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Influence of temperature and application rate on efficacy of a diatomaceous earth formulation against *Tribolium castaneum* adults

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ABSTRACT

Unsanitary storage bins can harbor grain-infesting insects, including the red flour beetle, *Tribolium castaneum* (Herbst). In a previous study involving heat treatment of empty bins, temperatures in the range of 50–55 °C for 2–4 h were effective in completely killing stored-product insects. Previous research in flour mills showed improved efficacy in killing stored-product insects by using diatomaceous earth (DE) dusts at temperatures below 50 °C. In the current study, the efficacy of a diatomaceous earth formulation (DiaFil® 610) applied to concrete arenas, to simulate floor of empty bins, was examined at three application rates (0, 2.5 and 5.0 g/m^2) to control *T. castaneum* adults at five constant temperatures (28, 36, 42, 44, and 46 °C). Ten adults of *T. castaneum* were placed on individual untreated and DE-treated concrete arenas for 4, 8, 12, and 24 h at each of the five temperatures. The efficacy of DE against *T. castaneum* adults increased with an increase in temperature and exposure time. Generally more adults died at 5.0 g/m^2 when compared with 2.5 g/m^2. In 2.5 and 5.0 g/m^2 DE treatments, exposure for 12 h at a temperature of 42 °C resulted in 73–77% mortality of adults with 100% mortality observed after 24 h. At 44 and 46 °C, 100% mortality of adults was observed after 24 h of exposure at both DE rates. At these two temperatures, the high mortality in untreated arenas (controls) at 8, 12, and 24 h exposures ranged from 27 to 100% confounding the true effects of DE. Our results suggest that combined use of DE and temperatures below 50 °C can be used as an integrated approach for controlling insects in empty bins prior to storage of newly-harvested grain.

1. Introduction

Several species of stored-product insects have been reported from empty bins (Chao, 1954; Wright, 1991; Reed et al., 2003; Arthur et al., 2006; Hagstrum et al., 2008). Removal of grain and grain debris from empty bins prior to storing newly-harvested grain can help in reducing insect numbers in stored grain (Reed et al., 2003; Arthur et al., 2006). Some stored-product insects are long-lived, and removal of residual grain and grain debris, which serves as their food, may not be sufficient to control them. The use of an approved insecticide (Arthur and Subramanyam, 2012) after sanitation of empty bins is shown to provide effective control of insects. Bridgeman (1994) conducted tests with an amorphous silica (Dryacide®, A & R McLaughlin Private Limited, Wembley Downs, Western Australia) applied to storage surfaces at 6–8 g/m^2 in four 100 m long × 15 m wide × 5 m high rectangular storage structures in Australia. Treatment efficacy was verified by using 30 flour-baited cardboard traps (Wright, 1991) in each storage facility. Trapping three weeks before sanitation and three weeks after sanitation showed no significant difference in the percentage of traps with beetles and psocids. However, after application of Dryacide®, trapping over the next 11 weeks showed a decrease in the percentage of traps with beetles and psocids from 18 to 3% and from 90 to 40%, respectively.

Clean, empty bins can also be treated with several alternatives to chemical insecticides. Two approved alternatives to chemical insecticides include the use of diatomaceous earth or DE and the use of high temperatures (Subramanyam and Roesli, 2000; Tilley et al., 2007; Subramanyam et al., 2011), or a combination of DE and heat (Dowdy and Fields, 2002).

There are numerous studies documenting the effectiveness of DE dusts against stored-product insects, mostly on grains (McGaughey, 1972; Korunić et al., 1996; Subramanyam and Roesli, 2000; Kavallieratos et al., 2005, 2010; Vardeman et al., 2007). There are limited published studies examining the efficacy of heat

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treatment of empty bins against adults of stored-product insects. Tilley et al. (2007) reported 100% mortality of adults of the red flour beetle, *Tribolium castaneum* (Herbst), lesser grain borer, *Rhyzopertha dominica* (F.), and rice weevil, *Sitophilus oryzae* (L.), by raising temperatures of the bin's floor to a minimum of 50 °C for up to 2–4 h. Moog and Maier (2007) reported 77–91% mortality of adults of the maize weevil, *Sitophilus zeamais* (Motschulsky), when exposed for 3 h at 55 °C in the plenum area of empty bins. The mortality of *T. castaneum* adults in the plenum area at this temperature and exposure time was 72–87%. However, similar exposure in areas 1.83 m above the plenum resulted in 100% mortality of both species. The authors inferred that the lack of uniform distribution of hot air at the plenum may have resulted in less than 100% mortality of beetles.

