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Mechanisms for tolerance to diatomaceous earth between strains of *Tribolium castaneum*

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Abstract

Fourteen strains of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) had mortalities ranging from 5 to 100% when exposed to diatomaceous earth at 600 ppm for seven days. The most tolerant strain had a lethal dose for 50% of the population (LD_{50}) of 413 ppm and the most susceptible strain had a LD_{50} of 238 ppm. Adults of the tolerant strain were lighter (2.0 mg) than the susceptible strain (2.6 mg). Tolerant adults lost water at lower rate (6 μg h^{-1} than susceptible adults (12 μg h^{-1}), when held in wheat treated with 600 ppm diatomaceous earth for 24 h, than held at 5% r.h. with no food. Tolerant adults that were not exposed to diatomaceous earth lost water at a lower rate (3 μg h^{-1}) than susceptible adults (5 μg h^{-1}). Both strains, exposed and not exposed to diatomaceous earth died when their water content was between 33 and 37% of their total weight. Insects taken directly from the cultures had 52% (tolerant) and 53% (susceptible) of their total weight as water. Tolerant adults moved slower through grain and across filter paper than susceptible adults. Tolerant adults avoided wheat treated with diatomaceous earth at concentrations as low as 75 ppm, whereas the adults from the susceptible strain did not avoid diatomaceous earth, even at 600 ppm. The consequences of a strain tolerant to diatomaceous earth is discussed with respect to the use of diatomaceous earth to control stored-product insect infestations.

Introduction

Since the 1950's, synthetic insecticides have been the method of choice to control stored-product insects (Subramanyam & Hagstrum, 1995; White & Leesch, 1995). Nevertheless, there is an increasing concern over worker exposure, detrimental effects on the environment, reduced efficacy due to resistant insect populations, and pesticide residues in the finished food product. There are a number of alternatives to chemical control of stored-product insects, including low or high temperature (Fields, 1992), sanitation, biological control (Flinn et al., 1996; Snelson, 1987), and natural products (Golob et al., 1982).

One natural product that has had increasing use in the last decade is diatomaceous earth. Diatomaceous earth is a soft rock that is the fossilised remains of unicellular algae called diatoms. Depending upon the geological source, it is almost pure amorphous silicon dioxide, which is non-toxic to mammals (IARC, 1997). Different geological sources have different efficacies (Korunic, 1998). Diatomaceous earth removes the insect's cuticular waxes, and insects die from desiccation (Ebeling, 1971). For protection of seed, it is usually mixed into the grain as a powder. It is inert, and as long as the grain remains dry (below 15% moisture content), it will not lose its activity.

Stored-product insects show a wide range of susceptibility to diatomaceous earth (Fields & Korunic, 2000). Under laboratory selection experiments, the tolerance to diatomaceous earth could be increased two-fold with the coleopterans *Cryptolestes ferrugineus* (Stephens) and *Rhyzopertha dominica* (Fabrius) and 1.6-fold with *Tribolium castaneum* (Herbst) (Korunic & Ormesher, 1998). The mechanisms for this increased tolerance were not studied. Resistance to chemical insecticides is a widespread phenomenon. There are several stategies to delay or prevent the development of resistance in insect populations (Subramanyam & Hagstrum, 1995), and an understanding of the mechanism of resistance would be useful to prevent widespread resistance to diatomaceous earth in stored-product populations.

Our objective is to examine the susceptibility of several strains of *T. castaneum* to diatomaceous earth, and to understand the mechanism responsible for the differences in susceptibility.

Materials and methods

Diatomaceous earth. We used Protect-It® diatomaceous earth (Hedley Techologies Inc., Canada) in all tests. It is a mixture of fresh water diatomaceous earth with 10% silica aerogel (Korunic & Fields, 1998), which contains over 87% amorphous silicon dioxide, 3% Al_2O_3, 1% Fe_2O_3, less than 1% CaO, MgO, TiO_3, P_2O_3, and 3 to 6% water. The median particle size is 5.4 μm, specific gravity is 0.20, pH is between 5.5 and 5.7 in a 10% slurry (Korunic et al., 1996).

