Evaluation of a new insecticide formulation (F2) as a protectant of stored wheat, maize, and rice ☆

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Abstract

A new commercial formulation, F2, was evaluated as a protectant of stored wheat, stored maize, and stored rough (paddy) rice. This formulation comprises the technical active ingredients 0.03% deltamethrin, 0.37% piperonyl butoxide, and 0.95% chlorpyriphos-methyl, plus 10% mineral oil and 88.0% of the diatomaceous earth Protect-It®. Tests were conducted with dust and slurry formulations at 50 and 100 ppm, 57% and 75% relative humidity, and 22°C, 27°C, and 32°C. On wheat, survival of the lesser grain borer, *Rhyzopertha dominica* (F.), ranged from 0% to 30.0%, survival of the rice weevil, *Sitophilus oryzae* (L.), was 0–6.2%, and survival of the red flour beetle, *Tribolium castaneum* (Herbst), was 0–97.5%. Few F_1 adults of any of the three species were found in the treated samples. Survival of the maize weevil, *Sitophilus zeamais* (Motschulsky), on treated corn was 0–32.5%, while survival of *T. castaneum* was 0–88.7% in the 50-ppm dust and slurry treatments, and 0–51.4% in the 100-ppm treatments. Again, few F_1 adults of either species were found in treated maize. Survival of *R. dominica* on treated rough rice averaged 0–4.1% and survival of *S. oryzae* on treated rice was 0–48.8%, but the majority of weevils that survived were in one replicate. F_1 adults in the treatments ranged from 0 to 24.4. Results show that the combination insecticidal product F2 was extremely effective on all three commodities at the rate of 100 ppm, as either a dust or slurry, and could be used as a commodity protectant.
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1. Introduction

There is renewed interest in using natural products to control insects in many agricultural environments, including post-harvest ecosystems. An example of a natural product is diatomaceous earth (DE), which is composed of fossilized deposits of diatoms that are either marine or freshwater in origin. In recent years there have been several popular articles (Quarles and Winn, 1996), reviews (Golob, 1997; Korunic, 1998; Subramanyam and Roesli, 2000), and research reports (Nielsen, 1998; Arthur, 2000a, b; Fields and Korunic, 2000a, b; Mewis and Ulrichs, 2001) regarding availability, efficacy and performance of various commercial DE products. An extensive list regarding current registrations of all inert dust products within the United States can be found in Subramanyam and Roesli (2000).

Although current commercial DE products are considerably more effective than older formulations (Subramanyam et al., 1998), inert dusts applied to grain can sometimes affect the physical properties of grain, including flow rate and test weight (Korunic et al., 1996, 1998). Higher rates are required to kill some insect species, such as the lesser grain borer, *Rhyzopertha dominica* (F.), compared to more mobile stored-grain pests (Fields and Korunic, 2000a). Also, high relative humidity (r.h.) and grain moisture content can have a negative effect on efficacy of DE (Golob, 1997; Korunic, 1998; Arthur, 2000a, b; Fields and Korunic, 2000a), thereby requiring higher rates or additional applications for control. One possibility for reducing the problems associated with DE as an insecticide is to reduce the application rate and use low rates of conventional chemicals combined with the DE.

Hedley Technologies Inc. (Mississauga, Ont., Canada) has developed a new formulation of a grain protectant that is a combination of DE with conventional insecticides, all of which are currently registered throughout much of the world for direct application to stored commodities. However, all of the ingredient insecticides may not be registered in any one particular country. This product, called F2, comprises the technical active ingredients deltamethrin, piperonyl butoxide, and chlorpyriphos-methyl, plus mineral oil and DE. One hundred ppm of the formulation contains 88 ppm of the DE Protect-It®, 0.95 ppm chlorpyrifos-methyl, 0.37 ppm piperonyl butoxide (PBO), 0.03 ppm deltamethrin, 10 ppm mineral oil, and 0.65 ppm inert ingredients. The objectives of this study were to: (1) assess the efficacy of F2 to control insect pests in stored wheat, stored maize, and stored rice; and (2) evaluate efficacy under different environmental conditions.

2. Materials and methods

2.1. Experiment 1: hard red winter wheat

Approximately 16 kg of wheat were removed from cold storage at 4°C and allowed to warm at about 25°C for several days. The wheat was then subdivided by placing 2 kg of wheat in each of eight 3.8-l glass jars, and about 10% of the wheat from each jar was cracked and ground in a blender, and returned to the jar. The jar was hand-tumbled for about 30 s to ensure even distribution of the cracked wheat. Wheat from four of the jars (8 kg) was treated as follows.

Two application methods of F2, a dry dust and a slurry, and two rates, 50 and 100 ppm for each, along with an untreated control, were evaluated in this test (five treatments total). Individual replicate treatment lots for each concentration consisted of 333 g wheat, of which approximately 10% consisted of cracked and ground wheat. The dry dust was applied at the rate of 50 and 100 ppm by placing the 333 g of wheat into a 0.95-l glass jar, and adding either 16.7 mg of dust for the 50-ppm treatment or 33.5 mg of dust for the 100-ppm treatment. Jars were hand-tumbled for about 30 s to evenly distribute the dust. The slurry for the 50-ppm rate was formulated by placing 250 mg of F2 dust in each of four 5-ml volumetric flasks, adding water to reach the 5-ml level, and thoroughly mixing the solution. For each replicate, 333 g of wheat was spread out in a thin layer on a piece of $0.62 \times 0.31 \, m^2$ heavy white paper, and a Badger 100 artists' airbrush (Franklin Park, IL, USA) was used to spray an aliquot of 0.33 ml solution on the wheat. This gave the desired concentration of 50 ppm per each replicate of 333 g. After the wheat was sprayed, it was placed in a 0.95 l glass jar, and shaken as described for the dust. Procedures were repeated for the 100-ppm slurry by placing 500 mg of F2 in the 5-ml vial, and formulating the solutions and treating the wheat as described above. The untreated control replicate was sprayed with 0.33 ml of tap water. Each of the five treatments was subdivided into nine vials, each containing 30 g of wheat, and the remainder of the wheat was discarded.

