Standardised testing for diatomaceous earth

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Abstract

Over the last decade there has been a renewed interest in diatomaceous earth (DE) as a grain protectant because of concerns of insecticide residues in grain, worker exposure to insecticides and resistant insect populations. At the last two meetings of the International Working Conference on Stored Product Protection there was a discussion of the problems encountered in testing DE. A protocol was developed, and the efficacy of four DE samples was tested, as an admixture to wheat, against laboratory reared cultures of 7 to 21-day-old unsexed adult *Sitophilus oryzae* (L.), rice weevil, (CSIRO strain 418), and *Tribolium castaneum* (Herbst), the red flour beetle, (CSIRO strain 4). Four different laboratories used this protocol to evaluate four DE samples. One laboratory conducted a rapid assessment of DE samples that uses physical characteristics to predict insecticidal activity. One laboratory tested the DE samples as surface treatments applied both as a dust and as a slurry.

In general, there was good agreement between laboratories, although one laboratory had significantly higher mortality than the others. The possible reasons for this are discussed. Efficacy in grain bioassay was not correlated with efficacy in the surface bioassay. We make recommendations for a standard protocol for testing DE and further work.

Introduction

The grain industry needs to reduce its reliance on synthetic pesticides because of withdrawal of insecticides from the market, resistant populations and consumer concerns over insecticide residues. Diatomaceous earth (DE)-based insecticides are finding increased use as stored commodity protectants because of these concerns. DE is obtained from geological deposits of diatomite, which are fossilised sedimentary layers of microscopic algae called diatoms. DE, made up mainly of SiO_2, works as an insecticide through physical mechanisms. The fine DE dust absorbs wax from the insect cuticle, causing death due to desiccation (Ebeling, 1971; Golob, 1997; Korunic, 1998; Fields and Korunic, 2002).

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The main advantages of DE are its low-toxicity to mammals and its stability. However, several problems limit its widespread use: reduction of the bulk density and flowability of grain; dusty to apply; low efficacy against some insects; and reduction in efficacy at high moisture contents.

Several factors affect the efficacy of DE: relative humidity (Fields and Korunic, 2000); temperature (Fields and Korunic, 2000); geological source (Korunic, 1998); insect species (Carlson and Ball, 1962; Desmarchelier and Dines, 1987; Fields and Korunic, 2000); insect life stage (Subramanyam et al., 1998; Mewis and Reichmuth, 1999); strain of insect (Riguax et al., 2001); grain type (La Hue, 1972); and insect density (Arthur, 2002; Z. Korunic, unpublished data). The goal of the project reported here was to develop a standard protocol that could be used as a base line for studying DE. The discussions began at the Sixth International Working Conference on Stored Product Protection in Canberra, Australia and more concrete plans were made at the Seventh International Working Conference on Stored Product Protection in Beijing, China.

Methods

Test insects

Sitophilus oryzae (L.) (CSIRO strain 418), the rice weevil, and *Tribolium castaneum* (Herbst), the red flour beetle (CSIRO strain 4) were used in the experiments. *Sitophilus oryzae* was reared on whole wheat between 12 and 14 % m.c. at 25±1°C, 60±10% r.h. *Tribolium castaneum* was reared on 95% wheat flour with 5% brewers yeast mixture at the same temperature and humidity.

Uninfested, clean wheat of known origin was conditioned to 13% m.c. by water addition, and held in sealed glass jars or plastic bags at 25°C for two weeks. Each replicate had 100 g of wheat, with 50 unsexed 7 to 21-day-old adults held in a 200 mL jar. *Sitophilus oryzae* were tested on whole kernel wheat. The necks of the jars for *S. oryzae* were treated with Fluon (liquid Teflon) to prevent escape. *Tribolium castaneum* were tested with whole-kernel wheat with 1% (by weight) cracked kernel wheat. The cracked kernel wheat was sieved over a 1-mm mesh sieve to remove any flour. Each test centre used a locally available wheat.

