Effect of five diatomaceous earth formulations against Tribolium castaneum (Coleoptera: Tenebrionidae), Oryzaephilus surinamensis (Coleoptera: Silvanidae) and Rhyzopertha dominica (Coleoptera: Bostrychidae)

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**Abstract** Laboratory bioassays were conducted to determine the effect of food source on the survival of *Tribolium castaneum* Herbst, *Oryzaephilus surinamensis* L. and *Rhyzopertha dominica* F., after exposure to five diatomaceous earth (DE) formulations: Protect-It\(^a\), Insecto\(^b\), Perma-Guard\(^c\), Dryacide\(^d\) and SilicoSec\(^e\). Adults of these species were exposed to DEs at the rate of 0.5 mg/cm\(^2\) for 1 day on filter paper inside plastic Petri dishes. After exposure, the initial mortality was counted and live individuals of the three species were held for a week in glass vials containing 50 mg wheat flour, rice and whole wheat, respectively. In the second experiment, after 1 day exposure to DEs, beetles were transferred to Petri dishes without food and held for a week to determine if the presence of food source would decrease the mortality of beetles. Experiments were carried out at 27°C and 55% RH in the dark. The initial mortality in both of the experiments reached 100% for the three species exposed to Protect-It\(^a\) and in the case of *R. dominica* and *O. surinamensis* exposed to Dryacide\(^d\). In contrast, low level of mortality (< 10%) was observed for *T. castaneum* exposed to Perma-Guard\(^c\) and Insecto\(^b\). The mortality after the post-treatment period on food was decreased for the three species exposed to Perma-Guard\(^c\) and in the case of *T. castaneum* and *R. dominica* exposed to Insecto\(^b\) and SilicoSec\(^e\). Adults of *O. surinamensis* were the most susceptible followed by *R. dominica* and 100% adult mortality was obtained, whereas *T. castaneum* were the least susceptible beetles to DEs. Protect-It\(^a\) and Dryacide\(^d\) were the most efficient DE formulations and can be used effectively in a stored grain integrated pest management program.

**Key words** diatomaceous earth, initial mortality, food, delayed mortality, *Oryzaephilus surinamensis*, *Rhyzopertha dominica*, *Tribolium castaneum*

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**Introduction**

In stable, well organized societies, infestations of storage pests are mostly held under control. However, the costs of keeping insects under control are significant and damaging infestations can occur if mistakes or neglect occur. Over the life of a given batch of commodity, protection against insect attack may account for several percent of its value (Rees, 2004). For control of stored grain insect pests several methods exist, but nowadays researchers seek reduced-risk methods. Diatomaceous earth (DE) has long been known as a potentially useful grain protectant because it is safe to use, does not affect grain end-use quality, provides long-term protection and is comparable in cost to...
other methods of grain protection and dust formulations are more likely to be adopted in developing countries (Korunic et al., 1996; Korunic, 1998; Athanassiou et al., 2005a, b; Moras et al., 2006; Ziaee et al., 2007).

Diatomaceous earths are composed of amorphous silicon dioxide and formed from fossilized diatoms (single-celled algae). They have low mammalian toxicity, and their use is compatible with other, reduced-risk, integrated pest management (IPM)-based control methods in storage facilities (Subramanyam & Roesli, 2000). DE particles adhere to the body of the insect and damage the protective waxy layer of the insect cuticle by absorption, and to a lesser degree, by abrasion. Death is caused from loss of water from the insect’s body (Ebeling, 1971). Apart from treating the entire mass of the grain commodity, DEs can be used for surface treatment of bulk-stored grains. White and Loschiavo (1989) conducted studies in which the effects of exposure interval, type and quantity of food after exposure to silica aerogel on the efficacy of different DE formulations applied on an inert surface were investigated. Extensive comparisons among different DEs against stored-grain insect species have been carried out by other researchers as well (Fields & Korunic, 2000; Athanassiou et al., 2004, 2005; Kavallieratos et al., 2005; Athanassiou, 2006; Vayias et al., 2006a, b; Athanassiou et al., 2007; Ziaee et al., 2007).

Arthur (2000a) carried out an experiment to determine the effects of temperature and relative humidity on the toxicity of Protect-It® placed on filter paper inside plastic Petri dishes against T. castaneum and T. confusum. He confirmed that mortality of both species increased with exposure interval. Furthermore, Arthur (2000b) investigated the impact of food source on the survival of T. castaneum and T. confusum. He reported that accumulated food material reduces the residual efficacy of the DE treatment.

