Standardized testing for diatomaceous earth[1]

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Abstract

Over the last decade there has been a renewed interest in diatomaceous earth 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 diatomaceous earth. A protocol was developed, and the efficacy of four diatomaceous earth samples was tested as an admixture to wheat against laboratory reared cultures of 7 to 21-day old unsexed adult *Sitophilus oryzae* (Linnaeus), 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 diatomaceous earth samples. One laboratory conducted a rapid assessment of diatomaceous earth samples that uses physical characteristics to predict insecticidal activity. One laboratory tested the diatomaceous earth 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 to efficacy in the surface bioassay. We make recommendations for a standard protocol for testing DE and further work.

1. Introduction

The grain industry needs to reduce its reliance on synthetic pesticides because of insecticide deregulation, 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 fossilized 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).

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

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(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 (La Hue, 1972) and insect density (Arthur, 2002; Korunic, unpublished data). The goal of this project 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. So it is our pleasure to present this work at the Eighth International Working Conference on Stored Product Protection in York, England.

2. Methods

2.1. Test insects

Sitophilus oryzae (Linnaeus) (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. (moisture content) at 25±1°C, 60±10% r.h. (relative humidity). *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., and held in sealed glass jars or plastic bags at 25°C for 2 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; Sylvia Allen and Alan Mclaughlin used Australian Standard White wheat, Paul Fields used Hard Red Spring Wheat from Manitoba, Tanya Stathers used Organic English Winter Wheat.

2.2. 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, ON, 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.

2.3 Grain Bioassay

The DE concentrations tested against *S. oryzae* were: 0; 100, 200; 400, 600 and 800 ppm. While the DE concentrations tested against *T. castaneum* were: 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 2 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 - Sylvia Allen (Australia 2), Canberra, Australia; CRC - Paul Fields (Canada), Winnipeg, Canada; and NRI - Tanya Stathers (UK) Kent, U.K. 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 7 days the contents of each jar were poured onto a tray and the number of live and dead noted. After 14 days the grain was sieved, all adults removed, the number of dead and live 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 7 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.

2.4. Surface Bioassay

For the slurry application, 0.15 mg of DE was placed in the center of a clean glass Petri dish (140 mm diameter). 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 very fine artists paint brush or a gloved fingertip may be used to assist the dispersion of the deposit. Evaporate the water by placing the dish in an oven (~ 80°C) and rotate the dish approximately every 5 minutes until the water has evaporated. It is important that the Petri dishes are extremely clean as clean Petri dishes allow the water to spread evenly when placed in the center of the dish. Petri dishes were cleaned with detergent (Decon 90) or chromic acid.

For the dust application, 0.4 mg of DE was placed in a plastic Petri dish (140 mm base and 147 mm top), 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 Perti dish top was fitted within the experimental rh environment. Control insects were placed into untreated Petri dishes. After 24 h insects were assessed as: live, if able to move normally and to respond to stimuli; as 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 number 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 $1g/m^2$ and slurries at $6g/m^2$ were replicated four times. .

2.5 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 approximately 1 day, compared to 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.

2.6 Data analysis

All data was analyzed using Analysis of Variance (ANOVA, SigmaStat 1.0). To

equalize variances, mortality data was 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 a *F*-ratio test.

3. Results

3.1. Grain bioassay

After 7 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 labs, 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 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 U.K. 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}.

3.2. Surface bioassay

The surface bioassay showed that the 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.

3.3. 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 only with selected DE samples. Bioassay testing is needed because the rapid assessment method can not 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 then other 3 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} than predicted by the rapid assessment.

3.4. Comparison of DEs between laboratories

Comparing the results between laboratories and methods of assessment, there is 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 is agreement that Protect-It and INSECTO are equal and that Dryacide DE and Perma-Guard are less effective. For *T. castaneum* at 14 days, most laboratories considered Protect-It the most effective, followed by Dryacide DE and INSECTO, which are more effective than Perma-Guard

The ranking by the rapid assessment also classes Perma-Guard as the least effective DE in the group. Although Protect-It is 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 is often ranked most effective by the bioassay method, but sometimes it is equal to INSECTO and Dryacide DE.

The ranking by the surface bioassay puts Perma-Guard as the least effective as with the other assays. Dryacide DE is 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 x dose) (Table 5). Therefore laboratories with smaller variations between replicates would have smaller error mean squares. 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/

4. Discussion

4.1. 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, fifty 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 DE's tested here using wheat at 13% m.c. held at 25°C and 60% r.h. *Sitophilus oryzae* should be used, however we strongly recommend a second insect preferably *T. castaneum* also be used, as the ranking of DE 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 in the Canadian laboratory. The differences seen between the Canadian laboratory and the other laboratories could be due to wheat type, rearing conditions, handling of insects, or some other undetermined factor.

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.

4.2 Other factors that affect DE efficacy

There are a number of factors that are not controlled in the outlined protocol, 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 not impossible to obtain the strain of each insect used by the laboratories in this study from CSIRO. Also, if there are differences between strains, researchers are more interested in determining efficacy against a local strain rather 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 ones we tested.

