in stored grain is conventionally achieved by using fumigation and residual insecticides. However these methods are not always efficient and accepted by the consumers. The residues of the active ingredients can affect worker safety and represent risk of contamination of the storage facility and environment. In addition, resistance of insect populations to insecticides may also occur. In fact, this has become a problem in some parts of the world where few active ingredients are available. In order to control the resistant populations, the use

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of a mixture of insecticides has become common practice, although this procedure is forbidden by Brazilian laws (Beckel, 2004).

Nowadays, alternative methods, such as the use of resistant bean varieties (Wanderley et al., 1997; Mazzonetto, 2002), artificial cooling (Moreira, 1994; Pinto Jr., 1999), inert dusts (Subramanyam and Roesli, 2000; Lorini et al., 2002), natural enemies (Kistler, 1985), repellent plants oils and powders (Oliveira and Vendramim, 1999; Mazzoneto and Vendramim, 2003) and other integrated measures of handling pests are becoming more common.

Diatomaceous earth (DE) is a silica dioxide inert dust extracted from fossil deposits of diatomaceous algae skeletons (Subramanyam and Roesli, 2000; Jayas, 1995). Its action is based on abrasion and adsorption of lipids from the epicuticle, killing the insect by dehydration (Chiu, 1939) when about 60 % of water or 30 % of the total corporal mass is lost (Ebeling, et al. 1966). The insecticidal effect of DE depends on the porosity and size of the skeleton particles (Ebeling, 1971) and is affected positively by denser pilosity and insect mobility (David and Gardiner, 1950). As DE does not depend on metabolic reactions, insects do not develop genetic resistance (Ebeling, 1971). Moreover, other advantages of DE in comparison to the products currently used (such as chemical insecticides) are the low toxicity, long lasting protection and its easy and safe application (Atui et al., 2003).

Diatomaceous earth has shown to be a promising biocontrol method and many studies are now available on the effectiveness of this inert dust against a great variety of storage grain pests (Jayas et al., 1995; Pinto Jr., 1999; Subramanyam and Roesli, 2000). Infestations of some species of Coleoptera have been controlled using dosages of 0.75 and 1.0 g of DE/kg of grain, such as *Sitophilus oryzae* (Linnaeus) (Curculionidae), *Tribolium castaneum* (Herbst) (Tenebrionidae), *Oryzaephilus surinamensis* (Linnaeus) (Silvanidae) and *Cryptolestes ferrugineus* (Stephens) (Cucujidae) and in dosage of 0.75 of DE/kg of beans for *Acanthoscelides obtectus* Say (Chrysomelidae, Bruchinae) (Pinto Jr. 1999). In contrast, studies on the performance of the Mexican bean weevil in beans treated with DE do not exist, despite the fact that *Z. subfasciatus* is a very important bean pest.

The objective of this research was to evaluate the use of DE under different temperatures to control *Z. subfasciatus* in stored beans, as an alternative to residual insecticides.

## Materials and methods

The bioassays were carried out in the Departmento de Zoologia of the Universidade Federal do Paraná (UFPR), Curitiba, Brazil. Adults of *Z. subfasciatus* were obtained from the Laboratório de Sistemática e Bioecologia de Coleoptera (Insecta), UFPR, Centro Nacional de Pesquisa Arroz e Feijão (Embrapa), Goiás, Brazil and Mercado Municipal de Curitiba, Paraná. The insects were reared in their usual host, *P. vulgaris*, commercial variety carioca. The cultivar Juriti was used, because of its excellent acceptability by *Z. subfasciatus* (Teixeira and Zucoloto, 2003). The DE used was the Brazilian brand KeepDry® with at least 86 % of amorphous silica dioxide ($SiO_2$), particles of approximately 15 $\mu$m and apparent density of 200 g/L. Beans were homogenized with DE concentration of 0.50, 0.75 and 1.00 g/kg. This was done by mixing 300 g of beans in a plastic bag with the respective DE concentration and manually shaking each sample vigorously for approximately 2 minutes until homogeneous distribution of DE in grain mass. Then, each sample was subdivided in six sub-samples of 50 g each. The same procedure was followed for the control (concentration of 0.00 g of DE/kg). Sub-samples were placed in plastic flasks with 200 mL and each one was infested with five couples of virgin *Z. subfasciatus*, with age of up to 24 hours. To obtain these specimens, beans were previously infested with males and females for 24 hours. Individual bean seeds contained only one egg attached on it by a gelatin capsule and kept at 30 ºC, 70 ± 10 % r.h. in total darkness until adult emergence (Sari et al., 2003).