The aim of the current study was: (i) to compare the efficacy of different DE formulations applied on an inert filter paper against T. castaneum, O. surinamensis and R. dominica; and (ii) to determine whether the presence of food can affects survival of T. castaneum, O. surinamensis and R. dominica after exposure to DEs.

**Materials and methods**

**Insects**

Adults of T. castaneum, O. surinamensis and R. dominica were used in the tests. The T. castaneum adults were taken from a culture that was kept on wheat flour plus 5% brewers yeast (by weight); adults of O. surinamensis were taken from a culture that was kept on rolled oats and 5% brewers yeast (by weight); and R. dominica adults were taken from a culture that was kept on whole wheat; at 28°C, 65% ± 5% RH and continuous darkness. All species were kept in laboratory cultures for > 3 years. Adults used in the experiments were < 14 days old.

**DE formulations**

Five commercially available DE formulations were used in the tests:

1. Protect-It® is a mixture of freshwater DE (Hedley Technologies Inc., Mississauga, ON, Canada) with 10% silica aerogel to enhance insecticidal activity. It contains approximately 83.7% amorphous silicon dioxide, 5.6% Al2O3, 2.3% Fe2O3, 0.9% CaO, 0.3% MgO, and 1.9% other oxide (e.g. TiO2, P2O5), and 3%–5% moisture content (m.c.). The median particle size is 5 μm (Korunic & Fields, 1995).

2. Insecto® is a marine DE (Natural Insects Products, Inc., Orange, CA, USA) with 10% food-grade bait. It has 87% (w/w) amorphous silicon dioxide, with 2%–4% m.c., and a chemical composition of approximately 3% Al2O3, 1% Fe2O3, <1% CaO, MgO, TiO2, and P2O5. The median particle size is approximately 8.2 μm (Subramanyam et al., 1994).

3. The freshwater DE Perma-Guard™ D-10 (Perma-Guard™ Inc., Albuquerque, NM, USA) contains 93% SiO2, 3% Al2O3, 1.3% Fe2O3, 1.1% CaO, 0.6% Na2O, 0.3% K2O and 0.2% TiO2 and maximum 4.5% m.c. The median particle size is approximately 11.7 μm (Arnaud et al., 2005).

4. The marine DE Dryacide® (Dryacide USA LLC, San Diego, CA, USA), is a white dust that contains 91%–92% amorphous silicon dioxide, 3%–5% clay minerals and 3%–5% m.c. with a mean particle size of 13–15 μm (A. McLaughlin, personal communication).

5. SilicoSec® is a freshwater formulation of DE obtained from Biofa GmbH, Münisingen, Germany and it is composed of 92% SiO2, 3% Al2O3, 1% Fe2O3 and 1% Na2O. The median particle size is 8–12 μm (Athanassiou et al., 2005b).

**Post-treatment period on food**

A standard plastic Petri dish with an external radius of 10 cm, internal radius of 8.8 cm and an area of 62 cm² served as the exposure arena. Beetles were exposed for a day to five DE formulations at the rate of 0.5 mg/cm². Therefore 31 mg of each DE formulation was placed on filter paper inside plastic
Petri dishes. The dishes were shaken for a minute to distribute the DE which due to static electricity tends to stick to the filter paper. After shaking, the Petri dishes were left undisturbed for at least 1 minute in order to settle the DE particles on the filter paper. Subsequently, 15 adults were introduced in each dish and the dishes were covered with lids. Petri dishes were placed in the incubator set at 27oC and 55% RH in the dark. The desired RH was maintained by using a saturated salt solution of sodium bromide, as recommended by Greenspan (1997). Each treatment of DEs plus untreated dishes containing filter paper alone were replicated four times for each species (24 Petri dishes per species). After a day of exposure to DEs, insects were assessed as live if able to move normally and to respond to stimuli or as dead if unable to do either and initial mortality was recorded; then live individuals of T. castaneum, O. surinamensis and R. dominica were held for a week in glass vials containing 50 mg wheat flour, rice and whole wheat, respectively under the same conditions. The moisture content of commodities was measured by drying 10-gram samples of each commodity in a ventilated oven at 110oC. The moisture content of wheat flour, rice and wheat was 13.4%, 12.8% and 11.3% m.c., respectively. The level of 55% RH corresponds to moisture content values ≈ 13% for wheat flour, 11.5% for rice and 12.5% for rice, respectively (Pixton, 1967; Pixton & Warburton, 1971; Henderson & Pixton, 1982). After 1 week, the insects were discarded from the samples and the number of live and dead individuals was assessed as above.

