Effectiveness of Insecto®, a new diatomaceous earth formulation, in suppressing several stored-grain insect species[1,2]

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Abstract

Insecto® is registered in the United States for use on stored grain and empty grain-holding facilities to control insects. The effectiveness of Insecto® in suppressing populations of six economically important stored-grain insect species during an 8.2-month test period was studied in 12 metal barrels using 109-kg lots of wheat per barrel. The six species used in tests were the lesser grain borer, *Rhyzopertha dominica* (F.); rice weevil, *Sitophilus oryzae* (L.); sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); flat grain beetle, *Cryptolestes pusillus* (Schönherr); and red flour beetle, *Tribolium castaneum* (Herbst). There were three Insecto® treatments and a control (untreated wheat) treatment. Each treatment was replicated three times. In Insecto® treatments 1 and 2, the top 27.2 and 54.4 kg of wheat, respectively, were treated with Insecto® at the labeled rate of 0.5 g/kg of grain. In Insecto® treatment 3, all 109 kg were treated with 0.5 g of Insecto®/kg of grain. In Insecto® treatments 1, 2, and 3, the grain surface in each barrel was top dressed with 152.6, 101.9, and 20.0 g of Insecto® per square meter, respectively.

Thirty adults of each species were introduced into each of the barrels. After adult introduction, beetle adults were sampled with six Storgard WB probe II traps per barrel at approximately monthly intervals during the 8.2-month test period. Numbers of each species captured in traps in two days were counted. In untreated wheat, *R. dominica* was the most abundant species followed by *S. oryzae*, *C. ferrugineus*, *O. surinamensis*, *T. castaneum*, and *C. pusillus*. Differences in number of beetles captured in traps among the treatments, and the overall percent reduction in trap catch in Insecto® treatments relative to the control treatment, indicated that the three Insecto® treatments were equally effective in suppressing insects. Insecto® effectively suppressed *C. ferrugineus* and *C. pusillus* populations (99.5 to 100% suppression), followed by *O. surinamensis* (94.8 to 97.1%), *S. oryzae* (82.5 to 93.4%), *T. castaneum* (57.4 to 98.7%), and *R. dominica* (55.5 to 70.4%). There were 1.4 to 2.4 times fewer damaged kernels in Insecto® treatments than in the control treatment. In the laboratory, adults of these six insect species and one additional species (merchant grain beetle, *Oryzaephilus mercator* [Fauvel]), were highly susceptible (96 to 100% mortality) when exposed for 14 days to wheat or barley treated with 0.5 g of Insecto®/kg of grain. At an Insecto® rate of 1.0 g/kg of wheat or barley, 100% mortality of the same insect species occurred within seven days.

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[2] Mention of a proprietary product does not constitute an endorsement for its use by the University of Minnesota.

Introduction

Inert materials, such as diatomaceous earth and silica aerogel dusts, have been tested for stored-grain insect control by numerous investigators (Parkin 1944; LaHue 1965, 1967, 1978; White et al. 1966; Redlinger and Womack 1966; McGaughey 1972; le Patourel 1986; Desmarchelier and Dines 1987; Aldryhim 1990, 1993). These inert dusts damage or adsorb the water-resistant epicuticle of insects, inducing death by dehydration (Alexander et al. 1944; Carlson and Ball 1962; Ebeling 1971).

In the United States, diatomaceous earth dusts have been evaluated for suppressing insects in stored shelled maize (Redlinger and Womack 1966), wheat (Strong and Sbur 1963; White et al. 1966; LaHue 1965, 1967, 1978), and rice (McGaughey 1972). The diatomaceous earth formulations tested were Kenite®, Perma-Guard® (LaHue 1965; Redlinger and Womack 1966), and Dicalite IG 3 (Strong and Sbur 1963). The effectiveness of these dusts on various insect species was variable, and high rates (2–4 g of dust/kg of grain) were necessary to obtain satisfactory (≥ 95% mortality) insect control. Prior to the 1980s, for stored-grain insect control, the Kenite and Perma-Guard labels recommended a rate of 3.5 g of dust/kg of grain. At this high rate, flowable properties of the grain were affected, test weight was reduced, grain quality was lowered, and excessive amounts of dust were produced during handling. Therefore, these dusts were not widely used or accepted by the grain industry for insect control. The milling industry also was reluctant to accept grain treated with diatomaceous earth because of possible damage to milling machinery.

The development of resistance in stored-grain insects to the organophosphate grain protectants [malathion, pirimiphos-methyl, and chlorpyrifos-methyl] (Subramanyam et al. 1989; Zettler and Cuperus 1990), and to the commonly used grain fumigant phosphine (Zettler 1991), coupled with consumer concern for pesticide residues in processed cereal grains (National Research Council 1993), has prompted us to explore newer diatomaceous earth formulations as alternatives to pesticides for stored-grain insect control.

Insecto® is a new diatomaceous earth formulation registered by the United States Environmental Protection Agency in June 1984. Registered uses of Insecto® include application to empty grain-holding facilities at the rate of 5 g/m^2, and to grain at a rate of 0.5–1.0 g/kg of grain. Insecto® is an off-white powder containing 86.7% (by weight) of amorphous silica (silicon dioxide) and ≤ 3% of crystalline silica. The formulation also contains 10% of food-grade additives. The physical characteristics of the formulation are as follows: mean particle diameter, 6.89 µm; mean particle size, 1.0–34.3 µm; retention on 325 mesh, 0.5%; oil adsorption capability, 175% by weight; pH, 7.0; bulk density, 0.128 g/cm^3; specific gravity, 2.3; and moisture, 7.8%.

