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Effects of Age and Sex on Mortality of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) Exposed to INSECTO®-treated Wheat

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ABSTRACT: INSECTO is a commercial diatomaceous earth dust registered in the US for grain treatment at 0.5 to 1 g/kg rates. In the laboratory, male and female adults of different specific ages of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae), were exposed for 7 days to hard red winter wheat treated with 0.25 g/kg of INSECTO at 30°C and 65% RH. The mortality of *T. castaneum* adults was similar between the sexes, but varied with adult age. Adults that are 0.5 and 2.5-days-old, and those that were 64.5-days old, were about 3 to 6 times more susceptible to INSECTO than 4.5 to 32.5-day-old adults. Mortality of 4.5 to 32.5-day-old adults was similar (4.5 to 5.5%) Therefore, when evaluating the efficacy of diatomaceous earth dusts on *T. castaneum*, unsexed adults between 4.5 and 32.5 days should be used in bioassays to reduce variation in mortality data attributable to adult age.

KEY WORDS: *Tribolium castaneum*, Diatomaceous earth, Stored grain, age-specific mortality

Introduction

Inert dusts are materials that contain silica, such as diatomaceous earth, silica aerogels, acid-activated kaolin, and clays (Golob, 1997; Korunic, 1998). These materials abrade and adsorb the water resistant epicuticular lipid layer layers of insects, causing death by desiccation (Ebeling, 1971). There are several commercial formulations of diatomaceous earth dusts currently registered for use on stored grain and for treating empty grain storage facilities (Subramanyam and Roesli, 2000).

David and Gardiner (1950) and Subramanyam and Roesli (2000) described biological and environmental factors affecting the action of inert dusts, including diatomaceous earth dusts, on adults of stored-product insects. These include physical properties of diatomaceous earth particles (hardness, specific surface, size, and shape) (Chiu, 1939; Ebeling, 1971; Melichar and Willomitzer, 1967; Korunic, 1997), qualitative and quantitative differences in cuticular lipid composition among insects (Ebeling, 1971), cuticular texture and morphology (David and Gardiner, 1950), nutritional status of insects (starved versus fed) (Vrba *et al.*, 1983; Loschiavo, 1988), retention of dust on insects (le Patourel *et al.*, 1989), duration of contact with the dust (Singh, 1981; Arthur, 2000), and grain temperature and moisture or ambient relative humidity (Arthur, 2000). David and Gardiner (1950) mentioned that the susceptibility of insects exposed to dust insecticides, including inert dusts, may be influenced by adult age and sex, but they did not provide any experimental data. Generally, when evaluating the efficacy of diatomaceous dusts on stored-product insects, adults of mixed ages and sexes or unsexed adults of a specific age are used.

To our knowledge, the influence of adult age and sex on susceptibility of stored-product insects exposed to a diatomaceous earth is unknown. Therefore, laboratory experiments were conducted by exposing various ages of male and female adults of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae) to INSECTO, a commercial diatomaceous earth dust registered in the United States.

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This paper presents results of research only. Mention of a proprietary product name does not constitute an endorsement by the United States Department of Agriculture or Kansas State University.

Materials and Methods

Cultures of *T. castaneum*, reared on a diet of whole-wheat flour plus 5% brewers yeast, were maintained at the Grain Marketing and Production Research Center, Manhattan, Kansas. Pupae were collected daily from the culture and sexed (Park, 1934). The male and female pupae were placed into separate 0.95-liter jars with a layer of diet. Adult males and females that emerged from pupae were placed in 0.95-liter jars with a layer of diet. These were held at 30.5 ± 0.4°C and 65 ± 10% RH. Using this method, we were able to obtain male and female adults of different median ages (±0.5 day) as follows: 0.5, 2.5, 4.5, 8.5, 16.5, and 32.5 days, post-eclosion. Sufficient male pupae were not available to set-up the 64.5-days-old cohort; however, we had adequate numbers of female pupae to setup the 64.5-day-old cohort. The adults were aged on the diet until they were used in the tests.