Diatomaceous earths

Four diatomaceous earths were tested: Dryacide DE (A & R McLaughlin Pty Ltd, PO Box 38, Scarborough, WA 6922, Australia, the DE used for Dryacide® before processing to

increase activity), INSECTO (Natural Insecto Products, Inc., 221 Sherwood Place, PO Box 12138, Costa Mesa, CA 92627, USA, commercial sample), Perma-Guard™ (Perma-Guard, Inc., PO Box 25282, Albuquerque, NM 87125 USA, commercial sample) and Protect-It® (Hedley Technologies Inc., Unit 5, 2601 Matheson Blvd, Mississauga, Ontario, L4W 5A8, Canada, commercial sample). The DE samples were bagged by a third party at CSIRO, coded and sent to participants, to enable the tests to be run blind.

Grain bioassay

The DE concentrations tested against *S. oryzae* were: 0, 100, 200; 400, 600 and 800 ppm, and those against *T. castaneum*: 0, 200, 400, 600, 800 and 1000 ppm. The appropriate weights of DE were added to 300 g of grain. The grain and DE were shaken in jars by hand for two minutes. After mixing, the treated grain was divided into three 100-g samples, one for each replicate. These concentrations were used in the following three laboratories: CSIRO, Canberra, Australia (Australia 2); CRC, Winnipeg, Canada; and NRI, Kent, UK. Alan McLaughlin (Australia 1), Scarborough, Australia used the following concentrations: *S. oryzae*; 0, 300, 450, 600 and 800 ppm; *T. castaneum*; 0, 450, 550, 700 and 900 ppm. Following the addition of insects to each jar, the jars were kept at 25±1°C and 60±10% r.h. for the remainder of the bioassay.

After seven days the contents of each jar were poured onto a tray and the numbers of live and dead insects noted. After 14 days, the grain was sieved, all adults removed, the numbers of dead and live insects noted, and the grain returned to the jar for offspring production. The jars with the grain and the immatures were returned to 25°C, 60% r.h. After seven weeks for *S. oryzae* and 10 weeks for *T. castaneum* (dated from beginning of experiment), the grain in each jar was sieved and the number of adults counted to estimate offspring production.

Surface bioassay

For the slurry application, 0.15 µg of DE was placed in the centre of a clean glass Petri dish (140-mm diameter, 17-mm sidewall). The DE was mixed with 1.5 mL of water by rotating the dish by hand to obtain an even deposit over the base and sides of the dish. A artists fine paint brush or a gloved fingertip may be used to assist the dispersion of the deposit. The water was evaporated by placing the dish in an oven (~ 80°C) and rotating it approximately every five minutes until the water has evaporated. It is important that the Petri dishes be extremely clean, as clean Petri dishes allow the water to spread evenly when it placed at the centre of the dish. Petri dishes were cleaned with detergent (Decon 90) or chromic acid.

For the dust application, 0.4 µg of DE was placed in a plastic Petri dish (base, 140 mm with 17.5-mm sidewall; top 147-mm diameter with 9.0-mm sidewall), the Petri dishes were joined top to top or bottom to bottom with a strip of parafilm and the dish was shaken and tapped to distribute the DE evenly between both Petri dishes. Static electricity causes the DE to stick to the plastic Petri dishes.

Thirty unsexed adult *T. castaneum* or *S. oryzae* were placed in a Petri dish base. The treated Petri dish top was fitted within the experimental relative humidity environment. Control insects were placed into untreated Petri dishes. After 24 h insects were assessed as follows: *live*, if able to move normally and to respond to stimuli; *dead*, if unable to do either; or as *moribund*, if able to respond to stimuli, but unable to move normally. Insects were transferred to 40 g of wheat in 100-mL jars, and the jars sealed with filter paper and hot wax. The neck of each jar was ringed with Fluon to prevent the escape of insects. Jars were held at 25°C, 60% r.h. After 7 days, the insects were shaken out of the wheat and the numbers of live and dead assessed as above. Insects were normal or clearly dead after the seven-day holding period. The four DE samples applied as both dusts at 1 g/m^2 and slurries at 6 g/m^2 were replicated four times. These experiments were carried out by A. McLaughlin.