There is very little information documenting the activity of Insecto® against stored-grain insects. Therefore, a small-scale field test was designed to determine effectiveness of Insecto® in suppressing populations of six economically important stored-grain insect species in wheat during an 8.2-month test period, and to assess kernel damage at the end of the test period. In the laboratory, susceptibility of the same six insect species and one additional species was evaluated by exposing adults to Insecto®-treated wheat and barley.

Materials and Methods

Small-scale field test

Experimental treatments and insect introduction

Experiments were conducted in 12 metal barrels of 208.2 L volume using 109 kg of wheat per barrel. The barrel diameter was 58.4 cm. The wheat was of 13.0% moisture, and contained 0.12% foreign matter. Four treatments, replicated three times, were assigned to the 12 barrels. The four treatments were: untreated wheat (control), and Insecto® treatments 1, 2, and 3. In Insecto® treatment 1, the top 27.2 kg layer of wheat was treated with 0.5 g of Insecto®/kg of grain, and the grain surface was top dressed with 152.6 g of Insecto®/m^2. In Insecto® treatment 2, the top 54.4-kg layer of wheat was treated with 0.5 g of Insecto®/kg of grain, and Insecto® was applied to the grain surface at the rate of 101.9 g/m^2. In Insecto® treatments 1 and 2, the quantity of top dressed Insecto® corresponds to the amount that was not applied (at the rate of 0.5 g/kg of grain) to 75% and 50% of the grain mass, respectively. In Insecto® treatment 3, all the wheat (109 kg) was treated with 0.5 g of Insecto®/kg of grain, and the surface was dusted with an additional 20.0 g of Insecto®/m^2. In Insecto® treatment 3, the actual Insecto® rate (based on amounts applied to the grain and the grain surface) was 0.55 g/kg of grain.

On 22 January 1992, wheat was treated with Insecto®, by passing the grain and the required amount of Insecto® into a seed-treating device (L. R. Jacobs Seed Mixer, Serial No. 390, Yellow Springs, Colorado). The wheat and Insecto® were passed through the device twice to ensure uniform coverage of kernels with the dust. Insecto® sprinkled on the grain surface was admixed with kernels by slightly disturbing the grain surface with a wooden stick.

On 3 February, 180 unsexed 2–4-week-old adults of six insect species (30 adults per species) were introduced via the surface into each of the barrels. Barrels were tightly covered with reusable and removable lids made from plastic garbage bags and an adhesive (packaging) tape. The six insect species were: lesser grain borer, *Rhyzopertha dominica* (F.); rice weevil, *Sitophilus oryzae* (L.); sawtoothed grain beetle, *Oryzaephilus surinamensis* (F.); rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); flat grain beetle, *Cryptolestes pusillus* (Schönherr); and red flour beetle, *Tribolium castaneum* (Herbst). *Rhyzopertha dominica* and *S. oryzae* were reared on whole wheat kernels; *T. castaneum* was reared on whole wheat flour + 5% [by weight] Brewer's yeast diet; *C. ferrugineus*, *C. pusillus*, and *O. surinamensis* were reared on rolled oats + 5% Brewer's yeast diet. All insect species were obtained from the Department of Entomology, Kansas State University, Manhattan, Kansas.

Treatment efficacy was verified by sampling each barrel with six Storgard WB Probe II traps (see Subramanyam et al. 1993) on six different occasions. Insects were sampled 22 (February 25), 80 (April 23), 106 (May 19), 143 (June 25), 212 (September 2), and 247 (October 7) days after adult introduction. In each barrel, three traps were placed at the barrel bottom, and three just below the grain surface. At each depth, one trap was placed at the barrel centre, and two traps were placed mid-way between the barrel centre and barrel edge along a north–south transect. Except for the June 25 trapping, traps were left in the grain for 2 days. During June 25 trapping, traps were in the grain for 7 days to increase the probability of insect detection (see Cuperus et al. 1990). Adults captured in each trap were separated by species and counted.

Analysis of trap catch data

Statistical analysis was performed on individual and combined species' trap catch data. Raw data (z) were transformed to log (z + 1) scale to stabilise variances. A split-split-plot analysis of variance [ANOVA] (SAS Institute 1988) was used to determine differences in number of insects captured among treatments, sampling occasions, and between the top and bottom trap positions within barrels. Only these three effects were considered important in this study, and information on interactions is therefore not presented or discussed. The insect counts were made repeatedly on the same wheat in barrels, and trap catches of insects in the top and bottom portions of wheat in each barrel were assumed to be independent. In spite of these design limitations, analyzing the trap catch data using a split-split-plot ANOVA was still legitimate (Dr Frank B. Martin, School of Applied Statistics, University of Minnesota; pers. comm). On each sampling occasion, differences in trap catch among control and Insecto® treatments were determined by one-way ANOVA and orthogonal contrasts (SAS Institute 1988). Percent reduction (suppression) in mean trap catch of insects in Insecto®-treated wheat (TRT) relative to catch in the control treatment (CON) was calculated as follows: $100 \times (1-[TRT \div CON])$. Because all treatments were sampled over time, a significant and consistent reduction ($\geq$ 90%) in insect catch in Insecto®-treated wheat compared with catch in the control treatment indicated that Insecto® was effective in killing introduced adults and/or in suppressing population growth by killing progeny of the introduced adults.

Grain sampling

On 19 March and 10 July, five samples were withdrawn from each barrel with a 12-compartment grain trier, by probing the grain in the barrel centre and in each cardinal direction. In each cardinal direction, samples were taken mid-way betwen the barrel centre and barrel edge. The trier was inserted into the grain at a 10° angle until the its bottom touched the barrel bottom. All five grain samples from a barrel were pooled, and each pooled sample was placed in a Ziplock® bag for laboratory analysis. Grain samples were processed within 6 hours of collection. The weight of the pooled sample among barrels averaged 590 g. Each pooled sample was passed over a 2.1 mm round-holed sieve to separate live adults. Live adults in each sample were counted. Adult data were transformed to logarithmic scale and subjected to one-way ANOVA and orthogonal contrasts to determine differences among treatments. Percent reduction in insect numbers in Insecto® treatments relative to the control treatment was calculated as explained above.