The plastic flasks were placed in rearing chambers at temperatures of 15, 20, 27 and 30 ºC, at 70 ± 10 % r.h. and 24 h of scotophase, with six replicates for each treatment. Samples were examined daily, and dead specimens were counted, sexed and then discarded and live specimens counted, sexed and returned to the same plastic flask. Specimens were considered dead when they did not react to the touch of a metal clamp (Pinto Jr., 1994). These data were analyzed using variance analysis, Tukey test at 5% probability, and linear regression, using Statistica version 6.0, (Microsoft Statsoft release in 2002).

## Results

At 15 and 20 ºC, the average mortality of the females and males was significantly different from the control in DE concentrations of 0.75 and 1.00 g/kg, except for the third day (Tables 1, 2, 3). At the highest temperatures (27 and 30 ºC) and at the highest DE concentrations (0.75 and 1.00 g/kg), the increase of exposure time to DE was followed by an increase in mortality. At these temperatures, significant differences had already been observed in the average of female and male mortality at the third day of exposure. On the third day of exposure, for 0.75 and 1.00 g of DE/kg of bean, the mortality at 27 ºC was approximately 70 % for females and 90 % for males. At 30 ºC, mortality was 90 % for females and 100 % for males. On the fifth day, no significant differences were observed for *Z. subfasciatus* mortalities at temperatures above 15 ºC and at concentrations of 0.50, 0.75 and 1.00 g/kg, except for females at 30 ºC at 0.75 and 1.00 g/kg where the mortality was 100 %. On the tenth day, mortality was about 100 % for all temperatures, at all concentrations, except for the control at 15 and 20 ºC.

Determination coefficients ($R^2$) showed that temperature and DE concentrations have high influence on *Z. subfasciatus* mortality of females and males (Figure 1). Low mortality was observed in the control for females and males in lower temperatures. With the use of DE in all