We did not control 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 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% r.h.), 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 humidity and the rate that silica particles are picked up by beetles. 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, Grade 2 and 3 and Extra Strong Red Spring wheat, Grade 1 (85%, 84% and 86%, respectively). *T. castaneum* mortality was significantly lower on Ontario soft feed wheat (23%) followed with 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 Grade 1, 2 and 3 (91%, 82% and 91%), Amber Durum Grade 2 (91%) and Extra Strong Red Spring wheat Grade 1 and 2 (both 84%). As Fields used Hard Red Spring Wheat and the other groups used softer winter wheats, this may be one reason that the Canadian laboratory had higher mortalities than the other laboratories.

4.3 Future work

We did not control the rearing conditions for the test insects, and this may be one of the reasons that the Canadian lab had much lower survival for *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 (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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References

Aldryhim, Y.N., 1993. Combination of classes of wheat and factors affecting the efficacy of amorphous silica dust, Draycide, against *Rhyzopertha dominica* (F). Journal of Stored Product Research 29, 271-275.

Arthur, F.H., 2002. Survival of *Sitophilus oryzae* (L.) on wheat treated with diatomaceous earth: impact of biological and environmental parameters on product efficacy. Journal of Stored Product Research 38, 305-313.

Carlson, S.D., Ball, H.J., 1962. Mode of action and insecticidal value of a diatomaceous earth as a grain protectant. Journal of Economic Entomology 55, 964-970.

Desmarchelier, J.M., Dines, J.C., 1987. Dryacide® treatment of stored wheat: its efficacy against insects, and after processing. Australian Journal of Experimental Agriculture 27, 309-312.

Ebeling, W., 1971. Sorptive dusts for pest control. Annual Review of Entomology 16, 123-158.

Fields, P.G., Korunic, Z., 2000. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. Journal of Stored Products Research 36, 1-13.

Fields, P.G., Korunic, Z., 2002. Post-harvest insect control with inert dusts. In: Pimentel, D. (Ed.), Dekker Encyclopedia of Pest Management. Marcel Dekker, New York, pp 650-653.

Golob, P., 1997. Current status and future perspectives for inert dusts for control of stored product insects. Journal of Stored Products Research 33, 69-79.

Korunic, Z., 1997. Rapid assessment of the insecticidal value of diatomaceous earths without conducting bioassays. Journal of Stored Products Research 33, 219-229.

Korunic, Z., 1998. Diatomaceous earth, a group of natural insecticides. Journal of Stored Products Research 34, 87-97.

La Hue, D., 1972. The retention of diatomaceous earths and silica aerogels on shelled corn, hard winter wheat and sorghum grain. Agricultural Research Service, United States Department of Agriculture, ARS 51044, 8pp.

McGaughey, W.H., Speris, R.D., Martin, C.R., 1990. Susceptibility of classes of wheat grown in the United States to stored-grain insects. Journal of Economic Entomology 83, 1122-1127.

Mewis, I., Reichmuth, C. 1999. Diatomaceous earths against coleoptera granary weevil *Sitophilus granarius* (Curculionidae), the confused flour beetle *Tribolium confusum* (Tenebrionidae) and the mealworm *Tenebrio molitor* (Tenebrionidae). In: Zuxun, J., Quan, L., Yongsheng, L., Xianchang T., Lianghua, G., (Ed.), Proceedings of the 7th International Working Conference of Stored Product Protection, Beijing, China. Sichuan Publishing House of Science & Technology, Chengdu, pp. 765-780.

Nielsen, P.S., 1998. The effect of a diatomaceous earth formulation on the larvae of *Ephestia kuehniella* Zeller. Journal of Stored Products Research 34, 113-121.

Pomeranz, Y., Czuchajowska, Z., Shogren, M.D., Rubenthaler, G.L., Bolte, L.C., Jeffers, H.C., Mattern, P.J., 1988. Hardness and functional (bread and cookie-making) properties of U.S. wheats. Cereal Food World 33, 297-304.

Rigaux, M., Haubruge, E., Fields, P.G., 2001. Mechanisims for tolerance to diatomaceous earth between strains of *Tribolium castaneum.* Entomologia Experimentalis et Applicata 101, 33-39.

Subramanyam, Bh., Madamanchi, N., Norwood, S., 1998. Effectiveness of INSECTO applied to shelled maize against stored-product insect larvae. Journal of Economic Entomology 91, 280-286.

Table 1. The LD_{50} (ppm) of the four DE's tested in four different laboratories[1].

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 (*)
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 (*)
	INSECTO		184 (*)	124 (102, 145)	191 (160, 220)
	Perma-Guard		986 (*)	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)

[1] 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

* $g \leq 0.90$, confidence intervals could not be calculated

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 DE's either as a dust at 1g/m^2 and 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	
	1 d	7 d	1 d	7 d	1 d	7 d	1 d	7 d
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 DE's are indicated by different letters, Student-Newman-Keuls multiple range test ($p > 0.05$). All data was transformed with an arcsin of the square root of the proportion dead to equalize variances.

Table 3. The comparison of the four DE's 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 (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

* bulk density of untreated Hard Red Spring wheat – 81.20 ± 0.11 kg/hL
For a given test, differences between DE's are indicated by different letters, Student-Newman-Keuls multiple range test ($p > 0.05$).

Table 4. The rankings[1] of four DE's 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	a b	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	a b			
	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			

1. In decreasing order of efficacy for a given lab and measure, significant differences between DE's 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 x 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 was transformed with an arcsin of the square root of the proportion dead to equalize variances between doses.