Previous research has shown that heat treatments in combination with DE increased mortality of stored-product insects. Dowdy (1999) reported mortality of unfed and fed adults of *T. castaneum* adults exposed to untreated glass Petri dishes and dishes treated with 5 g/m^2 of four DE dusts at 34 and 50 °C and 65% r.h. The DE formulations used were Concern® (Necessary Organics, Inc., New Castle, Virginia, USA), Natural Guard® (VPG Co-op Gardening Group, Inc., Bonham, Texas, USA), Insecto® (Natural Insecto Products, Inc., New Castle, Virginia, USA), and Protect-It® (Headley Technologies, Vancouver, British Columbia, Canada). Exposure of unfed insects for 15–30 min to 34 °C alone resulted in 0–1.3 and 42.5–55.0% mortality, when mortality assessments were made 1 d and 7 d after exposure, respectively (Dowdy, 1999). A similar exposure to 50 °C resulted in 1.3–28.8 and 51.3–65.0% when assessments were made 1 and 7 d after exposure, respectively. Adults that were fed or had access to food showed reduced mortalities that ranged from 0 to 1.3% and 0–56.3%, irrespective of whether observations were made 1 or 7 d after exposure. Protect-It® was the most efficacious dust producing 91.3–100% mortality of unfed adults after a 15–30 min exposure to 34 and 50 °C when mortality was assessed 1 d after exposure. The mortality with the other three DE dusts was greater at 50 °C compared to 34 °C, and the mortality of adults ranged from 8.9 to 76.3% based on mortality 1 d after exposure. However, all dusts produced 97.5–100% mortality at both temperatures when mortality of adults was assessed after 7 d. Mortality of adults that were unfed never reached 100%, irrespective of the temperature, exposure time, and post-mortality assessment time, except for adults exposed for 30 min to Protect-It® at 50 °C. These results suggest that sanitation, in conjunction with heat and DE, is more effective than heat alone or DE plus heat. Additionally, this study also showed delayed mortality effects associated with heat alone and heat plus DE. Fields et al. (1997) reported that in an oat mill, completely mortality of adults of the confused flour beetle, *Tribolium confusum* Jacquelin du Val, occurred when temperatures reached 47 °C after 32–38 h, but in the presence of DE complete mortality of adults occurred when temperatures reached 41 °C. Dowdy and Fields (2002) evaluated heat in combination with application of Protect-It® applied at 0.3 g/m^2 to second and third floor surfaces of a pilot flour mill subjected to a heat treatment against *T. confusum* adults. The benefits of DE were only evident on the south side of the second floor where temperatures did not quickly reach 47 °C. At the end of the heat treatment, adults exposed to partially and fully treated DE floor surfaces had 50 and 75% mortality, respectively, compared to 15% mortality of those exposed to heat alone. Ebeling (1994) showed that the time required for 100% mortality of the German cockroach, *Blatella germanica* (L.), at a temperature of 43.3 °C in the presence of a silica aerogel, a synthetic silica, was reduced from 147 to 41 min.

To our knowledge, there are no published studies that investigated the combined efficacy of DE and a range of temperatures on concrete surfaces, such as those found in empty bins. The combination of these treatment methods would involve lower energy inputs to obtain temperatures lethal to insects (Fields et al., 1997). Eliminating stored-product insects in empty bins prior to storage of newly-harvested grain, along with additional integrated pest management methods, such as bin sanitation, can increase the profitability and quality of stored grain in a more sustainable and environmentally friendly manner.

In the present investigation, laboratory experiments were designed to examine the influence of five temperatures below 50 °C, two DE application rates to concrete arenas, and four exposure times on mortality of *T. castaneum* adults. Concrete arenas in 9-cm Petri dishes simulated the floor of empty bins.