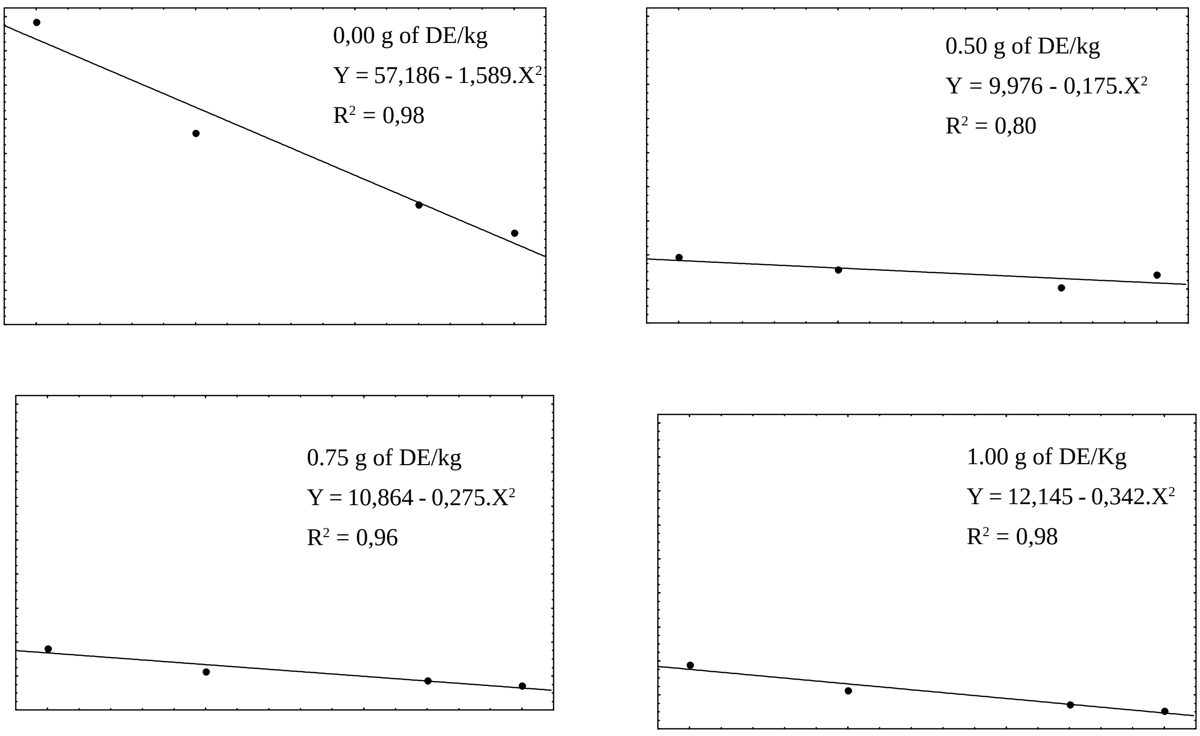


**Figure 1.** Sobrevivência *Z. subfasciatus* em feijão carioca tratados com diferentes concentrações de terra de diatomácea e armazenados em diferentes temperaturas.

concentrations, a much higher mortality was verified even at the lowest temperature.

## Discussion

The mortality levels (Tables 1, 2, 3) were higher in higher temperatures since the beginning of the exposure period. Mortality percentages were higher than those obtained by Pinto Jr. (1994) for *A. obtectus*. In general, the results confirm that higher dosages of DE require less exposure time than lower dosages as observed by Pinto Jr. (1994) for *A. obtectus*. Some Coleoptera species as *S. oryzae*, *R. dominica*, *T. castaneum* and *T. confusum* Jacquelin du Val (Coleoptera, Tenebrionidae) are more susceptible to DE at temperatures below 20 ºC, while *Sitophilus granarium* and *R. dominica* are more susceptible to DE at 30 ºC (Aldryhim, 1990, 1993; Nickson et al., 1994), as is *Z. subfacsiatus*.

Insects are more active in high temperatures, from 28 to 33 °C (Subramanyam and Roesli, 2000, Arthur 2000) and since mobility is essential for the effectiveness of DE (David and Gardiner, 1950). The increase in temperature, concentration of DE and time of exposure are surely responsible for the gradual reduction of *Z. subfasciatus* development and survival.

**Table 1.** Porcentage (± EP) of female and male mortality of *Z. subfasciatus* in carioca beans, until the third day of exposition at different concentrations of DE in different temperatures.

| | Temperature (ºC) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| DE | 15 | | 20 | | 27 | | 30 | |
| (g/kg) | Females | Males | Females | Males | Females | Males | Females | Males |
| 0.00 | 0.00 a | 0.00 a | 0.00 a | 0.00 a | 0.00 a | 3.40 ± 3.33 a | 13.40 ± 4.00 a | 3.40 ± 3.33 a |
| 0.50 | 6.60 ± 4.22 a | 20.00 ± 5.16 b | 0.00 a | 0.00 a | 50.00 ± 13.60 b | 86.60 ± 4.22 b | 33.40 ± 4.22 b | 53.40 ± 4.22 b |
| 0.75 | 0.00 a | 0.00± a | 40.00 ± 9.55 b | 53.40 ± 16.10 b | 70.00 ± 12.38 b | 90.00 ± 4.47 b | 96.60 ± 3.33 c | 100.00 c |
| 1.00 | 10.00 ± 4.47 a | 6.60 ± 4.22 a | 40.00 ± 8.94 b | 56.60 ± 9.55 b | 73.40 ± 12.29 b | 90.00 ± 4.47 b | 83.40 ± 6.15 c | 100.00 c |

Means followed by different letters differ significantly at $p<0.05$, as assessed by Tukey's Significance Test.