Variation between strains of T. castaneum. Adults from 14 strains (Table 1) of *T. castaneum*, were tested for their susceptibility to diatomaceous earth. All tests and rearing were carried out at 30 ± 1 °C and 70 ± 5% r.h., unless stated otherwise. Strains were reared in the dark, on wheat flour and brewer's yeast (20:1 w:w). Pupae were sexed (Lange, 1967) and males and females were reared separately. For each treatment, 600 ppm (300 mg) of diatomaceous earth was added into a jar containing 500g of grains of wheat (cv Camp Rémy, spring wheat) and then mixed by hand. Untreated grain was used as a control. For each strain and sex, 50 insects, aged between one and two weeks, were introduced in each jar with two or three replicates depending on the strain. After seven days, the grain was sieved, the number of live and dead insects was noted, and all grain, dust, and insects replaced in the jars. After 21 days, mortality was assessed a final time. Mortality data was analysed by ANOVA. A Gupta test was used to distinguish differences between sex and strains concerning the diatomaceous-earth- tolerance.

Dose response to diatomaceous earth. We chose the most tolerant (Abidjan) and the most susceptible (Georgia) strains to examine the basis for the differences in susceptibility to diatomaceous earth. To obtain more detailed information on the levels of susceptibility to diatomaceous earth, we ran a dose response test with the two strains. For the tolerant strain 0, 400, 600, 1000, and 2000 ppm diatomaceous earth was used. For the susceptible strain 0, 100, 200, 400, and 600 ppm diatomaceous earth was used. The diatomaceous earth was placed in the jar with the wheat, and mixed by placing the jar on a mechanical roller for 10 min. For each concentration, there was 200 g of wheat (cv AC Barrie, Canadian Hard Red Spring) with 50 unsexed adults, aged between one and two weeks. There were three repetitions/treatment. After seven days, the grain was sieved and the mortality noted. The LD_{50} (lethal dose for 50% of the population) and LD_{95} (lethal dose for 95% of the population) of the two strains for diatomaceous earth was calculated using Polo-PC (Roberston & Preisler, 1992).

Rate of water loss. Fifteen live adults of each strain were removed from the culture medium and immediately weighted (Metler M3 Fisher Scientific, Canada, accuracy ± 1 μg). Insects were then dried during 24 h in an oven at 60 °C after which they were weighed again to determine their dry weight. Water content was the difference between the fresh weight and the dry weight divided by the fresh weight.

Fifteen adults of both strains were held for 24 h on wheat treated with 600 ppm of diatomaceous earth and untreated wheat. A grain moisture content of 16% was chosen to minimise the mortality during the first 24 h. The adults were gently sieved from the wheat, and placed in small individual aluminium dishes, without food. They were held in the dark, at 25 ± 1 °C, inside a desiccator with $CaCl_2$ to reduce the relative humidity to less than 5%. The dishes containing the adults were weighed every 2 h, until all the diatomaceous earth treated individuals were dead, after which measurements were taken every 6 h until all the remaining adults had died. The room where the insects were weighed varied between 22–25 °C and 23–51% r.h. We analysed the mass loss that we assume to be the rate of water loss with linear regression. The cuticular permeability was calculated as follows: P = L/(SV) (Hadley, 1994) where P is cuticular permeability, L = water loss (μg h^{-1}), S = surface area (cm^2), V = vapor pressure difference (25 °C, 5% r.h. it is 23.46 Torr). The surface area of insects was estimated using Meeh's formula: $S = kW^{0.667}$ (Hadley, 1994) where S = surface area, W = weight, and k = 6.7 ±

Table 1. Mortality of *T. castaneum* males and females adults after 7 and 21 days on wheat grains treated with 600 ppm of diatomaceous earth at 30 °C, 70% r.h., listed in order of tolerance at 7 days