Rapid assessment

An assessment of the four DEs was conducted according to Korunic (1997) by Korunic. This method measures the physical attributes of the DE and predicts the efficacy against *S. oryzae* and *T. castaneum*. The procedures take about a day, compared with several weeks with the insect bioassays. The following physical attributes were measured: pH of DE in water, reduction in bulk density with 50 ppm of DE added to wheat, tapped density of DE and adherence to wheat.

Data analysis

All data were analysed using analysis of variance (ANOVA, SigmaStat 1.0). To equalise variances, mortality data were transformed using the square root of the arcsin of the proportion dead. The lethal dose for 50% of the population (LD_{50}) was estimated using probit analysis (Polo PC). To estimate if there was significantly more variation between laboratories, we used an *F*-ratio test.

Results

Grain bioassay

After seven days the mortality was not great enough in all of the tests to estimate the LD_{50} (Table 1). The assessment after 14 days allowed the estimation of the LD_{50} for most of the DEs in most of the laboratories, as well as giving smaller confidence intervals. *Sitophilus oryzae* was more susceptible than *T. castaneum* to DE. In general, there was good agreement on the LD_{50} between laboratories, except that the Canadian laboratory had a lower LD_{50} than the other laboratories for the *S. oryzae*. There was greater variation in the estimates for Perma-Guard™, possibly because the doses tested were too low to give a good estimate of the LD_{50}.

The amount of DE required to reduce the offspring by 50% was lower than the DE required to reduce the parent survival to 50%. It is difficult to compare the offspring assessment between laboratories, because Australia 1 was unable to complete this part of the test, only the UK laboratory had any appreciable progeny for *T. castaneum*, and the data for *S. oryzae* from Australia 2 laboratory did not fit the probit model well enough to give good estimates of the LD_{50}.

Surface bioassay

The surface bioassay showed that a dust application at 1 g/m^2 was more effective than a slurry application at 6 g/m^2 (Table 2). As in the grain bioassay, *S. oryzae* was more susceptible than *T. castaneum* to DE. Dryacide DE performed better than the other DEs and Perma-Guard™ performed worse than the other DEs.

Table 1. The LD_{50} (ppm) of four diatomaceous earths tested in four different laboratories.[a]

Measure	DE	Lethal dose for 50% of the test insects (ppm) confidence intervals in brackets			
		Australia 1	Australia 2	Canada	U.K.
Sitophilus oryzae 7 days	Dryacide DE	870 (805, 962)	775 (732, 841)	408 (386, 430)	891 (671, 1962)
	INSECTO	687 (609, 784)	1200 (787, 6055)	370 (299, 441)	671 (622, 733)
	Perma-Guard™	1095 (912, 1602)	–	599 (478, 822)	–
	Protect-It®	666 (603, 736)	573 (496, 656)	289 (255, 324)	558 ([b])
Tribolium castaneum 7 days	Dryacide DE	–	780 (705,879)	801 (779, 824)	827 (760, 919)
	INSECTO	–	703 (665, 743)	1240 (1117, 1528)	930 (816, 1168)
	Perma-Guard™	–	–	–	1536 (1236, 2489)
	Protect-It®	–	562 (537, 586)	730 (673, 788)	638 (586, 685)
Sitophilus oryzae 14 days	Dryacide DE	637 (575, 705)	580 (490, 719)	269 (242, 297)	609 (477, 887)
	INSECTO	405 (342, 458)	404 (253, 658)	143 (134, 151)	330 (301, 358)
	Perma-Guard	651 (583, 733)	1611 (1072, 4862)	210 (192, 228)	593 (536, 645)
	Protect-It®	430 (399, 459)	381 (293, 480)	140 (128, 153)	332 (293, 372)
Tribolium castaneum 14 days	Dryacide DE	940 (850, 1150)	570 (478, 670)	483 (448, 516)	589 (542, 639)
	INSECTO	1012 (882, 1457)	453 (417, 487)	489 (466, 512)	483 (378, 544)
	Perma-Guard™	–	1623 (1221, 3890)	757 (701, 845)	902 (834, 1003)
	Protect-It®	770 (732, 816)	336 (255, 409)	344 (303, 378)	462 (418, 499)
Sitophilus oryzae Offspring	Dryacide DE		–	180 (114, 243)	420 ([b])
	INSECTO		184 ([b])	124 (102, 145)	191 (160, 220)
	Perma-Guard™		986 ([b])	240 (206, 273)	340 (273, 425)
	Protect-It®		–	129 (85, 167)	167 (113, 217)
Tribolium castaneum Offspring	Dryacide DE				210 (131, 269)
	INSECTO				205 (127, 262)
	Perma-Guard™				243 (140, 320)
	Protect-It®				121 (20, 185)