Estimating kernel damage

On the last day of the experiment (9 October), five trier samples were withdrawn from each barrel in the manner described above. The weight of the pooled sample among barrels averaged 485 g (range among barrels, 354–665 g). Each sample was passed over a 2.1-mm round-holed sieve to separate dockage and grain/Insecto® dust. The amount of dockage was weighed (wet weight), and this weight was expressed as a proportion (or percentage) of the total sample weight. Proportions were transformed to angular values, and subjected to one-way ANOVA and orthogonal contrasts (SAS

Institute 1988) to determine differences among treatments. Each pooled sample was passed through a Boerner divider to obtain a working sample of 10 g. All kernels in the 10 g sample were checked for insect damage. Kernels with shallow or deep holes in the germ or endosperm or both were counted as damaged. Treatment differences were determined by one-way ANOVA and orthogonal contrasts (SAS Institute 1988).

Laboratory tests

In the laboratory, Insecto® was applied to wheat and barley at three rates (two label rates [0.5 and 1.0 g/kg of grain] and one above the higher label rate [1.5 g/kg of grain]) to determine effectiveness against adults of five to seven stored-grain insect species.

Effectiveness of Insecto® on wheat

In trial I, wheat of 12.7% moisture was treated with Insecto® to give rates of 0.5, 1.0, and 1.5 g/kg of grain. The required amount of Insecto® and grain were placed in a plastic barrel, which was tumbled on a ball-mill roller for 10 minutes. Untreated grain served as control. One hundred grams of untreated and Insecto®-treated grain were placed in separate 0.47 L glass jars. Into each jar, 50 unsexed (2–4-week-old) adults of one of the following insect species were introduced: *C. ferrugineus*, *C. pusillus*, *O. surinamensis*, merchant grain beetle, *Oryzaephilus mercator* (Fauvel), *T. castaneum*, *R. dominica*, and *S. oryzae*. Except for *R. dominica*, there were eight jars per treatment. For *R. dominica*, there were six jars per treatment. After adult introduction, jars were closed with wire-mesh screens and filter paper lids, and kept in a chamber maintained at 27°C and 65% r.h. On day 7, half of the jars were selected at random, and mortality of insects in each jar was recorded. The remaining jars were checked on day 14. Percentage mortality of insects in Insecto® treatments was corrected for control mortality using Abbott's formula (Abbott 1925). For each species, corrected percentage mortality data were transformed to angular values and subjected to factorial ANOVA to determine differences among Insecto® doses, between exposure durations, and dose × exposure duration interaction. Significant differences among Insecto® doses or between exposure durations were determined by Bonferroni *t* tests (SAS Institute 1988). This analysis was performed only on *R. dominica* and *S. oryzae* data, because the 7-day mortality of the other species exposed to Insecto®-treated wheat was 100%.

In trial I, except for *S. oryzae*, 7-day mortality of the other species was > 10% in the control treatment. Therefore, trial II was run using untreated wheat (13% moisture), and wheat treated with 0.5 and 1.0 g of Insecto®/kg of grain. Unsexed 2–4-week-old adults of *C. ferrugineus*, *O. surinamensis*, *R. dominica*, *T. castaneum*, and *S. oryzae* were used in trial II. Wheat treatment and number of insects added to wheat were similar to trial I. Each treatment was replicated three times. Mortality assessments were made after 7 days. Insect mortality on untreated wheat was ≤ 11%. Data were not statistically analysed because of 100% mortality of all five species at the two Insecto® doses.

Effectiveness of Insecto® on barley

Barley (13.4% moisture) was treated with Insecto® as explained above to provide rates of 0.5, 1.0, and 1.5 g/kg of grain. Untreated barley served as control. About 100 g of untreated and Insecto®-treated barley (95% whole barley and 5% cracked barley) were placed in separate jars. Cracked barley was added to promote insect survival. Preliminary tests showed that on untreated barley without cracked kernels, > 40% mortality of insects occurred within 7 days. Cracked barley was treated separately with Insecto® and later added to 95% of whole barley. Insect species used in tests included: *S. oryzae*, *O. surinamensis*, *O. mercator*, *C. ferrugineus*, *C. pusillus*, and *T. castaneum*. Except for *C. pusillus*, each jar was infested with 100 adults of a species. In tests with *C. pusillus*, 50 adults were introduced into each jar because adequate number of adults could not be obtained from cultures. Infested jars were kept at 26°C and 50% r.h. for 7 or 14 days. At each dose and exposure duration, there were 3–4 jars. If the 7-day mortality of insects in at least one treatment was < 100%, a separate set of jars was sampled to determine 14-day mortality. Mortality of only *T. castaneum* and *S. oryzae* were checked on two separate occasions. Mortality of insects in Insecto® treatments was corrected for control mortality (Abbott 1925). Corrected mortality data (proportions) were transformed to angular values. One-way or factorial ANOVA (*T. castaneum* and *S. oryzae*) and Bonferroni *t* tests (SAS Institute 1988) were used to determine differences among treatments, between exposure durations, and treatment × exposure duration interaction.