2. Materials and methods

2.1. Insects

Cultures of *T. castaneum* were reared in the Stored-Product Insect Research and Education Laboratory at Kansas State University in the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, USA. Cultures were reared in 0.94-L glass jars filled with 250 g of a medium consisting of 95% organic, whole wheat flour (Heartland Mills, Marienthal, Kansas, USA) and 5% (by wt) brewer's yeast in growth chambers at 28 °C and 65% r.h. Jars were closed with metal lids fitted with filter papers and wire-mesh screens.

2.2. Concrete arenas

Ready-mix concrete (Rockite, Hartline Products Co., Inc., Cleveland, Ohio, USA) was mixed with tap water to make a slurry. The slurry was poured into plastic Petri dishes (Fisher Scientific, Denver, Colorado, USA) with a diameter of 9 cm, height of 1.5 cm, and surface area of ~62 cm^2. The ratio of grams of concrete mix to milliliters of water used to fill the Petri dishes was 2:1. The slurry was allowed to dry in the Petri dishes for 48 h before dishes were used in experiments.

2.3. Diatomaceous earth application

The DE formulation used for experiments was DiaFil610® (Imerys Minerals California, Inc., San Jose, California, USA). DiaFil® 610 is natural fresh water DE, white in color, and has an average particle size of 10 μm (Korunić, 1997). It has a surface area of 26–28 m^2/g. The DE moisture content was 3–5%. DE was applied at either 2.5 or 5.0 g/m^2, the recommended rate for application to empty storage bins for insect management, directly onto the concrete arenas. After adding DE, the Petri dishes were gently shaken in a counter clockwise manner to evenly distribute DE on concrete arenas. Control treatment (0 g/m^2) included concrete arenas that were not treated with DE.

2.4. Bioassays

Adults of *T. castaneum* used in experiments were separated from diet using a sieve with 841 μm openings (Seedburo Equipment Co., Chicago, Illinois, USA). Ten adults of 1–4-weeks of age and of mixed sex were aspirated and placed on untreated and DE-treated concrete arenas. After insect introduction, concrete arenas were placed inside incubators with a volume of 0.14 m^3 (Isotemp Standard Lab Incubator, Fisher Scientific, Denver, Colorado, USA) set at 28, 36, 42, 44, and 46 °C. The temperature and humidity levels were measured using HOBO® data loggers (Onset Computer Corporation, Bourne, Massachusetts, USA). Humidity levels at 28, 36, 42, 44, and 46 °C were an average of 65, 21, 20, 19, and 18% r.h., respectively.

Generally, at elevated temperatures humidity levels are around 22–25% (Mahroof et al., 2003; Subramanyam et al., 2011). At each temperature, 20 untreated concrete arenas and 20 arenas each treated with DE at 2.5 or 5.0 g/m^2 were placed in incubators. At each of the temperatures, a replication consisted of five arenas representing a DE application rate of 0, 2.5, or 5.0 g/m^2 that were sampled after 4, 8, 12, and 24 h of exposure. At each exposure time, all adults from five arenas (50 adults) were pooled and placed in 150 ml round plastic containers holding 30 g of *T. castaneum* diet. Containers were closed with perforated lids covered with a fine mesh to prevent insect escape but allow air diffusion. Care was taken when picking adults from DE-treated concrete arenas so as to not transfer any DE to insect diet in containers. Containers were then held at 28 °C and 65% r.h. for an additional 24 h before mortality assessments were made. To determine mortality, adults were separated from the flour in containers using an 841-μm sieve. Live and dead adults were counted, and percentage mortality was calculated based on number of dead adults out of the total. Each temperature, DE rate, and exposure time combination was replicated six times.

2.5. Data analysis

Mortality in DE treatments was corrected for control mortality using Abbott's formula (1925). Corrected mortality data were transformed to angular values and analyzed using the general linear model procedure in SAS (SAS Institute, 2008). A three-way analysis of variance (ANOVA) was run to determine significant differences ($P < 0.05$) in mortality due to the main and interactive effects of temperature, DE rate, and exposure time. Corrected mortality data by temperature and DE rate were analyzed using one-way ANOVA to determine significant differences ($P < 0.05$) among exposure times. If ANOVA was significant, the Ryan-Einot-Gabriel-Welsch (REGWQ) step-down, pairwise multiple comparison procedure was used for mean separation (SAS Institute, 2008).