[a] Australia 1 used the following concentrations: *S. oryzae*; 0, 300, 450, 600 and 800 ppm; *T. castaneum*; 0, 450, 550, 700 and 900 ppm. The other labs used: *S. oryzae*; 0, 100, 200, 400, 600, and 800 ppm; *T. castaneum*; 0, 200, 400, 600, 800, and 1000 ppm

[b] $g \leq 0.90$, confidence intervals could not be calculated

Rapid assessment

The rapid assessment method was developed as a preliminary screen of raw DE materials in order to select promising DE with a good insecticidal efficacy and to eliminate DE with low or no insecticidal efficacy. Different additives to DE (baits, silica gel etc.) may have some influence on the results of testing.

The results provide a rough prediction of the insecticidal efficacy of DE samples without conducting lengthy bioassays. Bioassays can then be performed on only selected DE samples. Bioassay testing is needed because the rapid assessment method cannot provide an accurate estimate of efficacy.

The rapid assessment showed that there were physical differences between the DEs tested (Table 3). The method predicted that sample No. 1 (Perma-Guard™) was less effective than the other three samples. There was good agreement between the rapid assessment prediction of the LD_{50} and the 14-day LD_{50} from the bioassay for *T. castaneum* (Table 1). We would expect the bioassays to have a lower LD_{50} as they were run at 13% m.c. and the rapid assessment predicts mortality at 14% m.c. For *S. oryzae,* there was good agreement between the Canadian bioassay and the rapid assessment prediction of the LD_{50}. For the other laboratories, the bioassays gave higher LD_{50} values than predicted by the rapid assessment.

Comparison of DEs between laboratories

Comparing the results between laboratories and methods of assessment, there was a general agreement that Perma-Guard™ was the least effective of all DEs tested (Table 4). If we examine the data after 14 days, when there is a better estimation of LD_{50}, for *S. oryzae* there was agreement that Protect-It® and INSECTO were equal, and that Dryacide DE and Perma-Guard™ were less effective. For *T. castaneum* at 14 days, most laboratories considered Protect-It® the most effective, followed by Dryacide DE and INSECTO, which were more effective than Perma-Guard™. The ranking by the rapid assessment also classed Perma-Guard™ as the least-effective DE in the group. Although Protect-It® was

estimated by the rapid assessment to be the most effective, there was a great deal of overlap between Dryacide DE, Protect-It® and INSECTO. Protect-It® were often ranked most effective by the bioassay method, but sometimes it was equal to INSECTO and Dryacide DE.

The ranking by the surface bioassay put Perma-Guard™ as the least effective, as with the other assays. Dryacide DE was ranked the most effective by the surface bioassay, though it rarely was ranked most effective in the other assays.

We estimated the variance between laboratories by comparing the error mean squares from a two-way ANOVA with DE type and dose and their interaction (DE type × dose) (Table 5). Therefore, laboratories with smaller variations between replicates would have smaller mean-square errors. In general, the variation between laboratories was similar, with the following exceptions: Australia 2 had a higher variation for *S. oryzae* at 14 days; and Canada had a lower variation in the *S. oryzae* offspring.

