Laboratory selection for resistance to diatomaceous earth\textsuperscript{1}.

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Abstract:

Three types of selection were used to test for the development of resistance to diatomaceous earth (DE). Insects were constantly cultured on wheat with low dose of DE, or insects were constantly exposed to DE with twice the low dose but only in the top half of the wheat. In the third method of selection, adults were exposed a high dose of DE for 10-14 days that killed approximately 50 to 80\% of the population approximately every 2-3 months. I tested 3 insects: \textit{Tribolium castaneum} (red flour beetle), \textit{Cryptolestes ferrugineus} (rusty grain beetle) and \textit{Sitophilus oryzae} (rice weevil).

Constant exposures to sublethal concentrations of DE, either mixed throughout the wheat or just the top half of the wheat, did not increase tolerance to DE over a 3-year period. Occasional high doses of DE that killed 50 to 80\% of the population increased the tolerance of all 3 insects tested. At the end of the 3 years of selection, \textit{T. castaneum} had a LD\textsubscript{50} of 1004 (831, 1143) ppm, (2 times the control), \textit{C. ferrugineus} had a LD\textsubscript{50} of 379 (321 - 501) ppm, (3 times the control), \textit{S. oryzae} had a LD\textsubscript{50} of 1286 (1198, 1375), (4 times the control). This work suggests that method of using diatomaceous earth will effect the rate that the DE-resistant populations develop.

Key words: Protect-It, application method, \textit{Tribolium castaneum}, \textit{Cryptolestes ferrugineus}, \textit{Sitophilus oryzae}, DE

1. Introduction

Resistance to synthetic contact insecticides by stored-product insects is a common problem throughout the world (Subramanyam and Hagstrum, 1996). Wide-spread use of contact insecticides have produced insect strains that are 2 to 200 fold more tolerant of insecticides, resulting in control failures and the eventual withdrawal of the insecticide from use. There is increased interest in diatomaceous earth (DE) because of populations resistant to synthetic insecticides, worker safety and concerned consumers (Ebeling, 1971; Golob, 1997; Korunic, 1998; Subramanyam and Roesli, 2000; Fields and Korunic, 2002). DE is obtained from geological deposits of diatomite, which are fossilized sedimentary layers of microscopic algae called diatoms. The fine DE dust, made up mainly of SiO\textsubscript{2}, absorbs wax from the insect cuticle, causing death due to desiccation.

Under laboratory selection with DE \textit{Tribolium castaneum} (Herbst) showed a 1.3 increase in tolerance after six generations, \textit{Rhyzopertha dominica} (L.) showed a 1.9 increase in tolerance.

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after seven generations and Cryptolestes ferrugineus (Stephens) showed a 2.2 increase after 5 generations (Korunic and Ormesher, 1996). There are a number of different methods to use DE; treating the entire grain bulk, treating the top and bottom 30-50 cm of grain, top dressing on top of the grain and treating empty structures. The objective of this study was to determine if tolerance of stored-product insects to DE increases depending upon the method of exposure to DE.

2. Materials and methods

Three insects were tested. The T. castaneum used was mixture of several strains that had been originally collected in Landmark MB in 1988 (21,200 adults), Steinback MB in 1989 (6300), Glenlea MB in 1988 (2600 adults) and Australia (174 adults, CSIRO strain 4). The C. ferrugineus used was originally collected from Manitoba and had been reared in the laboratory for over 20 years (39,000 adults). The Sitophilus oryzae (L.) used was a mixture of a laboratory strain (23,700 adults), and two strains that can reproduce on split peas originally collected from Burma (6000 adults, strain 85B from USDA-ARS Manhattan KS laboratory) and Trinidad (3000 adults, strain 86B from USDA-ARS Manhattan KS laboratory).

There were three types of selection. Insects were always held on sub-lethal concentrations of DE-treated wheat (Constant Low Doses). The starting concentrations were 200 ppm for T. castaneum, 30 ppm for C. ferrugineus and 100 ppm for S. oryzae. For the second type of selection, only the top-half of the wheat was treated (Top-Half Treated). Insects were always held in a jar that had the top half of the wheat treated with DE at twice the dose of the Constant Low Dose. The final selection method (Occasional High Doses) was similar to the one used by Korunic and Ormesher (1996). Every 2-3 months, 2,500 adults were placed on 2.5 kg of DE-treated wheat for 10-16 days, to obtain 50 to 80% mortality. Survivors were reared on untreated wheat. The starting concentrations were 500 ppm for T. castaneum, 100 ppm for C. ferrugineus, 300 ppm for S. oryzae. The concentrations of DE were increased in some cases to increase the selection pressure (Figure 1). Protect-It® was used for all tests (Hedley Technologies Inc., Unit 5, 2601 Matheson Blvd, Mississauga, ON, L4W 5A8, Canada, commercial sample). There were 3 replicates (jars) for each of the selection procedures, for each insect.