Origin	Date collected	Mortality ± S.E. (%)			
		7 days		21 days	
		Male	Female	Male	Female
Georgia (USA) (Susceptible)	1980	100 ± 0 a	100 ± 0 a	100 ± 0 a	100 ± 0 a
Waseco county, Minnesota (USA)	1982	99 ± 1 a	99 ± 1 a	100 ± 0 a	100 ± 0 a
Maff (UK)		85 ± 9 a	91 ± 3 a	100 ± 0 a	100 ± 0 a
Japan		89 ± 3 a	83 ± 3 a	99 ± 1 a	97 ± 3 a
Naphin (Philippines)	1976	54 ± 16 b	92 ± 2 a	100 ± 0 a	100 ± 0 a
Pakistan		57 ± 12 b	65 ± 4 b	91 ± 2 a	100 ± 0 a
Vancouver, British Columbia (Canada)	1976	31 ± 5 c	86 ± 10 a	96 ± 1 a	100 ± 0 a
Kansas (USA)		48 ± 22 b	46 ± 8 b	97 ± 1 a	96 ± 2 a
Nigeria	1961	32 ± 2 c	32 ± 6 c	92 ± 4 a	92 ± 5 a
Landmark, Manitoba (Canada)	1991	25 ± 9 c	19 ± 6 c	92 ± 2a	85 ± 5a
Argyle, Manitoba (Canada)	1992	20 ± 9 c	18 ± 4 c	71 ± 18 b	66 ± 14 b
Saint John, New Brunswick (Canada)	1976	8 ± 5 c	12 ± 3 c	88 ± 0 a	98 ± 2 a
Saint John, New Brunswick (Canada)	1976	7 ± 3 c	11 ± 3 c	84 ± 11 a	99 ± 1 a
Abidjan (Ivory Coast) (Tolerant)	1980	6 ± 2 c	4 ± 2 c	13 ± 4 c	60 ± 8 c

Means followed by the same letter are not significantly different. $n = 3$; 50 insects/repetition; Gupta test ($P < 0.05$).

Table 2. Comparison of several parameters (±S.E.) in relation with the rate of water loss in extremely dry conditions of individuals treated wit 600 ppm of diatomaceous earth (DE) treated and untreated individuals from two strains of *T. castaneum*; Georgia (susceptible) and Abidja (tolerant), at 25 °C; 5% r.h.

	Untreated		DE-treated		ANOVA (P)		
	Tolerant	Susceptible	Tolerant	Susceptible	Tolerant vs susceptible	Untreated vs DE-treated	Strain × treatment
Weight after 24 h in grains (mg)	1.98 ± 0.07 a	2.57 ± 0.04 b	1.80 ± 0.05 c	2.31 ± 0.06 d	<0.0001	0	0.46
Weight at death (% of the initial)	65 ± 1 ac	66 ± 1 bc	64 ± 1 ac	70 ± 2 b	0.0066	0.77	0.072
Water remaining at death (%)	34 ± 1 ab	35 ± 1 b	33 ± 1 ab	37 ± 1 c	0.0006	0.376	0.071
Permeability of cuticle (μg cm^{-2} h^{-1} Torr^{-1})	1.27 ± 0.07 a	1.52 ± 0.08 a	2.65 ± 0.30 b	4.24 ± 0.56 c	0.0009	<0.0001	0.039
Rate of water loss of live individuals (μg h^{-1})	3.1 ± 0.2 a	4.5 ± 0.2 b	6.1 ± 0.6 c	11.9 ± 1.5 d	< 0.0001	<0.0001	0.004
Rate of water loss of dead individuals (μg h^{-1})	11.5 ± 0.7 a	16.2 ± 1.4 ab	15.0 ± 1.4 a	24.0 ± 4.0 b	0.007	0.034	0.63
Duration of survival at 5% r.h. (h)	217 ± 11 a	194 ± 12 a	97 ± 11 b	49 ± 11 c	0.0019	<0.0001	0.26

Means in rows followed by the same letter are not significantly different. $n = 15$; Student Newman–Keuls test, $P < 0.05$.

0.4 (mean ± S.E.). To estimate k, six insects (three tolerant and three susceptible) were weighed and their surface area measured planimetrically. The data was analysed using an ANOVA. A Student Newman-Keuls test was used to distinguish differences between the strains. To normalize the premeability, rate of water loss of live and dead data were transformed using the arcsin square root transformation. The other data had a normal distribution and equal variance.