In the laboratory, 1300 g of hard red winter wheat (12.2% moisture) were treated with INSECTO at a rate of 0.25 g/kg of grain. This rate is well below the labeled rate of 0.5 to 1 g/kg (Subramanyam *et al.*, 1998). This rate was chosen to gauge effects of adult sex and age on susceptibility to INSECTO, because at the labeled rates, mortality of *T. castaneum* approaches 100% (Subramanyam *et al.*, 1994). For treatment, the grain and required amount of INSECTO were placed in a 3.8-liter glass jar that was tumbled on a ball-mill roller for 10 min. After 5 min, the jar was shaken briefly by hand before resuming tumbling on the ball-mill roller. Untreated grain (1300 g) served as the control treatment. About 650 g of untreated or INSECTO-treated grain were placed in separate 0.95-liter glass jars with wire-mesh and filter paper lids. Into each jar, 20 male or 20 female adults of a specific median age were introduced. Each treatment was replicated 5 times. After adult introduction, jars were closed with lids and held at 30.5 ± 0.4°C and 65 ± 10% RH without light. After 7 days the wheat was sieved using a 1.379 mm sieve (U.S. Standard Sieve No. 12) to count the number of live and dead adults. Mortality (expressed as a percentage) was determined based on the number of dead insects out of the total exposed.

The experiment was run using a randomized complete block design. The data, except for the 64.5-day-old females, were subjected to 3-way analysis of variance (ANOVA) using the PROC GLM procedure (SAS Institute, 1997) to determine the influence of main (treatment [control vs. treated], sex, and age) and interaction effects on adult mortality at $\alpha = 0.05$. If mortality was not significantly different between the sexes, male and female mortality data were pooled. The pooled data were subjected to 1-way ANOVA and Fisher's Protected Least Significant Difference (LSD) (SAS Institute 1997) to determine significant differences in mortality among 0.5 to 32.5-day-old adults. The 1-way ANOVA was performed only on adults exposed to INSECTO-treated wheat, because mortality of adults on untreated (control) grain was negligible. Similarly, 1-way ANOVA and LSD analysis were also performed on female mortality data to determine differences among 0.5 to 64.5 day-old adults.

Results and Discussion

The mean mortality among different ages (0.5 to 32.5 days) of male and female adults on untreated wheat ranged from 0–2% (mean ± standard deviation, SD = 0.33 ± 1.26). On INSECTO-treated wheat, corresponding mean mortality of male and female adults across the various median ages ranged from 2 to 34% (mean ± SD = 11.17 ± 12.05). The 3-way ANOVA showed that adults exposed to INSECTO-treated wheat had significantly higher mortality ($P < 0.05$) than those exposed to untreated wheat (Table 1). Mortality of adults also was highly significant ($P < 0.05$) among the different ages tested. However, males and females were equally susceptible to INSECTO ($P > 0.05$). The treatment × age interaction

Table 1. Analysis of variance (ANOVA) results showing the influence of main and interaction effects on mortality of adult *Tribolium castaneum*.

Source	Degrees of freedom	Mean square	*F*-value	*P*-value
Treatment[a]	1	3520.83	147.60	0.0001*
Age[b]	5	641.50	26.89	0.0001*
Sex	1	40.83	1.71	0.1939
Treatment × Age	5	580.83	24.35	0.0001*
Treatment × Sex	1	40.83	1.71	0.1939
Age × Sex	5	14.83	0.62	0.6835
Treat × Age × Sex	5	20.83	0.87	0.5021
Error	96	23.85		

[a]Mortality on untreated versus INSECTO-treated wheat.
[b]Analysis based on 0.5 to 32.5 day-old adult data.
*Significant ($P < 0.05$; 3-way ANOVA).

was the only significant interaction (Table 1). Because mortality of insects on untreated wheat was negligible, the significant interaction suggested that the mortality of adults exposed to INSECTO-treated wheat was not consistent across the different median ages tested.