**Post-treatment period without food**

All exposure conditions were the same as mentioned in experiment 1. Petri dishes were lined with filter paper and 31 mg of each DE placed on them. Each DE treatment was replicated four times for each insect and the untreated dishes containing filter paper alone served as a control with four replications (24 Petri dishes per species). Fifteen adults of each species were exposed to DEs for 1 day. Upon completion of the exposure, beetles were classified as live or dead and initial mortality was recorded; then live adults were transferred to new Petri dishes lined with filter paper alone; without food, and returned to the incubator for a week. After the 1-week holding period, the mortality of adults was measured. Trials were conducted at 27oC and 55% RH in the dark as described for the first experiment.

**Data analysis**

Control mortality was zero for insects and no corrections were necessary. The data were analyzed by using analysis of variance (ANOVA) and the t-test was used to determine significant differences between mortality of adults after the post-treatment period without food and on food for each species and DE formulation (SAS, 2000). To equalize variances, mortality percentage was transformed using the square-root of the arcsin. Means were separated by using the Tukey-Kramer (HSD) test at P = 0.05 (Snegecor & Cochran, 1989).

**Results**

**Post-treatment period on food**

The effect of DE formulation on adult mortality of T. castaneum, O. surinamensis and R. dominica after the post-treatment period on food and without food was significant at P < 0.01 level (Tables 1, 2). The initial mortality and percentage mortality of T. castaneum, O. surinamensis and R. dominica adults held for 1 week on the food source is presented in Table 3. Initial mortality ranged between 3.33% for T. castaneum exposed to Perma-Guard™ and 100% in the case of R. dominica and O. surinamensis exposed to Dryacide® and all three species exposed to Protect-It® were dead after 1 day of exposure. All adults of O. surinamensis were dead 1 week after their exposure to SilicoSec®.

**Post-treatment period without food**

Initial mortality and percentage mortality of beetles after the post-treatment period without food is recorded in Table 4.

### Table 1 ANOVA parameters for mortality levels of Tribolium castaneum, Oryzaephilus surinamensis and Rhizopertha dominica adults exposed to five diatomaceous earth (DE) formulations and after post-treatment period on food (df<sub>treatment</sub> = 4; df<sub>total</sub> = 19).

<table>
<thead>
<tr>
<th>Source</th>
<th>Insect species</th>
<th>1 day of exposure</th>
<th>1 week on food</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>DE formulation</td>
<td>T. castaneum</td>
<td>55.90</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>O. surinamensis</td>
<td>28.71</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>R. dominica</td>
<td>67.01</td>
<td>0.000</td>
</tr>
</tbody>
</table>

### Table 2 ANOVA parameters for mortality levels of Tribolium castaneum, Oryzaephilus surinamensis and Rhizopertha dominica adults exposed to five diatomaceous earth (DE) formulations and after post-treatment period without food (df<sub>treatment</sub> = 4; df<sub>total</sub> = 19).

<table>
<thead>
<tr>
<th>Source</th>
<th>Insect species</th>
<th>1 day of exposure</th>
<th>1 week without food</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>DE formulation</td>
<td>T. castaneum</td>
<td>137.23</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>O. surinamensis</td>
<td>102.70</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>R. dominica</td>
<td>454.60</td>
<td>0.000</td>
</tr>
</tbody>
</table>
After the post-treatment period without food, the mortality of *T. castaneum* exposed to DEs was 45% ± 2.4% to 100%, and for *R. dominica* mortality ranged between 86.6% ± 2.3% to 100%, whereas the entire population of *O. surinamensis* adults were dead after being held for 1 week without food (Table 4).

The comparison between mortality of beetles with and without food after the 1 week holding period for each species indicated that the presence of a food source may decrease the mortality of beetles (Table 5).

### Discussion

There are several reports on the increased survival of stored product beetles when they are given food after exposure to inert dusts. However, the magnitude of these effects can vary depending on the specific dusts, exposure conditions, type of food material and insect species (Arthur, 2000b; Athanassiou *et al.*, 2003, 2004, 2005b, 2007; Athanassiou & Kavallieratos, 2005; Kavallieratos *et al.*, 2005; Athanassiou, 2006).