The entire data set from the testing is available at <http://www.geocities.com/de_grain/>.

Discussion

Recommended protocol

We suggest that if future tests with DE follow the protocol outlined here, this will aid in the comparison of results between studies. We suggest the minimum protocol should be three replicates, 50 insects per replicate with, 7 to 21-day-old insects. We recommend the following concentrations of DE: 0, 300, 500, 700, 900 and 1100 ppm. Mortality assessment should be done at 7 and 14 days, with one of the DEs tested here, using wheat at 13% m.c. held at 25°C and 60% r.h. *Sitophilus oryzae* should be used, but we strongly recommend that a second species, preferably *T. castaneum*, also be used, as the rankings of DEs were not the same for both species. If other species are used, for example *Cryptolestes ferrugineus* which is very sensitive to DE, the concentrations used would have to be adjusted.

The rapid assessment is a good tool for screening large numbers of DE samples. However, bioassays are needed to provide a more-accurate estimate of efficacy. The rapid assessment was best at predicting results from the Canadian laboratory. This could be because the rapid assessment was initially correlated with bioassays done there. The differences seen between the Canadian laboratory and the other laboratories could be due to wheat type, rearing conditions, handling of insects, or other undetermined factors.

Efficacy in grain bioassay was not correlated to efficacy in the surface bioassay. Therefore, if surface treatment is an important target use for the DE tested, then surface bioassays must be used to select the best DE.

Other factors that affect DE efficacy

There are several factors that are not controlled in the outlined protocol, and which could affect efficacy. There are differences between insect strains. Riguax et al. (2001) found that there was two-fold difference in susceptibility to DE between *T. castaneum* strains. It is difficult, although

Table 2. The mortality (mean ± SEM) of *Sitophilus oryzae* and *Tribolium castaneum* held in Petri dishes for 1 day that have been treated with four diatomaceous earths (DE) either as a dust at 1g/m^2 or as a slurry at 6 g/m^2, and then placed on untreated wheat for 7 days.

DE	Mortality (%)							
	Sitophilus oryzae				*Tribolium castaneum*			
	Dust		Slurry		Dust		Slurry	
Dryacide DE	55 ± 3 a	99 ± 1 a	32 ± 5 a	95 ± 1 a	82 ± 3 a	96 ± 2 a	81 ± 5 a	98 ± 2 a
INSECTO	17 ± 2 c	98 ±1 a	1 ± 1 b	81 ± 2 b	2 ± 1 c	7 ± 2 c	1 ± 1 b	2 ± 1 b
Perma-Guard™	5 ± 1 d	82 ± 5 b	0 ± 0 b	29 ± 1 c	3 ± 1 c	13 ± 5 c	0 ± 0 b	0 ± 0 b
Protect-It®	45 ± 4 b	98 ± 1 a	1 ± 1 b	73 ± 1 b	31 ± 8 b	55 ± 5 b	0 ± 0 b	5 ± 4 b

For a given time, differences between DEs are indicated by different letters, Student-Newman-Keuls multiple range test ($p > 0.05$). All data were transformed with an arcsin of the square root of the proportion dead to equalise variances.

Table 3. The comparison of the four diatomaceous earths (DE) using a rapid assessment method (Korunic 1997).

Measurement	Dryacide DE	INSECTO	Perma-Guard™	Protect-It®
pH	6.60	5.70	9.30	5.75
Bulk density with 50 ppm DE[a] (kg/hL)	78.14 ± 0.06 a	78.57 ± 0.04 b	78.93 ± 0.05 c	78.03 ± 0.05 a
Bulk Density Reduction (kg/hL)	3.06	2.63	2.27	3.17
Tapped Density of DE (g/L)	219 ± 1 a	226 ± 3 a	297 ± 5 c	256 ± 4 b
Adherence to Wheat (%)	84.2 ± 0.3 a	86.1±.06 a	83.9 ± 1.0 a	87.2 ± 0.9 a
Predicted LD_{50} (ppm) of *S. oryzae*	Less than 400	Less than 400	400 to 700	Less than 400
Predicted LD_{50} (ppm) of *T. castaneum*	Less than 700	Less than 700	Less than 700	Less than 700
Insecticidal Efficacy for *S. oryzae*	2.4	7.9	21.3	–2.0
Insecticidal Efficacy for *T. castaneum*	10.1	14.0	24.4	5.9