Results

Small-scale field test

Mean trap catches of the six insect species during the 8.2-month test period are shown in Figure 1. The split-split-plot ANOVA indicated that mean trap catches of only *R. dominica* and *S. oryzae* adults among the four treatments were not significantly different ($F = 1.87–2.74$; $df = 3, 8$; $P > 0.11$). For all six species, mean trap catches over time were highly significant ($F = 4.08–66.56$; $df = 5, 40$ or $4, 20$ [only for *C. pusillus*]; $P < 0.005$). Trap position effect was significant only for *S. oryzae* ($F = 8.17$; $df = 1, 47$; $P = 0.006$) and *C. pusillus* ($F = 4.75$; $df = 1, 39$; $P = 0.035$). For these two species, the observed significant differences were a result of trap catch differences only in the control treatment. For example, in the control barrels, consistently more *S. oryzae* were captured in traps placed just below the grain surface than in traps at the barrel bottom. However, more *C. pusillus* adults in the control barrels were captured in traps at the barrel bottom than in traps placed below the grain surface.

Except for *C. pusillus*, adults of the other insect species were captured on all six sampling occasions only in traps in the control treatment (Fig. 1). In the control treatment, adults of *C. pusillus* were detected on the first, third, and fourth sampling occasions. In Insecto® treatment 1, adults of *C. pusillus* were detected by traps only on the fourth sampling occasion. In Insecto® treatments 2 and 3, the traps failed to detect *C. pusillus* adults on all six sampling occasions. In Insecto® treatments 1 and 2, *C. ferrugineus* was detected only on the fourth and fifth sampling occasions, and in Insecto® treatment 3 on second, fourth, and fifth sampling occasions. Adults of *R. dominica*, *S. oryzae*, *O. surinamensis*, and *T. castaneum* in the three Insecto® treatments were detected by traps on 3–5 sampling occasions.

One-way ANOVA and orthogonal contrasts of trap catch data indicated that differences among the four treatments varied with the insect species and sampling occasion (Table 1). Trap catches of *R. dominica* and *S. oryzae* among the four treatments were not significantly different from one another ($P > 0.05$) on five and four sampling occasions, respectively. On three of six sampling occasions, trap catches of *O. surinamensis* were not significantly different ($P > 0.05$) among the four treatments. In general, trap catches of insects in the three Insecto® treatments were lower than in the control treatment,

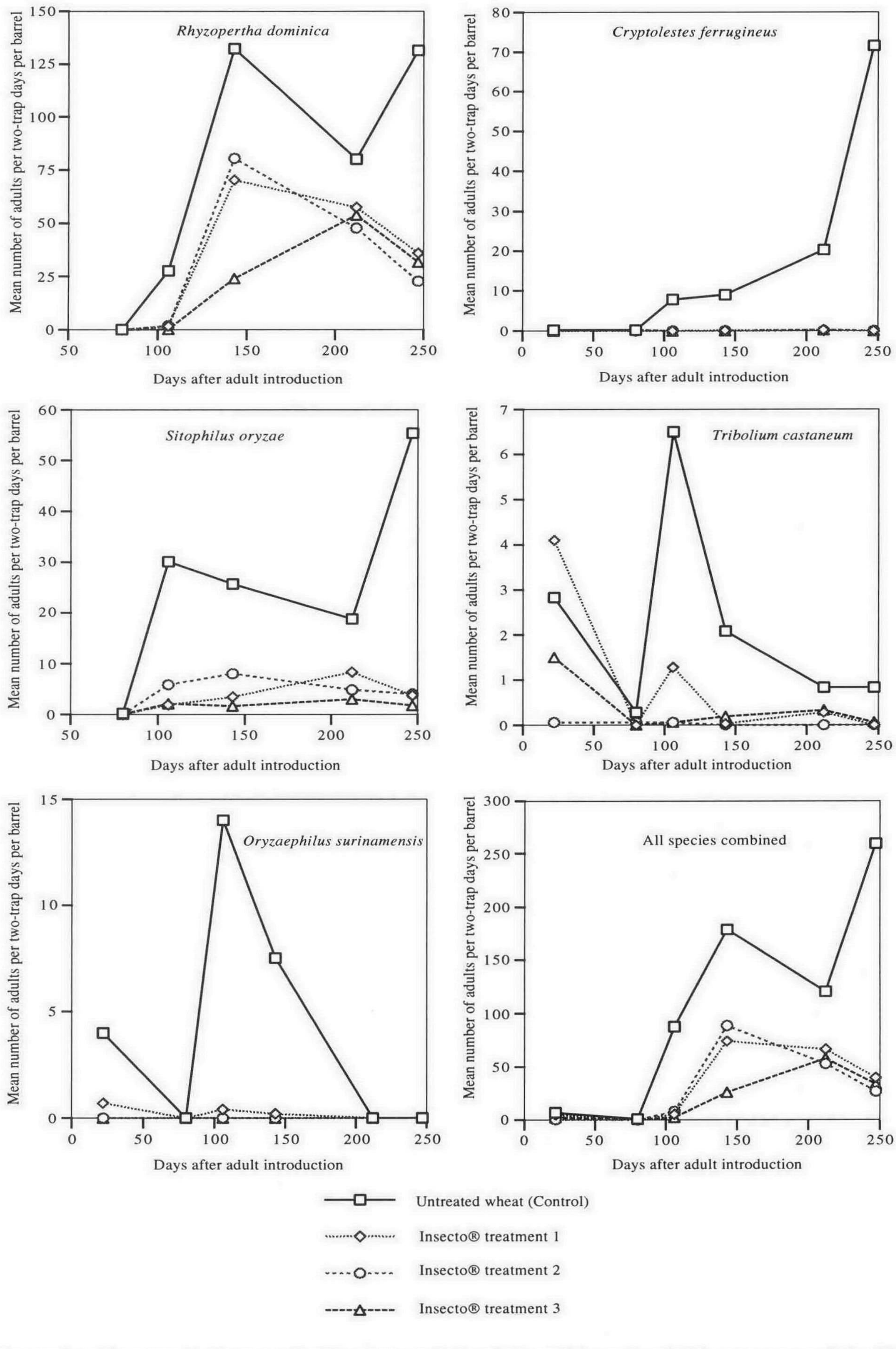

Fig. 1. Trap catches of insect species in untreated and Insecto-treated wheat during an 8.2-month period. Insects were sampled on 22, 80, 106, 143, 212, and 247 days after adult introduction. See text for *Cryptolestes pusillus* data. The combined species' graph includes data of all six insect species. Note: The y-axis scale is different for different species.