**Table 2.** Porcentage (± EP) of female and male mortality of *Z. subfasciatus* in carioca beans, until the fith day of exposition at diferent concentrations of DE in different teperatures.

| | Temperature (ºC) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| DE | 15 | | 20 | | 27 | | 30 | |
| (g/kg) | Females | Males | Females | Males | Females | Males | Females | Males |
| 0.00 | 3.40 ± 3.33 a | 0.00 a | 0.00 a | 0.00 a | 0.00 a | 3.40 ± 3.33 a | 20.00 ± 5.16 a | 10.00 ± 4.47 a |
| 0.50 | 13.40 ± 4.22 ab | 86.60 ± 6.67 c | 73.40 ± 4.22 b | 93.40 ± 4.22 b | 96.60 ± 3.33 b | 100.00 b | 63.40 ± 6.15 b | 93.40 ± 4.22 b |
| 0.75 | 43.40 ± 12.02 b | 96.60 ± 3.33 c | 96.60 ± 3.33 b | 100.00 b | 96.60 ± 3.33 b | 100.00 b | 100.00 c | 100.00 b |
| 1.00 | 50.00 ± 8.56 b | 50.0 ± 8.56 b | 93.40 ± 4.22 b | 100.00 b | 93.40 ± 4.22 b | 100.00 b | 100.00 c | 100.00 b |

Means followed by different letters differ significantly at $p < 0.05$, as assessed by Tukey's Significance Test.

**Table 3.** Porcentage (± EP) of female and male mortality of *Z. subfasciatus* in carioca beans, until de tenth day of exposition at different concentrations of DE in different temperatures.

| DE | Temperature (ºC) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 15 | | 20 | | 27 | | 30 | |
| (g/kg) | Females | Males | Females | Males | Females | Males | Females | Males |
| 0.00 | 6.60 ± 4.22 a | 0.00 a | 0.00 a | 3.40 ± 3.33 a | 100.00 a | 76.60 ± 8.03 a | 96.60 ± 3.33 a | 100.00 a |
| 0.50 | 100.00 b | 100.00 b | 100.00 b | 100.00 b | 100.00 a | 100.00 a | 96.60±3.33 a | 100.00 a |
| 0.75 | 100.00 b | 100.00 b | 100.00 b | 100.00 b | 100.00 a | 100.00 a | 100.00 a | 100.00 a |
| 1.00 | 100.00 b | 100.00 b | 100.00 b | 100.00 b | 100.00 a | 100.00 a | 100.00 a | 100.00 a |

Means followed by different letters differ significantly at $p < 0.05$, as assessed by Tukey's Significance Test.

## Conclusion

All DE dosages were efficient to control *Z. subfasciatus* in carioca beans, resulting in significant mortality at temperatures ranging from 15 to 30 °C. In the fifth day of exposure mortality was about 100 % in temperatures above 20 °C. DE at 0.75 and 1.00 g of DE/kg of beans is recommended for an effective control of *Z. subfasciatus*.

## Acknowledgments

The authors are thankful to Dr. Massaru Yokoyama (Embrapa Arroz e Feijão) for sending specimens of *Z. subfasciatus*, Dr. Edilson Batista de Oliveira (Embrapa Florestas) for help with the statistics analysis, Dra. Sonia Maria Noemberg Lazzari for valuable comments, and to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the grants to the authors.

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