The mortality data of 0.5 to 32.5-day age male and female adults exposed to INSECTO-treated wheat was pooled because of lack of significant differences between sexes. The pooled mean mortality ranged from 4.5 to 32.5% (Fig. 1). Adults of 2.5 days were more susceptible to INSECTO than any other age group (32.5 ± 7.9), and were significantly different from the 0.5 day-old adults (14.0 ± 7.7) ($F = 26.66$; d.f. = 5, 54; $P = 0.0001$). The mean mortality of 4.5 to 32.5 day-old adults was similar and ranged from 4.5 to 5.5 ($P > 0.05$), and significantly lower than 0.5 and 2.5 day-old adults. The cuticular lipid composition in a given insect species changes with age and stage, and after exposure to desiccant dusts (Howard *et al.*, 1995). We are unaware of any information on the changes in cuticular lipids of newly-emerged versus older *T. castaneum* adults to explain reasons for the increased mortality of 0.5 to 2.5-day-old adults. The mortality of insects exposed to desiccant dusts increases with increased water loss through the cuticle (Chiu, 1939; Ebeling, 1971). The reduced tolerance to water loss of newly emerged *T. castaneum* adults may be a plausible reason for the increased mortality of 0.5 to 2.5-day-old adults exposed to INSECTO.

It is unlikely that the amount of time the adults spent on the flour and yeast diet before being exposed to whole grain treated with INSECTO affected the results of the experiment. Diatomaceous earth does not cause mortality by ingestion, but by adsorption of the cuticular lipids, thereby causing desiccation. Mortality of control insects was negligible, indicating that the transfer from the flour diet to whole grain did not increase mortality.

The mean mortality (±SD) of 64.5-day-old female *T. castaneum* adults exposed to INSECTO-treated wheat was 27.0 ± 4.9, while mean mortality on untreated wheat was 0%. Mortality of females varied among the different median ages ($F = 7.69$; d.f. = 6, 28; $P = 0.0001$). The mortality of 64.5-day-old females was not significantly different ($P > 0.05$) from 0.5 or 2.5-day-old females, but was significantly higher than 4.5 to 32.5-day-old adults. Because 0.5 to 32.5-day-old male and female adults of *T. castaneum* responded similarly to INSECTO, 64.5-day-old males may be equally susceptible to INSECTO. Older adults are less vigorous, and may therefore succumb easily to environmental stresses, such as a desiccant dust exposure. This may explain the increased susceptibility of 64.5-day-old adults to INSECTO.

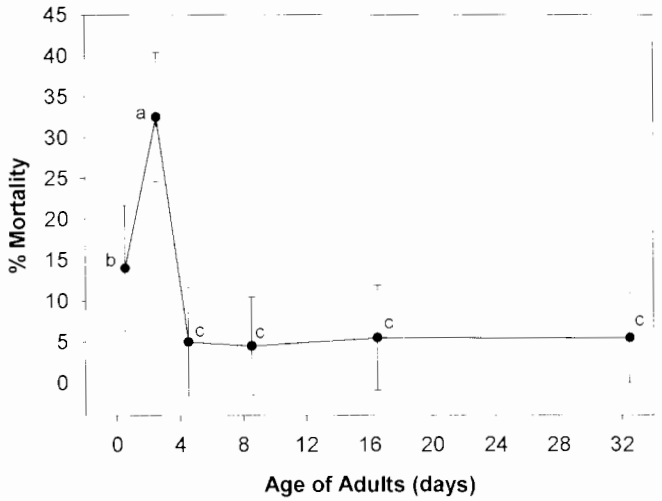

Fig. 1. Effects of age of adult *T. castaneum* on mortality induced by grain treated with INSECTO. Vertical bars indicate standard deviation of the mean. Means with the same letter are not significantly different ($P = 0.05$, d.f. = 54, LSD = 6.06).