[a] Bulk density of untreated hard red spring wheat – 81.20 ± 0.11 kg/hL
For a given test, differences between DEs are indicated by different letters, Student-Newman-Keuls multiple range test ($p > 0.05$).

Table 4. The rankings[a] of four diatomaceous earths tested in four different laboratories

Measure	Using grain bioassay data presented in Table 1								Using rapid assessment data from Table 3	Using surface bioassay data from Table 2	
	Australia 1		Australia 2		Canada		U.K				
Sitophilus oryzae 7 days	Protect-It®	a	Protect-It®	a	Protect-It®	a	Protect-It®		Protect-It®	Dryacide DE	a
	INSECTO	a	Dryacide DE	b	INSECTO	ab	INSECTO	a	Dryacide DE	Protect-It	b
	Dryacide DE	b	INSECTO	c	Dryacide DE	b	Dryacide DE	a	INSECTO	INSECTO	b
	Perma-Guard™	c	Perma-Guard™	d	Perma-Guard™	c	Perma-Guard™		Perma-Guard™	Perma-Guard™	c
Tribolium castaneum 7 days	–		Protect-It®	a	Protect-It ®	a	Protect-It ®	a	Protect-It®	Dryacide DE	*a*
	–		Dryacide DE	ab	Dryacide DE	b	Dryacide DE	a	Dryacide DE	Protect-It	b
	–		INSECTO	b	INSECTO	c	INSECTO	b	INSECTO	INSECTO	c
	–		Perma-Guard™	c	Perma-Guard™	d	Perma-Guard™	c	Perma-Guard™	Perma-Guard™	c
Sitophilus oryzae 14 days	INSECTO	a	Protect-It®	a	Protect-It®	a	INSECTO	a			
	Protect-It®	a	INSECTO	a	INSECTO	a	Protect-It®	a			
	Dryacide DE	b	Dryacide DE	a	Perma-Guard™	b	Perma-Guard™	b			
	Perma-Guard™	b	Perma-Guard™	b	Dryacide DE	b	Dryacide DE	b			
Tribolium castaneum 14 days	Protect-It®	a	Protect-It®	a	Protect-It®	a	Protect-It®	a			
	Dryacide DE	b	INSECTO	b	Dryacide DE	b	INSECTO	ab			
	INSECTO	b	Dryacide DE	b	INSECTO	b	Dryacide DE	b			
	Perma-Guard™	c	Perma-Guard™	c	Perma-Guard™	c	Perma-Guard™	c			
Sitophilus oryzae Offspring					INSECTO	a	Protect-It®	a			
					Protect-It®	a	INSECTO	a			
					Dryacide DE	ab	Perma-Guard™	b			
					Perma-Guard™	b	Dryacide DE	b			
Tribolium castaneum Offspring							Protect-It®	a			
							INSECTO	a			
							Dryacide DE	a			
							Perma-Guard™	a			

[a] In decreasing order of efficacy for a given lab and measure, significant differences between DEs are indicated by different letters.

Table 5. Error mean square from two-way ANOVA analysis of mortality at 7 and 14 days and offspring production (DE, dose and DE × dose as factors) and *F*-ratio ($p > 0.05$) tests for significant differences in variation between laboratories.