Movement. Sieves (depth = 5 cm, diameter = 20 cm, mesh opening = 2 mm) were filled in with 500 g of grain (thickness of the layer = 1 cm) and held 15 cm above a tray. One hundred adults of each strain were placed on a plate in the middle of the top surface of grain, and held there by an inverted Petri dish. The Petri dish was removed after 1 h, allowing the insects to leave the plate and enter the grain. There were ten replicates for each strain. The number of insects that passed through the grain mass after 24 h and 48 h was noted. Results were analysed by ANOVA.

Methods similar to Appel (1988) were used to measure horizontal movement. Adults of each strain were placed in a glass Petri dish lid (diameter = 16 cm), one adult/dish. The Petri dish was lined with black paper to allow us to see the insects under the infra red light and to allow the insects to move easily about the Petri dish. The dishes were held in a dark room, and observed with an infra red camera (WV-CD820, Panasonic Inc., Japan). A remote control system was used to move the camera, this minimised the disturbance to the insects. Sixty minutes after introducing the insects into the Petri dishes, the distance an insect moved in one minute was recorded. There were ten replicates for each strain. The experiment was run at room temperature (22–25 °C). Rates of movement (cm min^{-1}) were then compared by ANOVA.

Repellency. The repellency of diatomaceous earth was tested using an opaque choice chamber (Loschiavo, 1959). The chamber was cylindrical (height = 6 cm, diameter = 30 cm) with six pie-shaped sections and a raised platform in the centre. Three sections were filled with untreated wheat, and three were filled in with grain treated at 0, 30, 75, 150, 300 or 600 ppm of diatomaceous earth. Sections alternated treated and untreated to negate position artefacts. Strains were tested separately, with 150 insects/chamber, four replicates/concentration. Wheat at 16% moisture content was used to reduce the mortality due to desiccation. Adults were introduced to the platform by a hole in the top of the chamber. They were retained on the platform by an inverted Petri dish for 1 h, before the Petri dish was raised to allow the insects to enter the grain. After 24 h, the chamber was placed at −18 °C for 15 min to immobilise the insects, the grain removed from the sections, sieved, and the number insects in each section noted. Percentage of individuals found in the treated grains was analysed by ANOVA and means for each concentration were compared with a signification test with the theoretical value of 50% which would indicate no repellency.

Results

Mortality. There was a wide variation in the susceptibility of *T. castaneum* strains to diatomaceous earth (Table 1). After seven days, some strains had less than 10% mortality, whereas a few strains had over 95% mortality. Differences between strains are less after 21 days with most strains having over 95% mortality. In general there were no significant differences between the sexes, except for the strains from Vancouver (both 7 and 21 days, t-test, $P < 0.05$) and Pakistan, Saint John, and Abidjan (21 days only: t-test, $P < 0.01$). For the untreated controls, there was less than 0.2% mortality after seven days and less than 0.5% after 21 days for all strains. There was approximately a two-fold difference in the LD_{50} and the LD_{95} between the most susceptible strain (Georgia) and the most tolerant strain (Abidjan) (Figure 1).

Rate of water loss. When placed in dry conditions, the tolerant strain lost water at a lower rate than the susceptible strain, and these differences were even larger when adults had been held on grain treated with diatomaceous earth (Table 2, strain x treatment interaction $P = 0.004$). There was no difference between the strains in water content at the beginning of the experiment (tolerant: 52 ± 1 %, susceptible: 53 ± 1%, t-test, $P = 0.35$), nor large differences in the water content at death (34 to 37%, Table 2). Given the differences in the rate of water loss, it follows that the duration of survival would be different for untreated vs treated and tolerant vs susceptible strains (Table 2). Dead insects lost water at over three times the rate of live insects.

Movement. Tolerant insects moved more slowly vertically through the grain than the susceptible strain.