Table 1. Statistical differences in numbers of adults captured per trap per barrel in untreated wheat (control treatment) and wheat treated with Insecto (trt 1, trt 2, and trt 3) during each of the six sampling occasions.

Sampling occasion and insect species	Treatments compared	F(df)	P-value
February 25–27[a]			
R. dominica	Control, trt 1, trt 2, & trt 3	0.84 (3, 8)	0.508 ns
S. oryzae	Control, trt 1, trt 2, & trt 3	0.33 (3, 8)	0.802 ns
O. surinamensis	Control vs (trts 1, 2, & 3)	17.59 (1, 8)	0.003 *
C. ferrugineus	Control vs (trts 1, 2, & 3)	7.48 (1, 8)	0.026 **
C. pusillus	Control vs (trts 1, 2, & 3)	12.00 (1, 8)	0.009 **
T. castaneum	Control, trt 1, trt 2, & trt 3	3.32 (3, 8)	0.077 ns
	Trt 2 vs (trts 3 & 4)	6.68 (1, 8)	0.032 *
All species combined	Control vs (trts 1, 2, & 3)	5.38 (1, 8)	0.049 *
April 23–25[a,b]			
R. dominica	Control, trt 1, trt 2, & trt 3	30.33 (3, 8)	0.802 ns
S. oryzae	Control vs (trts1, 2, & 3)	21.37 (1, 8)	0.002 *
O. surinamensis	Control, trt 1, trt 2, & trt 3	0.68 (3, 8)	0.591 ns
C. ferrugineus	Control, trt 1, trt 2, & trt 3	1.48 (3, 8)	0.291 ns
T. castaneum	Control, trt 1, trt 2, & trt 3	1.73 (3, 8)	0.238 ns
All species combined	Control vs (trts 1, 2, & 3)	7.02 (1, 8)	0.029 *
May 19–21[a]			
R. dominica	Control vs (trts 1, 2, & 3)	45.68 (1, 8)	0.001 *
S. oryzae	Control vs (trts 1, 2, & 3)	17.10 (1, 8)	0.003 *
O. surinamensis	Control vs (trts 1, 2, & 3)	37.48 (1, 8)	<0.001 *
C. ferrugineus	Control vs (trts 1, 2, & 3)	46.93 (1, 8)	<0.001 *
C. pusillus	Control vs (trts 1, 2, & 3)	12.00 (1, 8)	0.009 *
T. castaneum	Control vs (trts 1, 2, & 3)	14.86 (1, 8)	0.005 *
All species combined	Control vs (trts 1, 2, & 3)	33.58 (1, 8)	<0.001 *
June 25–July 2[c]			
R. dominica	Control, trt 1, trt 2, & trt 3	1.16 (3, 8)	0.385 ns
S. oryzae	Control, trt 1, trt 2, & trt 3	2.90 (3, 8)	0.101 ns
O. surinamensis	Control vs (trts 1, 2, & 3)	209.65 (1, 8)	<0.001 *
C. ferrugineus	Control vs (trts 1, 2, & 3)	511.12 (1, 8)	<0.001 *
C. pusillus	Control vs (trts 1, 2, & 3)	39.54 (1, 8)	<0.001 *
T. castaneum	Control vs (trts 1, 2, & 3)	6.89 (1, 8)	0.030 *
All species combined	Control vs (trts 1, 2, & 3)	5.32 (1, 8)	0.050 ns
September 2–4[a,b]			
R. dominica	Control, trt 1, trt 2, & trt 3	0.39 (3, 8)	0.761 ns
S. oryzae	Control, trt 1, trt 2, & trt 3	0.85 (3, 8)	0.505 ns
O. surinamensis	Control, trt 1, trt 2, & trt 3	0.41 (3, 8)	0.751 ns
C. ferrugineus	Control vs (trts 1, 2, & 3)	30.53 (1, 8)	<0.001 *
T. castaneum	Control, trt 1, trt 2, & trt 3	1.67 (3, 8)	0.249 ns
All species combined	Control, trt 1, trt 2, & trt 3	0.86 (3, 8)	0.498 ns
October 7–9[a,b]			
R. dominica	Control, trt 1, trt 2, & trt 3	2.13 (3, 8)	0.175 ns
S. oryzae	Control, trt 1, trt 2, & trt 3	1.14 (3, 8)	0.391 ns
O. surinamensis	Control, trt 1, trt 2, & trt 3	1.00 (3, 8)	0.441 ns
C. ferrugineus	Control vs (trts 1, 2, & 3)	27.28 (1, 8)	<0.001 *
T. castaneum	Control vs (trts 1, 2, & 3)	18.10 (1, 8)	0.003 *
All species combined	Control vs (trts 1, 2 & 3)	26.08 (1,8)	<0.001*

ns, Not significant (P > 0.05; one-way ANOVA).
*Significant (P < 0.05; orthogonal contrasts).
**Trap catch in untreated wheat was significantly > 0 (P < 0.05; orthogonal contrasts).
[a]2-day trapping period.
[b]*Cryptolestes pusillus* adults were not detected by traps in any treatment.
[c]7-day trapping period.

and trap catches of insects among the Insecto® treatments were essentially similar (Figure 1).