I used Hard Red Spring Wheat at 14% m.c. for all experiments. For T. castaneum, 3% cracked wheat was added to all diets and 5% cracked wheat and 5% wheat germ were added to the wheat for rearing between treatments of the Occasional High Doses. For C. ferrugineus, 5% cracked wheat and 2% wheat germ were added to the Constant Low Doses and Top-Half Treated. For the Occasional High Doses only 5% cracked wheat was used during the selection and 5% cracked wheat and 5% wheat germ were added to the wheat for rearing between the treatments. For S. oryzae, 1% cracked wheat was added to all tests and rearings.

A dose response test with 6 to 9 concentrations of DE, 50 adults/replicate, 3 replicates per concentration, in 100 g of wheat at 14% m.c. was run approximately every 6 months. Polo-PC was used to estimate the LD₉₀ (lethal dose for 50% of the population) and LD₉₀. All tests and rearing were conducted at 30 ± 1°C, 60 ± 10% r.h. For the dose response test the following amounts of cracked wheat were added to the wheat, 3% for T. castaneum, 5% for C.
3. Results

Constant exposures to sub-lethal concentrations of DE, either mixed throughout the wheat or just the top half of the wheat, did not increase the resistance of insects to DE over a 3 year period. I believe the single high level of tolerance seen at 400 days for *C. ferrugineus* to be an artifact.

Occasional high doses of DE that killed 50 to 80% of the population increased the tolerance of all 3 insects tested. At the end of the 3 years of selection, *T. castaneum* had a LD$_{50}$ of 1004 (831, 1143) ppm, (2 times the control), *C. ferrugineus* had a LD$_{50}$ of 379 (321 - 501) ppm, (3 times the control), *S. oryzae* had a LD$_{50}$ of 1286 (1198, 1375) ppm, (4 times the control). At the end of the 3 years of selection, *T. castaneum* had a LD$_{90}$ of 1561 (1441, 1718) ppm, (2 times the control), *C. ferrugineus* had a LD$_{90}$ of 624 (521, 846) ppm, (3 times the control), *S. oryzae* had a LD$_{90}$ of 2276 (2055, 2564) ppm, (4.5 times the control).

4. Discussion

The development of resistance of only 2-fold would seriously limit the use of DE. The concentrations used already are limited by the reduction in bulk density (Korunic et al., 1998) and by cost.

I expect that if resistance can develop in laboratory populations that have a very narrow genetic diversity, populations resistant to DE could develop under commercial conditions. The following conditions would probably be required for this resistance to develop: 1. There would have be wide spread use of DE over several years. 2. There would have to be little migration of new insects from areas that are not treated with DE. 3. The DE would have to applied as entire bulk application. 4. The cost of DE resistance to the insect would not greatly reduce fitness.

For these strains, I do not know what is the mechanism of resistance. Rigaux et al. (2001) showed that a 2-fold difference in resistance to DE in *T. castaneum* strains was linked to behavioural differences. The resistant strain moved twice as slow as the susceptible strain, and it was repelled by DE at 75 ppm, whereas the susceptible strain still was not repelled at 600 ppm. I expect that the reduced movement would reduce the fitness of resistant strain under commercial conditions, but this is probably not a factor under laboratory conditions.

What are the ways that could be deployed to counter the development of DE resistant populations? I expect that the classic approach to resistant management would work to reduce the occurrence of DE resistant strains: alternation of control measures, the use of mixtures of DE and other insecticides and resistance monitoring (Subramanyam and Hagstrum, 1996).

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References


Figure 1. The concentrations of DE for the three types of treatment during the experiment. The concentrations of DE were increased in some cases to increase the selection pressure.
Figure 2. The changes in lethal doses of 50% of the population (LD$_{50}$ ± 95 confidence intervals) during selection for over 3 years.