Twenty 1- to 2-week-old mixed-sex adult *R. dominica* were placed in each of three vials for each treatment. Similarly, twenty 1- to 2-week-old mixed-sex adult rice weevils, *Sitophilus oryzae* (L.), and red flour beetle, *Tribolium castaneum* (Herbst) were placed as single species into the remaining two groups of three vials. Three separate humidity chambers were created in $26 \times 36.5 \times 15 \, cm^3$ plastic boxes, with waffle-type grids cut to fit the bottom (Perez-Mendoza et al., 1999; Arthur, 2001, 2002). The boxes were filled with about 750 ml of saturated NaBr to maintain an r.h. of 57%, which is approximately equivalent to 12.5% moisture content (m.c.) (Greenspan, 1977). One vial of each species from each of the four chemical treatments and the untreated control was placed on the waffle grid in each box (five treatments × three species = 15 vials in each box). The entire design was replicated on the remaining 8 kg of wheat, except humidity boxes were filled with saturated NaCl to maintain an approximate r.h. of 75%, approximately equal to 14.5% m.c. (Greenspan, 1977).

One box of vials for each humidity was placed into each of three temperature incubators set at 22°C, 27°C, or 32 ± 1°C. A HOBO recording device (Onset Computers, Pocasett, MA, USA) was put inside each of the six plastic boxes to monitor temperature and r.h.. The vials containing the insects were incubated for 3 weeks and then removed. Insects were sifted from each vial using a #12 mesh sieve (1.7 mm apertures) for *R. dominica* and a #10 mesh sieve (2 mm apertures) for *S. oryzae* and *T. castaneum*, and survival was assessed by touching the insects to elicit a response. If the insect did not move when prodded it was classified as dead. The wheat was returned to the vials, which were in turn placed back in the humidity boxes inside each incubator and held for an additional 8–10 weeks, depending on the temperature, to record F_1 emergence. F_1 adults of *R. dominica* and *S. oryzae* were counted, while larvae, pupae, and adults were counted for *T. castaneum*.

Because of the expected and obvious difference between the untreated controls and the four insecticidal treatments, results were analyzed separately for the untreated control and the treatments, with insect species, temperature and r.h. as main effects for the untreated control, and insect species, treatment, temperature and r.h. as main effects for the four insecticide treatments.

This method of analysis also allowed for more appropriate comparisons of the main effects of temperature and r.h. levels. Variables for analysis were initial adult survival (after the 3-week exposure period), the number of F_1 adults of *R. dominica* and *S. oryzae*, and the number of F_1 larvae, pupae, and adults of *T. castaneum*. The ANOVA, REG, and GLM procedures of the Statistical Analysis System (SAS, 1987) were used in the data analysis. Unless otherwise specified, statistical significance was determined at the 0.05 level.

2.2. Experiment 2: maize

The process used to prepare maize and apply insecticide were as described for wheat, except the species used in the bioassays were the maize weevil, *Sitophilus zeamais* (Motschulsky), and *T. castaneum*. Untreated and treated replicates were divided into six vials containing 25 g each. The remainder of the maize was discarded. Bioassays were conducted and F_1 adults of the two species were collected following the same procedures as described for wheat, except that a #8 mesh sieve (2.36 mm apertures) was used to collect the insects. Statistical analyses were also done as described for wheat, except that the Wilcoxon Rank Sum Test under the NPAR1WAY procedure of SAS was also used in the analysis because of the variation in the data set.

2.3. Experiment 2: paddy (rough) rice

Rice was treated as previously described for wheat and maize. Each individual replicate lot of 333 g contained 10% cracked rice, treatment procedures were the same as described earlier for maize in that the untreated and treated replicates were divided into 6 vials containing 20 g each, and the remainder of the rice was discarded. Insects used in the bioassays were *R. dominica* and *S. oryzae*. Humidity chambers were created and placed in the incubator, bioassays were conducted, and F_1 adults were collected using the same methods as described for wheat and maize. Statistical analyses were also done as described for maize, including non-parametric analysis when appropriate.

3. Results

3.1. Experiment 1: wheat

The overall ANOVA indicated that the main effect species was significant for survival on untreated wheat ($F = 3.3, \text{d.f.} = 2, 54, P = 0.04$), but neither of the other two main effects (temperature and r.h.) were significant nor were any interactions significant. However, even though the ANOVA was significant for species, multiple comparison analysis for differences among species was not significant ($P = 0.20$). Survival of *R. dominica*, *S. oryzae*, and *T. castaneum* on untreated wheat averaged $97.1 \pm 0.8\%$, $97.5 \pm 1.1\%$, and $94.0 \pm 2.2\%$, respectively.

The ANOVA for treated wheat showed a significant difference for the main effects species ($F = 206.6, \text{d.f.} = 2, 214, P < 0.01$), insecticide treatment (F2 dust or slurry, 50- and 100-ppm) ($F = 57.7, \text{d.f.} = 3, 214, P < 0.01$), and temperature ($F = 15.1, \text{d.f.} = 2, 214, P < 0.01$), but r.h. was not significant ($F = 3.6, \text{d.f.} = 2, 214, P = 0.06$). All interactions except species $\times$ r.h., r.h. $\times$ temperature, and species $\times$ r.h. $\times$ temperature were significant ($P < 0.05$). Survival of

R. dominica after 3 weeks of exposure ranged from 0% to 30.0%, and at two of the temperature/ r.h. combinations, 57% r.h./32°C and 75% r.h./27°C, survival was greatest in the 50-ppm slurry treatment (Table 1). Survival of *S. oryzae* after 3 weeks of exposure was 0–6.2% in the 50-ppm dust treatments and 0 in all other insecticide treatments (Table 1). More *T. castaneum* survived after 3 weeks of exposure on treated wheat compared to survival of *R. dominica* or *S. oryzae* (Table 1). Survival of *T. castaneum* in the four insecticide treatments ranged from 0% to 97.5%, and in general was greater in the 50-ppm dust and slurry treatments than in the 100-ppm treatments. Also, there was a temperature effect in that survival was greater at 27°C and 32°C compared to 22°C in the 50-ppm dust treatment, 57% r.h. and in the 50-ppm dust treatment at 75% r.h. In the 50-ppm slurry treatment at 75% r.h., more *T. castaneum* survived at 32°C than at 22°C and 27°C.