Suppression of insects in Insecto® treatments (based on trap catch in Insecto® treatments relative to insect catch in the control treatment) varied with the species, sampling occasion, and Insecto® treatment. Table 2 shows the average number of adults of each species captured across all six sampling occasions in untreated wheat (control treatment), and overall percent suppression of insects in the three Insecto® treatments. In untreated wheat, *R. dominica* was the most abundant insect species, followed by *S. oryzae*, *C. ferrugineus*, *O. surinamensis*, *T. castaneum*, and *C. pusillus* (Table 2). All three Insecto® treatments effectively suppressed *C. ferrugineus* and *C. pusillus* populations (≥ 99% suppression). *Oryzaephilus surinamensis* population suppression was consistent (95–97% suppression) among the Insecto® treatments. The suppression of *R. dominica* and *S. oryzae* among the Insecto® treatments was 56–70% and 83–93%, respectively. The suppression of *T. castaneum* was 98% in Insecto® treatment 2, and ≤ 84% in the other two Insecto® treatments.

Insects in grain samples

None of the grain samples removed on 19 March contained any insects. Grain samples removed on 10 July revealed the presence of four insect species in the control treatment (Table 3): *R. dominica*, *S. oryzae*, *C. ferrugineus*, and *T. castaneum*. In the Insecto® treatments, only *R. dominica* and *S. oryzae* adults were detected. The suppression of *R. dominica* and *S. oryzae* among the Insecto® treatments was 87–92% and 17–65%, respectively. In the Insecto® treatments, adults of *R. dominica* and *S. oryzae* were 11 to 19 and 4 to10 times higher, respectively, than the Federal Grain Inspection Standard for infested grain of 2 live insects/kg of grain.

Number of damaged kernels

The amount of extraneous matter (dockage, grain and Insecto® dust) in the control and Insecto®-treated wheat samples was similar ($P > 0.05$; Table 4). The combined weight of grain and Insecto® dust (a result of feeding by *R. dominica* and *S. oryzae* and dislodged Insecto® dust) in Insecto® treatments was perhaps compensated by an increased amount of grain dust in the control treatment. The number of damaged kernels was significantly higher ($P < 0.05$) in the control treatment (65 kernels/10 g sample) than in the three Insecto® treatments (27–42 kernels/10 g sample) (Table 4).

Laboratory tests

Effectiveness of Insecto® on wheat

In trial I, 7-day adult mortality of four insect species was 100% at all three Insecto® doses (Table 5). These four species

Table 2 Trap catch of insect species in untreated wheat (control treatment), and percentage suppression of insects in the three Insecto treatments.

Insect species	No. adults in the control treatment[ab]	Suppression in Insecto treatment (%)[cd]		
		1	2	3
R. dominica	371.6	55.5	58.9	70.4
S. oryzae	130.2	86.6	82.5	93.4
O. surinamensis	25.6	94.6	97.1	94.8
C. ferrugineus	109.1	99.7	99.9	99.6
C. pusillus	3.7	99.5	100.0	100.0
T. castaneum	13.4	57.4	98.7	84.0
All species combined	653.6	70.9	73.0	81.3

[a]Trap catches were averaged across all six sampling occasions.
[b]Trap catches are expressed as number of adults per two trap days per barrel.
[c]Percent suppression was calculated as (1 - [no. adults in Insecto trt ÷ no. adults in the control trt]) × 100
[d]Percent suppression was averaged across all six sampling occasions.

Table 3. Live adults of insect species recovered in grain samples in untreated (control) and Insecto-treated wheat.

Insect species	Number of adults (mean[a] ± SEM) per five trier samples per barrel in:			
	Control treatment	Insecto treatment		
		1	2	3
R. dominica[b]	76.3 ± 37.0	9.7 ± 3.8	9.7 ± 5.6	6.3 ± 4.1
S. oryzae[c]	17.3 ± 7.8	14.3 ± 11.9	6.3 ± 2.7	10.3 ± 8.8
C. ferrugineus[d]	1.7 ± 0.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
T. castaneum[d]	1.0 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
All species combined[b]	96.3 ± 34.5	24.0 ± 14.6	16.0 ± 2.9	16.7 ± 12.8

[a]Each mean is based on n = 3 grain samples. Each grain sample (590 g) was obtained from a barrel by probing the grain at five different locations with a 12-compartment trier (see text for details)
[b]The numbers of insects found in the control treatment were significantly greater than numbers found in Insecto treatments 1, 2, and 3 ($F = 9.67$; df = 1, 8; $P = 0.015$ for *R. dominica*, and $F = 3.08$; df = 1, 8; $P = 0.007$ for all treatments 1, 2, and 3 ($F = 9.67$; df = 1, 8; $P = 0.015$ for *R. dominica*, and $F = 3.08$; df = 1, 8; $P = 0.007$ for all species combined; orthogonal contrasts).
[c]The numbers of insects found among the control and Insecto treatments were not significantly different from one another ($F = 0.47$; df = 3, 8; $P = 0.711$; one-way ANOVA).
[d]The numbers of insects found in the control treatment were significantly greater than 0 ($F = 11.54$; df = 1, 8; $P = 0.009$ for *C. ferrugineus*, and $F = 10.40$; df = 1, 8; $P = 0.012$ for *T. castaneum*; orthogonal contrasts).

were: *C. ferrugineus*, *C. pusillus*, *O. surinamensis*, and *T. castaneum*. The 7-day mortality of *O. mercator*, *R. dominica*, and *S. oryzae* was 100% when exposed to wheat treated with 1.0 and 1.5 g of Insecto®/kg of grain. The 7-day mortality of *O. mercator* and *S. oryzae* adults exposed to 0.5 g of Insecto®/kg of grain was 97.7 and 99.5%, respectively. The mortality of *R. dominica* was ≈ 97% even after 14 days of exposure to wheat treated with 0.5 g of Insecto®/kg of grain. However, the mortality of *O. mercator*, *O. surinamensis*, or *R. dominica* among Insecto® doses was not significant ($P > 0.05$). In trial II, the mortality of all five insect species was 100% when exposed for 7 days to wheat treated with 0.5 and 1.0 g of Insecto®/kg of grain (Table 6).