This is the first paper examining the effect of adult sex and age of a stored-product insect species on mortality caused by diatomaceous earth. Our results showed that male and female *T. castaneum* were similar in their susceptibility to INSECTO. Therefore, when evaluating efficacy of diatomaceous earth dusts, using unsexed adults of *T. castaneum* in bioassays would not contribute to increased variation in mortality. However, adult age does affect variation in mortality. When conducting diatomaceous earth bioassays with *T. castaneum*, they should be between 4.5 to 32.5-days-old, so that the mortality among samples at a given rate would be consistent. Using adults that are between 0.5 to 2.5 days or 64.5 days could inflate the variation in the mortality. For example, when the mortality of adults (based on pooled male and female mortality data) was averaged across 0.5 to 32.5-day-old age groups, the mean mortality was 11.2 with a standard deviation (SD) of 12.1. This SD was two times higher than SD observed at any age group (see Fig. 1). Therefore, when conducting studies on the efficacy of diatomaceous earth, researchers should standardize *Tribolium castaneum* by age to reduce variability of their results.

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Literature Cited

Arthur, F. H. 2000. Toxicity of diatomaceous earth to red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae): effects of temperature and relative humidity. J. Econ. Entomol. 93:526–532.

Chiu, S. F. 1939. Toxicity studies of so-called "inert" materials with the rice weevil and the granary weevil. J. Econ. Entomol. 32:810–821.

David, W. A. L., and B. O. C. Gardiner. 1950. Factors influencing the action of dust insecticides. Bull. Entomol. Res. 41:1–61.

Ebeling, W. 1971. Sorptive dusts for pest control. Ann. Rev. Entomol. 16:123–158.

Golob, P. 1997. Current status and future perspectives for inert dusts for control of stored product insects. J. Stored Prod. Res. 33:69–79.

Howard, R. W., C. D. Howard, and S. Colquhoun. 1995. Ontogenetic and environmentally induced changes in cuticular hydrocarbons of *Oryzaephilus surinamensis* (Coleoptera: Cucujidae). Annals Entomol. Soc. Am. 88:485–495.

Korunic, Z., 1997. Rapid assessment of the insecticidal value of diatomaceous earths without conducting bioassays. J. Stored Prod. Res. 33:219–229.

Korunic, Z., 1998. Diatomaceous earths, a group of natural insecticides. J. Stored Prod. Res. 34:87–97.

le Patourel, G. N. J., M. Shawir, and F. I. Moustafa. 1989. Accumulation of mineral dusts from wheat by *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). J. Stored Prod. Res. 25:65–72.

Loschiavo, S. R. 1988. Availability of food as a factor in the effectiveness of a silica aerogel against merchant grain beetle (Coleoptera: Cucujidae). J. Econ. Entomol. 81:1237–1240.

Melichar, B., and J. Willomitzer. 1967. Bewerting der physikalischen insectizide. Sci. Paraceut. 2:589–597. Proc., 25th Cong. of Pharam. Sci., Prague, 1965.

Park, T. 1934. Observations on the general biology of the flour beetle *Tribolium confusum* Duval. Quart. Rev. Biol. 9:36–54.

SAS Institute. 1997. SAS/STAT software changes and enhancements through release 6.12. SAS Institute, Cary, NC.

Singh, J. 1981. Studies on the joint insecticidal action of synthetic pyrethroids and sorptive dusts. Ph.D. Thesis, University of London.

Subramanyam, Bh., N. Madamanchi, and S. Norwood. 1998. Effectiveness of INSECTO applied to shelled maize against stored-product insect larvae. J. Econ. Entomol. 91:280–286.

Subramanyam, Bh., and R. Roesli. 2000. Inert dusts. *In* Bh. Subramanyam, and D. W. Hagstrum (eds.), Alternatives to Pesticides in Stored-Product IPM, pp. 321–380. Kluwer Academic Publishers, Boston, MA.

Subramanyam, Bh., C. L. Swanson, N. Madamanchi, and S. Norwood. 1994. Effectiveness of INSECTO, a new diatomaceous earth formulation in suppressing several stored-grain insects. *In* E. Highley, E. J. Wright, H. J. Banks, B. R. Champ (eds.), Proceedings, 6th International Working Conference on Stored-Product Protection, Vol. 2, pp. 650–659. CABI, Wallingford, Oxon, U.K.

Vrba, C. H., H. P. Arai., and M. Nosal. 1983. The effect of silica aerogel on the mortality of *Tribolium confusum* (DuVal) as a function of exposure time and food deprivation. Can. J. Zool. 61:1481–1486.