Data for F_1 adults were analyzed separately by species because of the presence of larvae and pupae in bioassays conducted with *T. castaneum*. The number of F_1 adult *R. dominica* in untreated wheat was significant for main effect temperature ($F = 19.8, \text{d.f.} = 2, 18, P < 0.01$). The mean number ($\pm$ standard error) of F_1 adult *R. dominica* in untreated wheat held at 22°C, 27°C, and 32°C was 3.8 ± 3.6, 184.5 ± 57.1, and 435.2 ± 70.7, respectively, with each mean being significantly different from the other ($P < 0.05$). Few F_1 adults were found in the treated wheat. Only the main effect temperature was significant ($F = 4.1, \text{d.f.} = 2, 72, P = 0.02$), but the number of F_1's in treated wheat held at 22°C, 27°C, and 32°C averaged only 0, 0.2 ± 0.01, and 1.1 ± 0.6, respectively.

The numbers of F_1 adult *S. oryzae* in untreated wheat also differed significantly only for main effect temperature ($F = 29.8, \text{d.f.} = 2, 18, P < 0.01$). F_1 adults in untreated wheat held at 22°C, 27°C, and 32°C numbered 32.9 ± 15.2, 449.5 ± 56.0, and 333.6 ± 52.9, respectively, with fewer at 22°C compared to 27°C and 32°C ($P < 0.05$). Similar to *R. dominica*, few adults were found in the insecticide treatments. None of the main effects or interactions were significant, with the average over all treatments and conditions being only 1.6 ± 1.1.

F_1 production of all stages of *T. castaneum* in untreated wheat followed the same patterns as those observed for *R. dominica* and *S. oryzae*. For adults, only the main effect temperature was significant ($F = 13.7, \text{d.f.} = 2, 18, P < 0.01$). The mean number of F_1 adults in untreated wheat at 22°C, 27°C, and 32°C was 1.5 ± 1.0, 46.7 ± 6.8, and 114.6 ± 24.7, respectively, with each mean being significantly different from the other. For pupae, main effect temperature ($F = 7.3, \text{d.f.} = 2, 18, P < 0.01$) and the temperature $\times$ r.h. interaction was significant ($F = 4.8, \text{d.f.} = 2, 18, P = 0.02$). The number of pupae in wheat at 22°C, 27°C, and 32°C averaged 0, 7.7 ± 3.0, and 2.0 ± 0.9, respectively. For larvae, only the main effect temperature was significant ($F = 7.8, \text{d.f.} = 2, 18, P < 0.01$), and the number of larvae in wheat held at 22°C, 27°C, and 32°C averaged 0, 12.5 ± 3.9, and 22.5 ± 5.6, respectively. In the treatments, none of the main effects or interactions were significant for F_1 adults, and the overall average was 1.7 ± 1.2. Pupae (11.3 ± 6.3) and larvae (3.3 ± 1.7) were detected only in the 50-ppm dust treatment, 75% r.h./32°C, which biased the results of the ANOVA because of the 0 values in the remaining three treatments and temperature/r.h. combinations.

3.2. Experiment 2: maize

The ANOVA for survival on untreated maize showed significance for main effects species ($F = 13.5, \text{d.f.} = 1, 38, P < 0.01$) and r.h. ($F = 8.0, \text{d.f.} = 1, 38, P < 0.01$) but not for temperature or any interaction. Survival of *S. zeamais* was $97.5 \pm 1.0\%$ and $93.7 \pm 1.2\%$ at 57% and 75% r.h.,

Table 1
Survival (%, mean $\pm$ SEM) of parent *Rhyzopertha dominica*, *Sitophilus oryzae*, and *Tribolium castaneum* adults exposed for 3 weeks on wheat treated[a] with 50 or 100 ppm of F2 applied as a dust (D) or a liquid slurry (S)[b]; treated wheat was held at 57% or 75% r.h., and at 22°C, 27°C, or 32°C[c]