3. Results

The mortality of *T. castaneum* adults on untreated arenas at 28, 36, and 42 °C was <4% after 4–24 h of exposure (Table 1). At 44 °C, the mortality of adults was 1% at a 4 h exposure, but was between 27 and 63% at exposures of 8–24 h. Similarly, at 46 °C, mortality of adults was 19% at 4 h but was 48, 76, and 100% at 8, 12, and 24 h exposures, respectively. There were no significant differences in control mortality among exposure times at 28 and 36 °C ($F_{range} = 0.95–1.00$; df = 3, 20; $P \geq 0.4133$). However, significant differences were observed among exposure times at temperatures of 42, 44, and 46 °C ($F_{range} = 4.04–7.34$; df = 3, 20; $P_{range} = 0.0017–0.0212$).

The corrected mortality of *T. castaneum* adults exposed to 2.5 and 5.0 g/m^2 increased with an increase in temperature and exposure time. Three-way ANOVA (Table 2) showed that temperature, DE rate, and exposure time were significant ($P < 0.05$). Generally more adults died at 5.0 g/m^2 than at 2.5 g/m^2 (Figs. 1 and 2). At 44 °C, after 8 and 24 h, mortality in 2.5 g/m^2 treatment was 0.3–1.5% greater than in 5.0 g/m^2 treatment. Similarly, at 46 °C a 24 h exposure at both DE rates resulted in 100% mortality of adults. Except for the temperature and exposure time interaction, all two and three way interactions were not significant. The significant temperature and exposure time interaction indicated that the mortality responses over time at the different temperatures were not consistent.

One-way ANOVA of *T. castaneum* corrected mortality over time at 2.5 g/m^2 DE treatment at 28, 36, 42, 44, and 46 °C was significant ($F_{range} = 4.32–35.82$; df = 3, 20; $P_{range} = <0.0001–0.0168$).

Table 1
Mortality of *T. castaneum* adults on untreated concrete areas (control treatment) corresponding to elevated temperature and exposure time treatments.

Temperature (°C)	Exposure time (h)	Mean mortality ± SE (%)
28[a]	4	0.0 ± 0.0
	8	0.0 ± 0.0
	12	0.0 ± 0.0
	24	0.3 ± 0.3
36[b]	4	0.8 ± 0.7
	8	0.3 ± 0.3
	12	0.0 ± 0.0
	24	1.2 ± 0.8
42[c]	4	0.7 ± 0.7b
	8	0.7 ± 0.7b
	12	0.7 ± 0.4b
	24	3.7 ± 1.0a
44[c]	4	1.0 ± 1.0b
	8	26.7 ± 12.9ab
	12	44.0 ± 16.7a
	24	63.4 ± 12.2a
46[c]	4	19.3 ± 10.7c
	8	47.7 ± 17.8bc
	12	76.3 ± 16.5ab
	24	100.0 ± 0.0a

[a] There were no significant differences among exposure times ($F = 1.00$; df = 3, 20; $P = 0.4133$; by one-way ANOVA).
[b] There were no significant differences among exposure times ($F = 0.95$; df = 3, 20; $P = 0.4339$; by one-way ANOVA).
[c] At each temperature, means among exposure times followed by a different letter are significantly different ($P < 0.05$, by REGWQ test).

Table 2
Three-way ANOVA statistics showing main and interactive effective of temperature, DE rate, and exposure time on corrected mortality of *T. castaneum* adults.

Source	df	Mean square	*F*-value	*P*-value
Temperature	4	6.054	81.81	<0.0001*
Rate	1	0.340	4.59	0.0334*
Exposure time	3	7.660	103.51	<0.0001*
Temperature × rate	4	0.051	0.69	0.5997
Temperature × time	12	0.282	3.81	<0.0001*
Rate × time	3	0.008	0.11	0.9542
Temperature × rate × time	12	0.031	0.42	0.9545
Error	200	0.074		

*Significant ($P < 0.05$).