Dowdy (1999) exposed *T. castaneum* to different DE formulations.

### Table 3

Initial mean mortality and mortality (%) ± SEM of *Tribolium castaneum, Oryzaephilus surinamensis* and *Rhizopertha dominica* adults after post-treatment period on food.

<table>
<thead>
<tr>
<th>DE formulations</th>
<th><em>T. castaneum</em></th>
<th><em>O. surinamensis</em></th>
<th><em>R. dominica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day of exposure</td>
<td>1 week on food</td>
<td>1 day of exposure</td>
</tr>
<tr>
<td>Dryacide®</td>
<td>91.6 ± 7.2 a</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Perma-GuardTM</td>
<td>3.3 ± 4.1 c</td>
<td>31.1 ± 1.9 c</td>
<td>73.3 ± 2.9 c</td>
</tr>
<tr>
<td>Protect-It®</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Insecto®</td>
<td>8.3 ± 5.2 bc</td>
<td>30.0 ± 3.6 c</td>
<td>76.6 ± 2.9 c</td>
</tr>
<tr>
<td>SilicoSec®</td>
<td>33.3 ± 5.0 b</td>
<td>81.6 ± 5.7 b</td>
<td>93.3 ± 4.3 b</td>
</tr>
</tbody>
</table>

Means within columns followed by the same letter are not significantly different; –: 100% mortality was observed, Tukey-Kramer-HSD test at \( P = 0.05 \).

### Table 4

Initial mean mortality and mortality (%) ± SEM of *Tribolium castaneum, Oryzaephilus surinamensis* and *Rhizopertha dominica* adults after post-treatment period without food.

<table>
<thead>
<tr>
<th>DE formulations</th>
<th><em>T. castaneum</em></th>
<th><em>O. surinamensis</em></th>
<th><em>R. dominica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day of exposure</td>
<td>1 week without food</td>
<td>1 day of exposure</td>
</tr>
<tr>
<td>Dryacide®</td>
<td>91.6 ± 4.8 b</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Perma-GuardTM</td>
<td>6.6 ± 4.3 d</td>
<td>45.0 ± 2.4 b</td>
<td>66.6 ± 1.6 c</td>
</tr>
<tr>
<td>Protect-It®</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Insecto®</td>
<td>6.6 ± 0.0 d</td>
<td>60.0 ± 1.6 b</td>
<td>75.0 ± 2.0 c</td>
</tr>
<tr>
<td>SilicoSec®</td>
<td>36.6 ± 1.1 c</td>
<td>96.6 ± 4.1 b</td>
<td>86.6 ± 2.3 b</td>
</tr>
</tbody>
</table>

Means within columns followed by the same letter are not significantly different; –: 100% mortality was observed, Tukey-Kramer-HSD test at \( P = 0.05 \).

Dowdy (1999) exposed *T. castaneum* to different DE formulations.

### Table 5

Mortality (%) of *Tribolium castaneum, Oryzaephilus surinamensis* and *Rhizopertha dominica* after post-treatment period without food versus mortality (%) of beetles after post-treatment period on food.

<table>
<thead>
<tr>
<th>DE formulations</th>
<th><em>T. castaneum</em></th>
<th><em>O. surinamensis</em></th>
<th><em>R. dominica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day of without food</td>
<td>1 week</td>
<td>1 day of without food</td>
</tr>
<tr>
<td>Dryacide®</td>
<td>91.6 ± 4.8 b</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Perma-GuardTM</td>
<td>6.6 ± 4.3 d</td>
<td>45.0 ± 2.4 b</td>
<td>66.6 ± 1.6 c</td>
</tr>
<tr>
<td>Protect-It®</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Insecto®</td>
<td>6.6 ± 0.0 d</td>
<td>60.0 ± 1.6 b</td>
<td>75.0 ± 2.0 c</td>
</tr>
<tr>
<td>SilicoSec®</td>
<td>36.6 ± 1.1 c</td>
<td>96.6 ± 4.1 b</td>
<td>86.6 ± 2.3 b</td>
</tr>
</tbody>
</table>

Differences between mortality (%) of adults after post treatment period without food and on food for each species and DE formulation was determined by the *t* test (SAS, 2000). NC: cannot be calculated because the variance of data was zero; NS: no significant difference; * and ** indicate that mortality of adults without food > mortality on food at \( P < 0.05 \) and \( P < 0.01 \), respectively.