% Relative humidity	Treatment	Temperature (°C)		
		22	27	32
	Rhyzopertha dominica			
57	D-50	$0.0 \pm 0.0a$	$11.2 \pm 8.1a$	$1.2 \pm 1.2b$
	D-100	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$	$0.0 \pm 0.0b$
	S-50	$1.2 \pm 1.2a$	$8.7 \pm 5.1a$	$30.0 \pm 12.7a$
	S-100	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$	$1.2 \pm 1.2b$
75	D-50	$0.0 \pm 0.0a$	$3.7 \pm 2.4ab$	$0.0 \pm 0.0b$
	D-100	$3.7 \pm 2.4a$	$0.0 \pm 0.0b$	$0.0 \pm 0.0b$
	S-50	$2.5 \pm 1.4a$	$13.7 \pm 5.9a$	$5.0 \pm 2.0a$
	S-100	$1.2 \pm 1.2a$	$1.2 \pm 1.2b$	$0.0 \pm 0.0b$
	Sitophilus oryzae			
57	D-50	$0.0 \pm 0.0a$	$6.2 \pm 3.7a$	$1.2 \pm 1.2a$
	D-100	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$
	S-50	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$
	S-100	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$
75	D-50	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$
	D-100	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$
	S-50	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$
	S-100	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$	$0.0 \pm 0.0a$
	Tribolium castaneum			
57	D-50	$30.0 \pm 14.9ab$	$86.2 \pm 9.1aA$	$73.5 \pm 19.1aA$
	D-100	$0.0 \pm 0.0a$	$1.2 \pm 1.2b$	$0.0 \pm 0.0b$
	S-50	$52.5 \pm 17.9a$	$77.5 \pm 12.0a$	$76.2 \pm 9.0a$
	S-100	$11.2 \pm 8.1b$	$15.0 \pm 13.4b$	$21.2 \pm 18.1b$
75	D-50	$46.2 \pm 8.0aB$	$88.7 \pm 8.0aA$	$97.5 \pm 1.4aA$
	D-100	$5.0 \pm 5.0b$	$6.2 \pm 4.7b$	$5.0 \pm 5.0c$
	S-50	$1.2 \pm 1.2bB$	$15.0 \pm 3.5bB$	$57.5 \pm 5.9bA$
	S-100	$8.7 \pm 5.5b$	$22.5 \pm 15.8b$	$12.5 \pm 12.5c$

[a] Survival of *R. dominica*, *S. oryzae*, and *T. castaneum* on untreated wheat averaged $97.1 \pm 0.8\%$, $97.5 \pm 1.1\%$, and $94.0 \pm 2.2\%$, respectively.
[b] Means for treatment for each insect species and r.h. followed by the same lower-case letter are not significantly different ($P \geqslant 0.05$, Waller–Duncan k-ratio t-test).
[c] When means for temperature within treatment are significantly different, it is denoted by capital letters. No capital letters denote means were not significantly different ($P \geqslant 0.05$, Waller–Duncan k-ratio t-test).

respectively, while survival of *T. castaneum* was 100% and $98.3 \pm 0.9\%$, respectively. Although survival of *T. castaneum* was greater than survival of *S. zeamais* at both r.h. levels, the difference was so small that it could be considered as biologically insignificant.

The ANOVA for survival on treated maize showed significant differences for three main effects species ($F = 15.8$, d.f. $= 1, 144$, $P < 0.01$), insecticide treatment (F2 dust or slurry, 50- and 100-ppm) ($F = 9.9$, d.f. $= 3, 144$, $P < 0.01$), and r.h. ($F = 11.6$, d.f. $= 1, 144$, $P < 0.01$), but not for temperature ($F = 0.9$, d.f. $= 2, 144$, $P = 0.41$). All interactions except treatment $\times$ temperature, r.h. $\times$ temperature, and species $\times$ treatment $\times$ temperature were significant ($P < 0.05$). Survival of *S. zeamais* after 3 weeks of exposure was 0–32.5% in the 50-ppm dust and slurry treatments, and 0–28.7% in the 100-ppm treatments (Table 2). Although some means appeared to be greater than others, neither the standard ANOVA nor the Wilcoxon Rank Sum Test showed any differences among treatments within temperature and r.h. levels, or among temperatures within treatment and r.h. levels.

Survival of *T. castaneum* after 3 weeks of exposure was 0–88.7% in the 50-ppm dust and slurry treatments, and 0–54.1% in the 100-ppm treatments (Table 2). In some instances most of the survival came from one replicate, as evidenced by the large standard errors. Neither the ANOVA nor the Wilcoxon Rank Sum Test indicated a significant difference between treatments within temperature or between temperature within treatments at 57% r.h., however, both methods of analysis showed significant treatment effects at 75% r.h. Mixed results were observed at 22°C, but at 27° survival was greatest in the 50-ppm dust treatment, while at 32°C, survival was greater in the dust treatments than in the slurry treatments (Table 2).

Data for F_1's were analyzed separately by species, as was done for wheat. The number of F_1 adult *S. zeamais* in untreated maize was significant only for main effect temperature ($F = 8.8$, d.f. $= 2, 18$, $P < 0.01$). However, the number of F_1 adult *S. zeamais* produced on untreated maize was far less than the number of *S. oryzae* produced on untreated wheat. The mean number of F_1 adult *S. zeamais* in untreated maize held at 22°C, 27°C, and 32°C was 0.8 ± 0.4, 16.7 ± 5.6, and 1.1 ± 1.3, respectively. Few adults were produced in the treatments, only the main effect of r.h. was significant ($F = 4.1$, d.f. $= 1, 72$, $P = 0.048$), and the overall average at 57% and 75% r.h. was 0.5 ± 0.3 and 0.02 ± 0.02, respectively.

F_1 production of all stages of *T. castaneum* in untreated maize followed the same patterns evident for *S. zeamais*. For adults, only main effect temperature was significant ($F = 8.1$, d.f. $= 2, 18$, $P < 0.01$). The number of F_1 adults in untreated maize at 22°C, 27°C, and 32°C was 0.2 ± 0.2, 6.6 ± 4.1, and 20.6 ± 4.5, respectively, with each mean being significantly different ($P < 0.05$) from the other. For pupae and larvae, none of the main effects were significant, possibly because of variation in the data set. No pupae or larvae were found at 22°C, and the overall averages for numbers of each life stage were 3.4 ± 0.8 and 11.0 ± 3.6, respectively. For the treatments, none of the effects were significant for adults, with an overall average of 0.2 ± 0.1 across all treatments and combinations. No pupae or larvae were found in any of the treatment combinations.