Similarly, *T. castaneum* corrected mortality at 5.0 g/m^2 DE treatment over time at each of the five temperatures was significant ($F_{range} = 3.56–38.78$; df = 3, 20; $P_{range} = < 0.0001–0.0326$). The trends observed in mortality of adults at each of the five temperatures at 2.5 g/m^2 and 5.0 g/m^2 were similar (Figs. 1 and 2). Only the 24 h exposure at both DE rates produced significantly greater ($P < 0.05$) mortality at 28, 36, and 42 °C. Adult mortality at 44 and 46 °C at both the DE rates reached 99–100% after a 24 h exposure. However, the high control mortality at 44 and 46 °C in DE exposures, especially at 12 and 24 h (Table 1) confounded from truly gauging the effect of DE at these temperatures. Nevertheless, the laboratory results support that a combination of heat plus DE can increase mortality of *T. castaneum* adults at temperatures below 50 °C.

4. Discussion

DE dusts are typically composed of 80–93% silicon dioxide, as well as varying amounts of organic matter, clay minerals, magnesium carbonate, among others (Antonides, 1998; Subramanyam and Roesli, 2000; Shah and Khan, 2014). DE works by abrading the insect's cuticle, interfering with the water retention ability of the insect, and results in death through desiccation (Korunić, 1998;

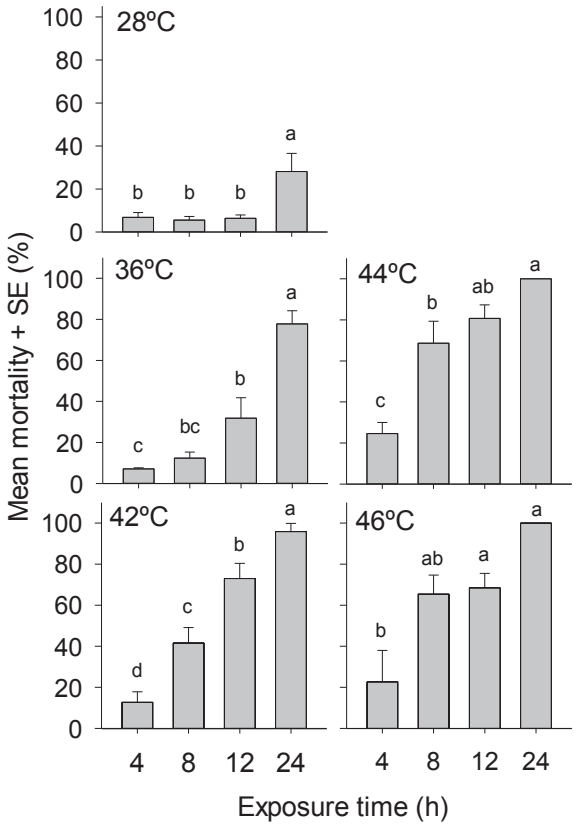

Fig. 1. Mean + SE corrected mortality of *T. castaneum* adults exposed for 4–24 h to 2.5 g/m^2 of DE on concrete arenas. At each temperature, means among exposure times followed by different letters are significantly different ($P < 0.05$, by REGWQ test).