Measurement	Error mean square			*F*-ratio test		
	Australia 2	Canada	U.K	Australia 2 vs Canada	Australia 2 vs U.K.	Canada vs U.K.
Sitophilus oryzae 7 days	0.0177	0.0112	0.0099	ns	ns	ns
Tribolium castaneum 7 days	0.0063	0.0047	0.0086	ns	ns	ns
Sitophilus oryzae 14 days	0.0276	0.0069	0.0084	*	*	ns
Tribolium castaneum 14 days	0.0077	0.0082	0.0079	ns	ns	ns
Sitophilus oryzae Offspring	23 275	7 525	21 842	*	ns	*
Tribolium castaneum Offspring	–	7.97	5.39	ns	–	–

Mortality data were transformed with an arcsin of the square root of the proportion dead to equalise variances between doses.

not impossible, to obtain from CSIRO the strain of each insect used by the laboratories in this study. Also, if there are differences between strains, researchers are more interested in determining efficacy against a local than an imported laboratory strain. We used commercial DEs, but the formulations and sources of commercial DE frequently change while the name remains the same. So although we recommend using one of the DEs tested here for comparison, there is no guarantee that the DE will have the same efficacy as the one we tested.

We did not account for differences in wheat, as we all used locally available wheats. Commodities differ in their physical and chemical properties. There are differences in the adherence of DEs to the surface of barley, maize, wheat, sorghum

and oat grains (La Hue, 1972). Pomeranz et al. (1988) found that kernel hardness was one of the most obvious differences between wheat classes and varieties. This property may have an influence on the level of infestation by insects. McGaughey et al. (1990) found that white wheat was the most susceptible to *R. dominica*, followed by durum, hard red winter wheat, hard spring wheat and soft red winter wheat. Aldryhim (1993) reported the importance of classes of wheat (durum, hard and soft wheat) on the efficacy of the DE Dryacide®. At low relative humidity (40% r.h.), the efficacy of Dryacide® against *R. dominica* was highest on durum, whereas at a higher relative humidity (60%), the efficacy was highest on hard wheat. His opinion was that two factors seem to contribute in this relationship: the degree of adhesion of silica particles to the different classes of wheat kernels at different relative humidities, and the rate that silica particles are picked up by beetles. Z. Korunic (unpublished data) investigated the effect of different classes and grades of wheat on the efficacy of DE. On different classes of wheat treated with Protect-It®, the mortality of *S. oryzae* was significantly lower on Ontario soft feed wheat, Canada prairie spring red wheat, and amber durum grade 2 (46%, 51% and 53%, respectively) in comparison with the mortality on hard red spring wheat Grades 2 and 3 and extra strong red spring wheat grade 1 (85%, 84% and 86%, respectively). *Tribolium castaneum* mortality was significantly lower on Ontario soft feed wheat (23%) followed by Canada prairie spring white wheat grade 1 (58%). Mortality was significantly higher on Canada prairie spring red wheat grade 1 (95%), on hard red spring wheat grades 1, 2 and 3 (91%, 82% and 91%), amber durum grade 2 (91%) and extra strong red spring wheat grades 1 and 2 (both 84%). As Canada (CRC) used hard red spring wheat in this study and the other groups used softer winter wheats, this may be one reason that the Canadian laboratory had higher mortalities than the other laboratories.

Future work

We did not control the rearing conditions for the test insects, and this may be one of the reasons that the Canadian laboratory had much lower survival of *S. oryzae* than the other laboratories. We suggest that the colonies be set up with approximately one adult per 5 g of wheat in a jar containing at least 500 g of wheat and be used as the first generation emerges. Age is a factor affecting susceptibility to DE, and it varies with insect species (A. McLaughlin, unpublished data).

Tribolium castaneum progeny emergence was low at all the test centres, even in the untreated controls. The use of a higher proportion of broken wheat kernels could have led to increased progeny development. The use of a *T. castaneum* culture reared on a wheat flour/yeast mixture might also have meant the insects were less adapted to exploiting the 1% cracked wheat test media. However, DE efficacy is reduced in wheat containing broken kernels, possibly due to the absorption of fatty acids from the broken kernels (Nielsen, 1998).

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