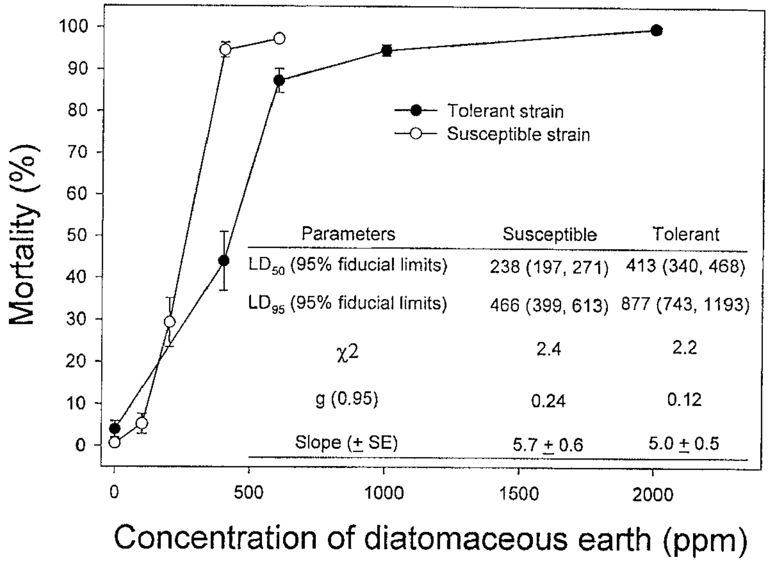

Figure 1. Mortality of *T. castaneum* originally from Georgia (USA) classed as susceptible and Abidjan (Ivory Coast) classed as tolerant. (Probit analysis: strains are significantly different: heterogeneity = 6.29, g = 0.086; probit lines are parallel (heterogeneity = 2.21, g = 0.034).

Table 3. Vertical and horizontal movement of diatomaceous earth susceptible (Georgia) and tolerant (Abidjan) strains of *T. castaneum*.

Variables	Strain	
	Tolerant	Susceptible
Movement through 500 g of wheat in 24 h (%)	52 ± 4 a	70 ± 3 b
Movement through 500 g of wheat in 48 h (%)	65 ± 4 a	84 ± 3 b
Rate of movement on a flat surface (cm/min)	37 ± 3 a	56 ± 4 b

Means in rows followed by the same letter are not significantly different, $n = 10$; *t*-test, $P<0.05$.

This test gives us an approximation of how fast insects move in grain during the exposure to diatomaceous earth in our trials. Also tolerant insects are slower (66% of susceptible) than the susceptible strain across a horizontal surface (Table 3).

Repellency. The tolerant strain avoided grain treated at 75 ppm or higher, whereas the susceptible strain never avoided the treated grain even at 600 ppm (Figure 2).

Discussion

Different insects have different susceptibility to diatomaceous earth (Fields & Korunic, 2000; Fields & Muir, 1995). This is the first study to show that there is wide variation in susceptibility to diatomaceous earth within one species. This may be one reason why researches have different results yet are using the same source of diatomaceous earth (Korunic & Subramanyam, pers. comm.). Such variation makes it difficult to compare studies done with different strains. Diatomaceous earth is not widely used commercially, so it is unlikely the differences between the strains are a response to selection pressure due to diatomaceous earth applications. Hence the tolerant strains had characteristics that made them pre-adapted to tolerate diatomaceous earth applications.

The rate of water loss is correlated with sensitivity to diatomaceous earth, a two-fold difference in sensitivity to diatomaceous earth correspondences to a two-fold difference between the strains in the rate of water loss of treated insects. This supports previous work showing that the mode of action of diatomaceous earth is desiccation (Ebeling, 1971). We can think of a number of adaptations that would increase an insect's tolerance to diatomaceous earth: increased feeding, changes in the cuticular waxes, better water reabsorption, avoidance of diatomaceous earth, reduced movement, and greater tolerance of low internal water