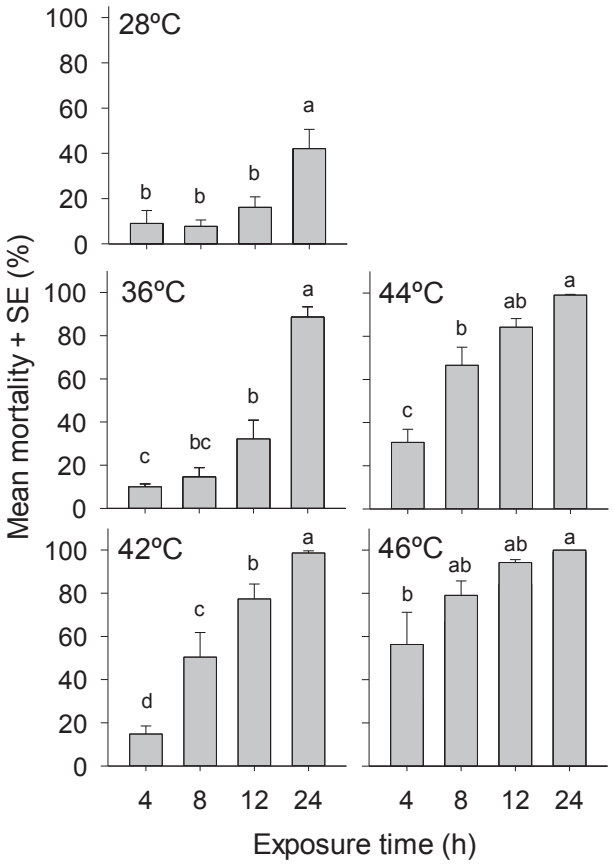

Fig. 2. Mean + SE corrected mortality of *T. castaneum* adults exposed for 4–24 h to 5.0 g/m^2 of DE on concrete arenas. At each temperature, means among exposure times followed by different letters are significantly different ($P < 0.05$, by REGWQ test).

Dowdy and Fields, 2002).

The use of elevated temperatures (50–60 °C) is a long-standing technology that is a safe and proven method to manage stored-product insects in empty bins and grain-processing facilities (Dosland et al., 2006; Subramanyam et al., 2011). Lethality in insects at high temperatures depends on both the temperature and exposure time (Evans and Dermott, 1981; Fields, 1992; Denlinger and Yocum, 1999; Mahroof et al., 2003). Death in insects exposed to elevated temperatures is due to quicker formation of lethal lesions, where the healing process that counters the lesions become less operative (Denlinger and Yocum, 1999). At elevated temperatures, insects' cuticular wax becomes more fluid, allowing loss of water, leading to death by desiccation (Hepburn, 1985). Additionally, insect's respiration, an indicator of overall metabolic rate, is adversely affected at elevated temperatures (Neven, 1998). At the cellular level, exposure of insects to elevated temperatures decreases hemolymph pH and ion concentration, denatures lipids, carbohydrates, proteins and nucleic acids, inactivates major glycolysis enzymes, and disrupts plasma membrane (Hochachka and Somero, 1984; Denlinger and Yocum, 1999; Neven, 2000).

In our study, the mortality of *T. castaneum* adults increased with increasing temperatures and exposure times. At temperatures of 28, 36, 42, and 44 °C the combined effect of DE and heat was better than heat alone. The use of DE at temperatures between 36 and 46 °C was shown to increase mortality of *T. castaneum* adults on concrete arenas compared to heat alone or DE treatment at 28 °C and 65% r.h. A DE treatment of 5.0 g/m^2 produced slightly greater mortality that varied with temperature and exposure time, than at 2.5 g/m^2, but the differences observed were not large enough to justify using the higher DE rate.

At 44 °C after a 24 h exposure, the DE treatments contributed to an additional 35–36% mortality of adults, because temperature alone provided 63% mortality. Unlike observations made by Tilley et al. (2007) at 50 °C, we observed 100% mortality of *T. castaneum* adults after a 24 h exposure to 46 °C alone on untreated arenas. Insects succumb to elevated temperatures greater than 35 °C, and at more extreme temperatures, the death of the insect is rapid. Fields, 1992). Our results support previous studies conducted at a few constant temperatures that the insecticidal effects of DE increase as the temperature increases (Arthur, 2000; Dowdy and Fields, 2002). The addition of DE to heated environments can result in a more rapid water loss from insects, especially at low humidities, resulting in faster death of insects (Mahroof et al., 2003). Furthermore, increased activity of insects at higher temperatures to seek cooler environments (Fields, 2006) may result in greater pick-up of DE particles, leading to rapid desiccation effects due to the combined effect of DE and heat. Our results suggest that it is possible to obtain effective control of *T. castaneum* adults by combining DE with temperatures of 44 or 46 °C. In practical heat treatments of empty bins (Tilley et al., 2007), unlike the laboratory experiments where temperatures remain constant, temperatures are dynamically changing over time. However, temperatures in the range of 44–46 °C can be maintained for several hours to obtain an effective kill of stored-product insects in the presence of DE when disinfesting empty bins. Additional studies are warranted in empty bins to determine minimum temperature-time combinations, with and without food, to show how the combined effect of DE and heat can be used for effective disinfestation under practical field conditions.

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