3.3. Experiment 3: rough (paddy) rice

The ANOVA for survival on *R. dominica* and *S. oryzae* on untreated rice was significant for main effects temperature ($F = 11.2$, d.f. $= 1, 38$, $P < 0.01$) but not for species and r.h., and all interactions were significant ($P < 0.05$). Survival of *R. dominica* was not significant with respect to temperature, and overall survival averaged $87.0 \pm 4.0\%$ at all treatment combinations. Survival of *S. oryzae* was significant with respect to temperature, and averaged 99.4 ± 0.6, 99.4 ± 0.6, and 53.7 ± 17.0 at 22°C, 27°C, and 32°C, respectively.

Table 2

Survival (%, mean ± SEM) of parent *Sitophilus zeamais*, and *Tribolium castaneum* adults exposed for 3 weeks on maize treated[a] with 50 or 100 ppm of F2 applied as a dust (D) or a liquid slurry (S)[b]; treated maize was held at 57% or 75% r.h., and at 22°C, 27°C, or 32°C[c]

% Relative humidity	Treatment	Temperature (°C)		
		22	27	32
	Sitophilus zeamais			
57	D-50	0.0 ± 0.0a	0.0 ± 0.0a	2.5 ± 1.4a
	D-100	2.5 ± 2.5a	0.0 ± 0.0a	1.2 ± 1.2a
	S-50	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
	S-100	28.7 ± 16.9a	15.0 ± 10.6a	6.2 ± 4.7a
75	D-50	32.5 ± 23.6a	26.0 ± 19.9a	1.3 ± 1.3a
	D-100	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
	S-50	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
	S-100	0.0 ± 0.0a	0.0 ± 0.0a	1.3 ± 1.3a
	Tribolium castaneum			
57	D-50	11.2 ± 5.1a	12.5 ± 4.8a	1.2 ± 1.2a
	D-100	0.0 ± 0.0a	1.2 ± 1.2a	0.0 ± 0.0a
	S-50	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
	S-100	15.5 ± 13.2a	37.5 ± 21.8a	0.0 ± 0.0a
75	D-50	2.6 ± 2.6aB	68.7 ± 13.0aA	88.7 ± 4.6aA
	D-100	18.7 ± 15.6a	22.5 ± 17.9b	54.1 ± 26.7a
	S-50	0.0 ± 0.0a	7.6 ± 5.9b	1.2 ± 1.2b
	S-100	16.6 ± 16.6a	0.0 ± 0.0b	0.0 ± 0.0b

[a] Survival of *S. zeamais* on untreated maize was 97.5 ± 1.0 and 93.7 ± 1.2 at 57% and 75% r.h., respectively; survival of *T. castaneum* was 100% and 98.3 ± 0.9%, respectively.

[b] Means for treatment for each species and r.h. followed by the same lower-case letter are not significantly different ($P \geqslant 0.05$, Waller–Duncan *k*-ratio *t*-test).

[c] When means for temperature within treatment are significantly different, it is denoted by capital letters. No capital letters denote means were not significantly different ($P \geqslant 0.05$, Waller–Duncan *k*-ratio *t*-test).

The ANOVA for survival on treated rice indicated significant differences for main effects species ($F = 9.5$, d.f. $= 1, 144$, $P < 0.01$), insecticide treatment ($F = 4.6$, d.f. $= 3, 144$, $P < 0.01$), and r.h. ($F = 5.1$, d.f. $= 1, 144$, $P < 0.01$), but not for temperature ($F = 0.9$, d.f. $= 2, 144$, $P = 0.41$). Only the species × treatment interaction was significant ($P < 0.05$). Survival of *R. dominica* after 3 weeks of exposure was 0–4.1% in the 50-ppm dust and slurry treatments, and 0–2.5% in the 100-ppm treatments (Table 3), with no significant differences with respect to treatment or temperature.

Survival of *S. oryzae* after 3 weeks of exposure was 0–24.4% in the dust treatments, and 0–48.8% in the slurry treatments (Table 3). However, in many cases where there was survival, nearly all of the survival occurred in one replicate, as evidenced by a standard error equal to the mean. Survival was usually 0 in the remaining three replicates. Although the overall ANOVA indicated significance for main effects treatment and temperature, the biases in the data were such

Table 3
Survival (%, mean $\pm$ SEM) of parent *Rhyzopertha dominica* and *Sitophilus oryzae* adults exposed for 3 weeks on rough (paddy) rice treated[a] with 50 or 100 ppm of F2 applied as a dust (D) or a liquid slurry (S)[b]; treated rice was held at 57% or 75% r.h., and at 22°C, 27°C, or 32°C[c]

% Relative humidity	Treatment	Temperature (°C)		
		22	27	32
		Rhyzopertha dominica		
57	D-50	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a
	D-100	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a
	S-50	1.2 $\pm$ 1.2a	1.2 $\pm$ 1.2a	2.5 $\pm$ 2.5a
	S-100	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	1.2 $\pm$ 1.2a
75	D-50	1.2 $\pm$ 1.2a	2.5 $\pm$ 1.4a	2.5 $\pm$ 2.5a
	D-100	0.0 $\pm$ 0.0a	1.2 $\pm$ 1.2a	2.5 $\pm$ 2.5a
	S-50	0.0 $\pm$ 0.0a	4.1 $\pm$ 2.5a	4.0 $\pm$ 2.6a
	S-100	0.0 $\pm$ 0.0a	1.2 $\pm$ 1.2a	0.0 $\pm$ 0.0a
		Sitophilus oryzae		
57	D-50	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a
	D-100	5.0 $\pm$ 5.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a
	S-50	1.2 $\pm$ 1.2a	17.5 $\pm$ 17.5a	8.8 $\pm$ 8.8a
	S-100	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	5.0 $\pm$ 5.0a
75	D-50	24.4 $\pm$ 24.6a	24.4 $\pm$ 24.6a	5.0 $\pm$ 3.5a
	D-100	1.2 $\pm$ 1.2a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a
	S-50	18.4 $\pm$ 15.1aB	10.6 $\pm$ 7.4bB	48.8 $\pm$ 14.4aA
	S-100	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a