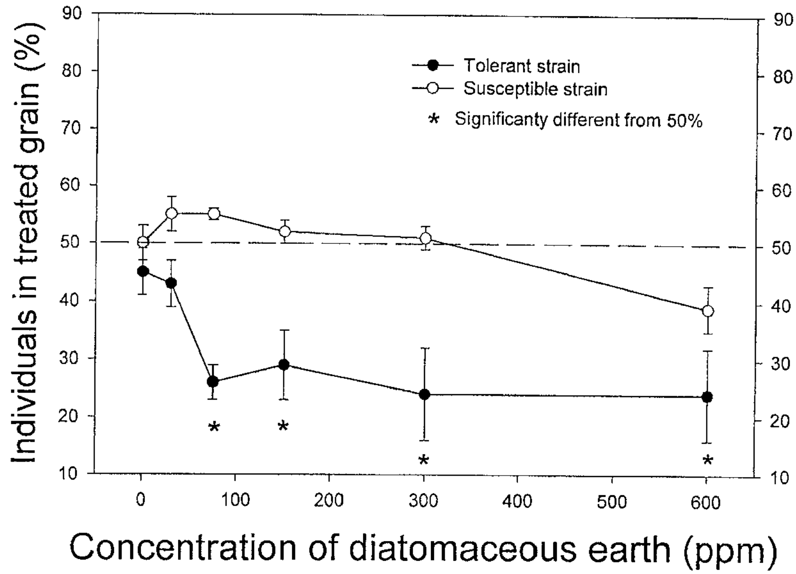

Figure 2. Behaviour of diatomaceous-earth-susceptible and diatomaceous-earth-tolerant strains of *T. castaneum* in wheat grains treated with different concentrations of diatomaceous earth. (150 insects/repetition; four repetitions/concentration/strain, * = $P < 0.05$, *t*-test).

levels. We have data that the tolerant strain has two of these adaptations: reduced movement and avoidance.

The tolerant strain moves slower than the susceptible strain. Less movement would cause less diatomaceous earth to come in contact with the insect's cuticle as it is moving through the grain, hence less damage and less water loss. Other insects have similar behavioural means of increasing tolerance to insecticides. Certain *Heliothis virescens* larvae respond to pyrethroids by decreasing their movement, thereby reducing their exposure to the insecticide and increasing survival (Sparks et al., 1989).

The tolerant strain also avoided grain treated with diatomaceous earth. Although we tried to mix the diatomaceous earth evenly throughout the grain sample, there may have been variation in the concentration of diatomaceous earth within the 200g of wheat used to expose the insects to diatomaceous earth. Tolerant strains would avoid these spots with high concentrations of diatomaceous earth, whereas the susceptible strain would not, increasing their exposure to diatomaceous earth. In a commercial setting it is very difficult to obtain an even mixing of any residual insecticide (White & Sinha, 1990; White & Leesch, 1995). Also, manufacturers recommend treating only part of the grain mass, so as to reduce the cost and the negative effects of diatomaceous earth treatment (Fields & Korunic, 2000).

There was a small but significant difference in the percentage of water loss that the strains could tolerate before dying. If this was a real difference between the strains, we would have expected to see similar differences in the insects held on untreated grain, which was not the case. A study of the fourteen strains is under way to determine if there is a correlation between qualitative or quantitative differences in the cuticular hydrocarbons and their sensitivity to diatomaceous earth.

The amount of water in insects vary widely among species, with an average of 69% (Hadley, 1994). Stored-product insects have lower amounts of water: 51% *Sitophilus oryzae* (L.) (Arlian, 1979), 53% *S. granarius* (L.), 52% *Cryptolestes ferrugineus* (Stephens) (Fields et al., 1998), and 52–53% *T. castaneum* (this study). The amount of water loss insects can tolerate before death also varies widely, 17 to 89%, according to species. *Sitophilus oryzae* dies after losing 56% of its water (Arlian, 1979), whereas we showed that *T. castaneum* dies after losing 30 to 36% of its water. The cuticular permeability of *T. castaneum* is similar to other insects from xeric habitats (Hadley, 1994). The rate of water loss is much higher after death, as observed by Wigglesworth (1945), demonstrating that water is actively retained by *T. castaneum*.

What are the implications of this study for the use of diatomaceous earth to control stored-product insects? Recommendations for dosages of diatomaceous earth done with sensitive strains could greatly underestimate the concentrations that are needed to control insects in granaries. One way to avoid this problem is to use insect populations that have been collected recently from granaries or food processing facilities. However, this will not address the problem of there being differences in strains from different regions. The tolerant strain in this study moved less than the sensitive strain. This could reduce the tolerant strain's fitness, and its spread to other locations, and hence reduce the spread of the resistance to diatomaceous earth to other populations.

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