Effectiveness of Insecto® on barley

The 7-day mortality of four insect species was 100% at all three Insecto® doses (Table 7). Factorial ANOVA indicated that the mortality *S. oryzae* adults was not significant among the three Insecto® doses ($F = 1.09$; df = 2, 12; $P = 0.368$) and between exposure durations ($F = 3.78$; df = 1, 12; $P = 0.076$). The dose × exposure duration interaction also was not significant ($F = 1.09$; df = 2, 12; $P = 0.368$). At each Insecto® dose, the mortality of *T. castaneum* adults between 7 and 14 days was not significant ($P > 0.05$). The only significant difference was on day 7 where the mortality of *T. castaneum* adults in 1.5 g of Insecto®/kg treatment was signifcantly higher ($P < 0.05$) than mortality in the 0.5 g/kg treatment.

Discussion

In our barrel study, Insecto® was applied to the grain surface and top grain layers to control infestations occurring via the grain surface. The wheat in each barrel was infested with 180 adults (1.7 adults/kg of grain) to simulate immigration of insects into newly harvested grain stored in bins. Infestation of newly harvested grain stored in empty, insect-free bins occurs via the grain surface. Initially, small numbers of insects colonise the grain surface, and with time, these insects increase in numbers and move to lower layers of the grain mass (Hagstrum 1989).

Our field test results cannot be compared with work conducted in the United States (e.g., Strong and Sbur 1963; LaHue 1965, 1967, 1978; Redlinger and Womack 1966; White et al. 1966; McGaughey 1972) on the long-term effectiveness of older diatomaceous earth formulations (Perma-Guard, Kenite, and Dicalite 3G), for several reasons.

Table 4. Amount of extraneous material and insect-damaged kernels in untreated (control) and Insecto-treated wheat at the end of the experiment.

Treatment	Extraneous material[a,b] (% mean [w/w] ± SEM)	Insect-damaged kernels[c] (mean [d,*] ± SEM)
Control	2.6 ± 0.6	64.7 ± 3.0
Insecto treatment 1	2.5 ± 0.3	27.2 ± 11.5
Insecto treatment 2	2.4 ± 0.7	46.3 ± 3.8
Insecto treatment 3	2.1 ± 0.9	41.7 ± 5.0

[a]Weight of broken kernels, weed seeds, grain, and Insecto dust in the pooled grain sample
[b]Differences among treatments were not significant ($P > 0.05$; one-way ANOVA)
[c]Number of insect-damaged kernels in a 10-g sample.
[d]Each mean is based on n = 3, 10-g samples.
*Difference in the number of kernels damaged between the control and Insecto treatments 1, 2, and 3 was highly significant ($F = 11.7$; df = 1, 8; $P = 0.001$; orthogonal contrasts).

Table 5. Trial I: Mortality of adults of seven insect species exposed to wheat treated with Insecto.

Insect species[a]	Exposure period (days)	Mortality (% mean[b] ± SEM) at Insecto dose:		
		0.5	1.0	1.5
			(g of Insecto/kg of grain)	
R. dominica[c]	7	94.1 ± 1.7	100.0 ± 0.0	100.0 ± 0.0
R. dominica[d]	14	97.2 ± 1.4	100.0 ± 0.0	100.0 ± 0.0
S. oryzae[e]	7	99.5 ± 0.5	100.0 ± 0.0	100.0 ± 0.0
S. oryzae	14	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
O. surinamensis	7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
O. mercator[f]	7	97.7 ± 1.3	100.0 ± 0.0	100.0 ± 0.0
C. ferrugineus	7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
C. pusillus	7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
T. castaneum	7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

[a]Mean mortality of insects on untreated grain was 21.0% for *R. dominica* on day 7 and 52.7% on day 14, 1.0% for *S. oryzae* on day 7 and 5.5% on day 14, 47.0% for *O. surinamensis*, 56.0% for *O. mercator*, 34.5% for *C. ferrugineus*, and 28.5% for *C. pusillus*.
[b]Except for *R. dominica*, each mean for the other species is based on $n = 4$ jars. For *R. dominica*, $n = 3$ jars. At each n, 50 adults were exposed to 100 g of untreated and Insecto-treated grain.
[c]Mortality at 0.5 g Insecto/kg of grain treatment was significantly lower than mortality at the other Insecto doses ($F = 36.32$; df = 2, 6; $P < 0.001$; one-way ANOVA).
[d]Mortality among Insecto doses was not significant ($F = 4.00$; df = 2, 6; $P = 0.079$; one-way ANOVA).
[e]Mortality among Insecto doses was not significant ($F = 1.00$; df = 2, 9; $P = 0.405$; one-way ANOVA).
[f]Mortality among Insecto doses was not significant ($F = 3.00$; df = 2, 9; $P = 0.10$; one-way ANOVA).