[a] Survival of *R. dominica* on untreated rice averaged 87.0 $\pm$ 4.0% (mean $\pm$ S.E.), survival of *S. oryzae* on untreated rice averaged 99.4 $\pm$ 0.6, 99.4 $\pm$ 0.6, and 53.7 $\pm$ 17.0 at 22°C, 27°C, and 32°C, respectively.
[b] Means for treatment for each species and r.h. followed by the same lower-case letter are not significantly different ($P \geqslant 0.05$, Waller–Duncan *k*-ratio *t*-test).
[c] When means for temperature within treatment are significantly different, it is denoted by capital letters. No capital letters denote means were not significantly different ($P \geqslant 0.05$, Waller–Duncan *k*-ratio *t*-test).

that neither the ANOVA nor the Wilcoxon Rank Sum Test showed any differences among treatments within temperature and r.h. levels, with the exception of 75% r.h., 32°C.

The numbers of F_1 adult *R. dominica* in untreated rice differed significantly only for main effect temperature ($F = 185.2$, d.f. $= 2, 18$, $P < 0.01$). The mean number of F_1 adult *R. dominica* in untreated rice held at 22°C, 27°C, and 32°C was 0.2 $\pm$ 0.1, 20.9 $\pm$ 4.0, and 122.0 $\pm$ 8.1, respectively, with each mean being different from the other ($P < 0.05$). Although the ANOVA for the number of F_1 adult *R. dominica* in treated rice was also significant only for main effect temperature ($F = 54.7$, d.f. $= 2, 72$, $P < 0.01$), few adult F_1's were produced in the treated rice. Averages for 22°C, 27°C, and 32°C were 0, 0.2 $\pm$ 0.01, and 0.8 $\pm$ 0.3, respectively.

The numbers of F_1 adult *S. oryzae* in untreated rice differed significantly for main effects temperature ($F = 9.3$, d.f. $= 2, 18$, $P < 0.01$) and r.h. ($F = 46.8$, d.f. $= 1, 18$, $P < 0.01$) but not the

Table 4
Number of F_1 adult ($\pm$SEM) *Sitophilus oryzae* produced when 20 parent mixed-sex adults were exposed for 3 weeks on rough (paddy) rice treated[a] with 50 or 100 ppm of F2 applied as a dust (D) or a liquid slurry (S)[b]; treated rice was held at 57% or 75% r.h.,[c] and at 22°C, 27°C, or 32°C[d]

	Temperature (°C)		
Treatment	22	27	32
D-50	4.2 ± 1.20a	8.2 ± 4.70ab	2.7 ± 2.2a
D-100	0.0 ± 0.0a	1.1 ± 0.7b	0.1 ± 0.1a
S-50	0.2 ± 0.2aB	24.4 ± 8.6aA	4.6 ± 2.2aB
S-100	0.0 ± 0.0a	8.7 ± 5.8ab	1.9 ± 1.0a

[a] The mean numbers of F_1 adult *R. dominica* in untreated rice held at 22°C, 27°C, and 32°C were 0.2 ± 0.1, 20.9 ± 4.0, and 122.0 ± 8.1, respectively; the numbers of F_1 adult *R. dominica* in treated rice were 0, 0.2 ± 0.01, and 0.8 ± 0.3, at each temperature, respectively; and the numbers of F_1 adult *S. oryzae* in untreated rice held at 22°C, 27°C, and 32°C were 14.9 ± 6.1, 45.2 ± 4.2, and 0.2 ± 0.2, respectively.

[b] Relative humidity was not significant ($P \geqslant 0.05$), and data were combined in analysis for treatment and temperature.

[c] Means for treatment followed by the same lower-case letter are not significantly different ($P \geqslant 0.05$, Waller–Duncan k-ratio t-test).

[d] When means for temperature within treatment are significantly different, it is denoted by capital letters. No capital letters denote means were not significantly different ($P \geqslant 0.05$, Waller–Duncan k-ratio t-test).

temperature × r.h. interaction. The mean numbers of F_1 weevils on untreated rice at 57% r.h., 22°C, 27°C, and 32°C were 14.4 ± 6.1, 45.2 ± 4.2, and 0.2 ± 0.2, respectively, with all means being different. The numbers of F_1's at 75% r.h., 22°C, 27°C, and 32°C were 16.5 ± 6.3, 68.0 ± 7.1, and 15.7 ± 5.5, respectively, with more at 27°C than at either 22°C or 32°C.

Main effects treatment ($F = 3.4$, d.f. $= 3, 72$, $P < 0.01$) and temperature were significant ($F = 8.1$, d.f. $= 2, 72$, $P < 0.01$) for F_1 adult *S. oryzae* in the treatments, but not r.h. or any interaction. This experiment with rice produced the only occurrence of an appreciable number of F_1's in any of the four insecticide treatments. However, there were few significant differences among the four treatments at each of the three temperatures, and the only significant difference among temperatures occurred in the 50-ppm slurry treatment (Table 4).

4. Discussion

Insects exposed to diatomaceous earth (DE) and other inert dusts are subject to physical and biological stresses, and combining desiccant dusts with other physical or chemical controls may either produce synergistic effects or reduce the amount of dust needed to control stored-product insects. Dowdy (1999) showed complete kill of *T. castaneum* exposed for 15–30 min to 5 g/m^2 of the commercial formulations Protect-It® or Insecto®, combined with high temperatures of 50°C, compared to 51–65% mortality at 50°C alone. Field trials have also shown the potential of combining DE and heat treatments (Fields et al., 1997).