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samples at the rate of 0.5 mg/cm² for a 15 and 30 minute duration at 34 and 50°C. After exposure, beetles were held for 1 week at 34°C and 60% ± 5% RH in empty dishes or on dishes with 500 mg of flour. He stated that the mortality during 7 days slowly increased, in comparison with the initial mortality. White and Loschiliao (1989) exposed O. mercator and T. confusum to silica aerogel at the rate of 0.72 mg/cm². The beetles were either starved after exposure or given 125 mg to 1 g of whole rolled oats, ground rolled oats, or ground breadcrumbs. All starved beetles were dead within 3 days, whereas the mortality of both species in the food treatments was greatly delayed. These findings were similar to the results of our experiment. The mortality of three species exposed to Protect-It® and Dryacide® and for O. surinamensis exposed to SilicoSec® after the 1-week holding period on food were 100% which was expected after the high level of mortality (> 90%) recorded after initial exposure. However, the insects that survived after 1 day of exposure may be able to remove the dust particles from the cuticular surface. Therefore, insects provided with a food source are going to survive far better than those left to starve. Chapman (1971) stated that stored-product beetles have well-developed cryptophagidial systems to remove water from dry foods such as cereals. At the same time, the physiological stress of an insect that can eat after exposure is far less than that of an insect left to starve and this will make the former far more capable in replenishing the lost protective waxy layer and terminating the water loss through the exposed cuticle. In our study comparing the mortality of beetles after the post-treatment period with and without food indicated that the mortality of beetles exposed to the most effective DEs (Protect-It® and Dryacide®) had not decreased, even when they are given food after exposure. In contrast, the mortality of adults was low, if they had access to food after exposure to the less effective DEs (Perma-Guard™, SilicoSec® and Insecto®).

Cook (2003) recorded complete mortality of T. castaneum when exposed to dishes treated with 10 g/m² SilicoSec® for 48 h, followed by a 7-day recovery period. Mason (1997) also reported 94% adult mortality against T. castaneum with 7 g/m² application of Protect-It®.

Arthur (2000b) emphasizes the importance of sanitation to reduce the occurrence of food material within the storage environment and to eliminate harborage sites and refuge when possible.

The effect of different DEs on various stored product insects has been studied by numerous researchers (Korunic, 1998; Athanassiou et al., 2003, 2004, 2005a, b, 2006, 2007; Athanassiou & Kavallieratos, 2005; Kavallieratos et al., 2005; Vayias et al., 2006a, b). Korunic et al. (1997) found that on the same commodity, there was a significant variation in the susceptibility of different insect species to the DE-based formulation Protect-It®. The insects, in order from the most susceptible to the least were Cryptolestes ferrugineus Stephens (Coleoptera: Cucujidae); O. surinamensis; Sitophilus granarius L. (Coleoptera: Curculionidae); Sitophilus oryzae L. (Coleoptera: Curculionidae); R. dominica; T. castaneum; and Prostephanus truncatus Horn (Coleoptera: Bostrichidae).

Several studies documented that Tribolium spp. are probably the most tolerant stored-product beetle species to DEs (Arthur, 2000a, b, Athanassiou et al., 2004, 2005a, Vayias & Athanassiou, 2004). However, according to Athanassiou et al. (2006), they have different susceptibilities to DEs. In the same study, strains from Denmark, United Kingdom and Germany were the most susceptible to Insecto®, Protect-It®, Protectot®, PyriSec® and SilicoSec® whereas the strain from Portugal was the least susceptible.

In this study, the DE Protect-It® and Dryacide® were the most efficient DE formulations against the three provided species, even in the presence of the food source. On the other hand, Perma-Guard™ and Insecto® were the least efficient DEs; therefore, high rates would be required to control infestations of stored product insect pests. Among the species tested, T. castaneum is less sensitive to DE dusts than R. dominica and O. surinamensis.

Further experiments will need to assess the efficacy of DEs on surfaces such as concrete and wood against the most tolerant insects to provide a more realistic challenge to DE efficacy. Farm-scale trials will also provide information on DE efficacy in the field and allow for dose recommendations to be provided. In conclusion, these laboratory tests have found that when applied to surfaces Protect-It® and Dryacide® were the most effective treatments against beetles with doses of 0.5 g/cm² producing 100% mortality in the insect species. These results would encourage the use of DE and inert dusts in general, as they appear to be among the most promising alternatives to pesticides in a stored-product IPM program.

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