Only a few literature reports document increased efficacy when combination treatments of desiccant dusts, particularly DE, are applied directly to stored grains. Published reports of tests

with silica gels show poor or mixed results (Subramanyam and Roesli, 2000), possibly because the intention of these tests was to enhance efficacy of insecticides rather than enhance efficacy of the dusts. Combination treatments of silica gels and organophosphates applied to wheat were not as effective as when the same rates of these insecticides were applied by themselves (Shawir et al., 1988). Barbosa et al. (1994) reported a synergistic effect of the silica gel Gasil 23D on deltamethrin but not pirimiphos-methyl, in combination trials in which the larger grain borer, *Prostephanus truncatus* (Horn), was exposed on treated shelled maize. In a recent study (Lord, 2001), combination treatments of DE and other sorptive dusts plus the fungal pathogen *Beauvaria bassiana* (Balsamo) were applied directly to wheat. In general, most of these combination treatments showed increased efficacy against *R. dominica* compared with *B. bassiana* applied alone, indicating some additive effects from the desiccant dusts.

Rhyzopertha dominica and *T. castaneum* are less susceptible to DE than other stored-product beetles (Korunic, 1998; Fields and Korunic, 2000a; Subramanyam and Roesli, 2000), and require either higher rates or longer exposure intervals for complete control. In this study, both the dusts and slurry applications of F2 gave virtually complete control of *R. dominica* on wheat. Although there were some treatment and temperature or humidity effects on survival after the 3-week exposure period, there were few F_1 adults in the treated wheat.

Although survival of *T. castaneum* on wheat treated with the two formulations and two rates of F2 was variable and extensive for some treatments, no F_1 adults were found in the treated wheat. Either the parent adults were unable to reproduce on the treated wheat, or the larvae died sometime during the developmental process. Most DE formulations are extremely effective against insect larvae, although susceptibility may decrease as the larvae age (Subramanyam et al., 1998; Mewis and Ulrichs, 2001). If *T. castaneum* was able to reproduce in these tests, it is likely that the early instars were killed before they could develop to the later stages where they would be less susceptible to the DE. Few *S. oryzae* survived on the treated wheat and no F_1 adults were produced. In previous tests in which *S. oryzae* was exposed on wheat treated with 300 ppm of the Protect-It® formulation of DE and held at 22°C, 27°C, and 32°C, 40%, 57%, and 75% r.h., all weevils were killed after 1 week at 40% and 57%, while exposure intervals of up to 3 weeks were needed for 100% kill at 75% r.h. (Arthur, 2002).

DE dusts may be more effective on wheat than on maize (Subramanyam et al., 1994). There are several reports of trials in tropical Africa (Barbosa et al., 1994; Gudrups and Golob, 2000) and in China (Ling et al., 2000) where different commercial formulations of DE applied to maize did not control stored-product insects, especially at higher relative humidities or moisture contents. In my tests, no *S. oryzae* survived exposure on wheat treated with F2, whereas some survival of *S. zeamais* occurred on treated maize. It is difficult to make a direct comparison because survival could also have been related to the different species in addition to any variation between the two commodities. Although no F_1 adult *S. zeamais* were found in treated maize, similar to results for *S. oryzae* in treated wheat, the number of F_1 *S. zeamais* in untreated control maize was much lower than corresponding F_1 *S. oryzae* in untreated wheat. This difference may have been related to the relative difference in the size of the maize kernel compared to a wheat kernel, the nutritional differences between maize and wheat, or the high oil content in maize.

Survival of *T. castaneum* on maize at the various treatment combinations was often greater at 75% compared to 57% r.h., indicating the expected reduction in compound efficacy at higher r.h.

levels (Golob, 1997; Korunic, 1998). A positive effect was noted in some instances with temperature, which is consistent with most studies involving efficacy trials with DE (Arthur, 2000b; Subramanyam and Roesli, 2000). However, this temperature effect is not always consistent. Fields and Korunic (2000b) reported lower mortality of *T. castaneum* exposed at 30°C compared to 20°C, the reverse for the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), and mixed results with *S. oryzae* depending on the specific formulation of DE. Previous studies have also reported difficulties in controlling *T. castaneum* on stored grains (Fields and Korunic, 2000a). Again, few F_1 of any stage were found in the treatments, indicating susceptibility of larvae to the F2 formulation, but it is difficult to assess reproductive inhibition because of the low number of F_1 adults in untreated maize compared to untreated wheat.

Little published information is available regarding efficacy of DE alone or in combination with other insecticides on rough rice, especially with newer formulations. Several publications (McGaughey, 1972; Ling et al., 2000) document difficulty in controlling insects on rough rice with rates <1000 ppm, and indicate that higher application rates may be necessary. In the current tests survival of *R. dominica* and *S. oryzae* did not exceed 5% on rough rice treated with 100 ppm of the dust or slurry formulation of F2. Again, it was difficult to assess treatment efficacy on reproduction because of the low numbers of F_1 adults of both *R. dominica* and *S. oryzae* in the controls held at 22°C and 32°C, compared to these same species on untreated wheat. Both of these insects are reared in laboratory colonies on wheat, which partially accounts for the fact that they did not reproduce as well on rough rice as they normally would on wheat. However, there were fewer F_1 on treated rice at 27°C, in contrast to untreated rice, and coupled with the obvious difference in survival, the treatments were obviously effective on rice.

One of the limitations of DE is its negative effects on physical properties of grain, and these effects seem to have consistent trends, with some variation, among the various grain crops (Korunic et al., 1996; Korunic et al., 1998). Some effects on physical properties can occur even when products are used at the recommended label rates, particularly at the higher rates required to kill *R. dominica* and *T. castaneum* (Korunic et al., 1998). Given the effects of desiccant dusts on the physical properties of grain, it may be more realistic to evaluate DE products for potential use as surface treatments rather than treatments to the entire grain mass. The results of this study show combinations of DE with conventional chemicals could be used to control insects in stored commodities. However, although there is tremendous potential in using DE in combination with other insecticidal products, regulatory approval must be obtained for each constituent product in the formulation mixture, as well as the combination. This process can vary from country-to-country, along with the economic and social costs required for registration. Also, commercial sources of DE vary greatly in efficacy and performance (Korunic, 1997; Fields and Korunic, 2000a), and results from one DE product are not necessarily transferable to others.

References

Arthur, F.H., 2000a. Toxicity of diatomaceous earth to red flour beetles and confused flour beetles: effects of temperature and relative humidity. Journal of Economic Entomology 93, 526–532.

Arthur, F.H., 2000b. Impact of food source on survival of red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae) exposed to diatomaceous earth. Journal of Economic Entomology 93, 1347–1356.

Arthur, F.H., 2001. Immediate and delayed mortality of *Oryzaephilus surinamensis* (L.) exposed on wheat treated with diatomaceous earth: effects of temperature, relative humidity, and exposure interval. Journal of Stored Products Research 37, 13–21.

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 Products Research 38, 305–313.

Barbosa, A., Golob, P., Jenkins, N., 1994. Silica aerogels as alternative protectants of maize against *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) infestations. In: Highley, E., Wright, E.J., Banks, J.H., Champ, B.R. (Eds), Stored-Product Protection: Proceedings of the Sixth International Working Conference on Stored-Product Protection, April 1994, Canberra, Australia. CAB International, Wallingford, UK, pp. 623–627.

Dowdy, A.K., 1999. Mortality of *Tribolium castaneum* exposed to high temperature and diatomaceous earth combinations. Journal of Stored Products Research 35, 175–182.

Fields, P., Korunic, Z., 2000a. 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., 2000b. Diatomaceous earth to control *Cryptolestes ferrugineus* (Coleoptera: Cucujidae) in stored barley in farm granaries. The Canadian Entomologist 132, 247–258.

Fields, P.G., Dowdy, A., Marcotte, M., 1997. Structural pest control: the use of an enhance diatomaceous earth product combined with heat treatment for the control of insect pests in food processing facilities. Environmental Bureau, Agriculture and Agri-Food Canada, and the US Department of Agriculture.

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.

Greenspan, L., 1977. Humidity fixed points of binary saturated aqueous solutions. Journal of Research of the National Bureau of Standards-Physics and Chemistry 81A, 89–96.

Gudrups, I., Golob, P., 2000. The use of inert dusts and insect growth regulators in Malawi to protect stored maize and red kidney beans from insect attack. In: Zuxun, J., Quan, L., Yongsheng, L., Xianchang, T., Lianghua, G. (Eds), Proceedings of the Seventh International Working Conference on Stored-Product Protection, October 1998, Beijing, China. Sichuan Publishing House of Science and Technology, Chengdu, PRC, pp. 730–737.

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 earths, a group of natural insecticides. Journal of Stored Products Research 34, 87–97.

Korunic, Z., Fields, P., Kovacs, M.I.P., Noll, J.S., Lukow, O.M., Demianyk, C.J., Shibley, K.J., 1996. The effect of diatomaceous earth on grain quality. Postharvest Biology and Technology 9, 373–387.

Korunic, Z., Cenkowski, S., Fields, P., 1998. Grain bulk density as affected by diatomaceous earth and application method. Postharvest Biology and Technology 13, 81–89.

Ling, Z., Zhanggui, Q., Korunic, Z., 2000. Field and laboratory experiments with Protect-It, an enhanced diatomaceous earth, in P.R. China. In: Zuxun, J., Quan, L., Yongsheng, L., Xianchang, T., Lianghua, G., (Eds), Proceedings of the Seventh International Working Conference on Stored-Product Protection, October 1998, Beijing, China. Sichuan Publishing House of Science and Technology, Chengdu, PRC, pp. 745–757.

Lord, J.C., 2001. Desiccant dusts synergize the effect of *Beauvaria bassiana* (Hyphomycetes: Moniliales) on stored-grain beetles. Journal of Economic Entomology 94, 367–372.

McGaughey, W.M., 1972. Diatomaceous earth for confused flour beetle and rice weevil control in rough, brown, and milled rice. Journal of Economic Entomology 65, 1427–1428.

Mewis, I., Ulrichs, Ch., 2001. Action of amorphous diatomaceous earth against different stages of the stored product pests *Tribolium confusum*, *Tenebrio molitor*, *Sitophilus granarius* and *Plodia interpunctella*. Journal of Stored Products Research 37, 153–164.

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.

Perez-Mendoza, J., Baker, J.E., Arthur, F.H., Flinn, P.W., 1999. Effects of Protect-It on efficacy of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) parasitizing rice weevils (Coleoptera: Curculionidae) in wheat. Environmental Entomology 23, 529–534.

Quarles, W., Winn, P., 1996. Diatomaceous earth and stored product pests. IPM Practitioner 18, 1–10.

SAS Institute, 1987. SAS/STAT Guide for Personal Computers, 6th Edition. Statistical Analysis System Institute, Cary, NC.

Shawir, M., Le Patourel, G.N.J., Moustafa, F.I., 1988. Amorphous silica as an additive to dust formulations of insecticides for control of stored product insects. Journal of Stored Products Research 24, 123–130.

Subramanyam, Bh., Roesli, R., 2000. Inert dusts. In: Subramanyam, Bh., Hagstrum, D.W. (Eds.), Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic Publishers, Boston, MA, pp. 321–379.

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.

Subramanyam, Bh., Swanson, C.L., Madamanchi, N., Norwood, S., 1994. Effectiveness of Insecto, a new diatomaceous earth formulation, in suppressing several stored-grain insect species. In: Highley, E., Wright, E.J., Banks, J.H., Champ, B.R., (Eds), Stored-Product Protection: Proceedings of the Sixth International Working Conference on Stored-Product Protection, April 1994, Canberra, Australia. CAB International, Wallingford, UK, pp. 650–659.