1) We used Insecto® at 0.5 g/kg of grain, whereas the older diatomaceous earth formulations were evaluated using very high rates (usually at 3.5 g/kg of grain; range, 1.75 –7.0 g/kg). Older diatomaceous earth formulations were effective only at high rates (Carlson and Ball 1962; Strong and Sbur 1963; McGaughey 1972).
2) Unlike our study, in previous work, a known number of insects was not added to untreated and diatomaceous earth-treated grain. Instead, insects (approximately 6500–7000 adults) were introduced periodically into an area near bins, and the insects were allowed to infest untreated or diatomaceous earth-treated grain. Perma-Guard and Kenite dusts repel insects (Redlinger and Womack 1966), and differences in insects present in untreated and treated grain could therefore be due to varying numbers of insects infesting the grain.
3) We used probe traps to sample insects, whereas in tests with older diatomaceous earth formulations, insects were sampled with grain-probing devices (e.g., grain trier and deep-bin cup probe). Comparison of our June–July trapping results (Figure 1) with the July grain-sampling results (Table 3) clearly indicated that traps were more sensitive than grain-sampling devices in detecting insects. Traps can detect insects when grain-sampling devices fail to detect them (Barak and Harein 1982; Subramanyam and Harein 1989). Therefore, we feel confident that the use of traps in our study gave an accurate indication of Insecto®'s activity in suppressing insects.
4) The older diatomaceous earth formulations were derived from freshwater diatoms; Insecto® is derived from saltwater diatoms.

Both the field and laboratory tests confirmed the high susceptibility of *C. ferrugineus* and *C. pusillus* to Insecto®. In laboratory tests (Bh. Subramanyam, unpublished data), all adults of *C. ferrugineus* and *C. pusillus* exposed to shelled maize treated with 0.5 g of Insecto®/kg of grain were killed within 2 days. Higher Insecto® doses (1.0 or 1.5 g/kg of grain) and longer exposure periods (7–14 days) were required to kill all *O. surinamensis*, *O. mercator*, and *T. castaneum* adults. Carlson and Ball (1962) reported that *C. pusillus* adults were more susceptible than seven other stored-grain insects when exposed for 14 days to 1.0 to 3.5 g of Perma -Guard/kg of grain.

The numbers of *R. dominica* and *S. oryzae* captured in traps in Insecto®-treated wheat were lower than in untreated wheat. However, in Insecto®-treated wheat, there were 22 –38 live *R. dominica* and 8 –20 live *S. oryzae* adults, respectively,/kg of wheat. Except for *R. dominica* and *S. oryzae*, the remaining four species are incapable of excavating healthy kernels. Therefore, in our study, the observed kernel damage (adult emergence and/or feeding holes) was caused by *R. dominica* and *S. oryzae*. There were 1.6–2.4 times more kernels damaged in untreated wheat than in Insecto®-treated wheat. In a 10 g wheat sample, there were 27–46 kernels, and this damage far exceeds the Federal Grain Inspection Standard of 32 insect-damaged kernels (IDK)/100 g of wheat. Wheat con-

Table 6. Trial II. Mortality of adults of five insect species exposed to wheat treated with Insecto.

Insect species[a]	Mortality (mean[b] ± SEM) at Insecto dose:	
	0.5	1.0
	(g of Insecto/kg of grain)	
R. dominica	100.0 ± 0.0	100.0 ± 0.0
S. oryzae	100.0 ± 0.0	100.0 ± 0.0
O. surinamensis	100.0 ± 0.0	100.0 ± 0.0
C. ferrugineus	100.0 ± 0.0	100.0 ± 0.0
T. castaneum	100.0 ± 0.0	100.0 ± 0.0

[a]Mean mortality of insects on untreated grain was 2.7% for *R. dominica*, 11.0% for *O. surinamensis*, and 0.0% for *C. ferrugineus* and *T. castaneum*.
[b]Each mean is based on $n = 3$ jars. At each n, 50 adults were exposed to 100 g of untreated and Insecto-treated grain.

Table 7. Mortality of adults of six insect species exposed to barley treated with Insecto.

Insect species[a]	Exposure period (days)	Mortality (% mean[b] ± SEM) at Insecto dose:		
		0.5	1.0	1.5
		(g of Insecto/kg of grain)		
S. oryzae	7	99.5 ± 0.5	100.0 ± 0.0	100.0 ± 0.0
S. oryzae	14	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
O. surinamensis	7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
O. mercator	7	100.0 ± 1.3	100.0 ± 0.0	100.0 ± 0.0
C. ferrugineus	7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
C. pusillus	7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
T. castaneum	7[c,d]	96.4 ± 1.5 a	99.6 ± 0.4 ab	100.0 ± 0.0 b
	14[e]	99.5 ± 0.5	100.0 ± 0.0	100.0 ± 0.0

[a]Mean mortality of insects on untreated grain was 4.3% for *S. oryzae* on day 7 and 38.0% on day 14, 4.3% for *O. surinamensis*, 20.0% for *O. mercator*, 55.7% for *C. ferrugineus*, 14.0% for *C. pusillus*, 5.7% for *T. castaneum* on day 7 and 29.0% on day 14.
[b]Except for *S. oryzae* and *T. castaneum*, for the other species, each mean is based on $n = 4$ jars For *S. oryzae* and *T. castaneum*, $n = 3$ jars. At each n, 100 adults (50 adults only for *C. pusillus*) were exposed to 100 g of untreated and Insecto-treated grain.
[c]Within a row, means followed by different letters are significantly different from one another ($P < 0.05$; Bonferroni t tests).
[d]Mortality of insects between 7 and 14 days was not significant at 0.5 g/kg treatment ($F = 5.73$; df = 1, 4; $P = 0.075$; one-way ANOVA), and at 1.0 g/kg treatment ($F = 1.00$; df = 1, 4; $P = 0.374$; one-way ANOVA).
[e]Mortality of insects among Insecto doses on day 7 or day 14, and between 7 and 14 days at each dose, was not significant ($P > 0.05$; see text for details).

taining ≥ 32 IDK/100 g is downgraded (U. S. Sample Grade) or rejected at the time of sale.

The selective treatment of grain layers with Insecto®, and application of varying amounts of Insecto® to the grain surface, did not have a differential effect on the introduced insect species, because trends in trap catch of the insect populations in the three Insecto® treatments were similar (see Fig. 1). Top dressing may be effective against moth larvae (Desmarchelier and Dines 1987), which confine their feeding close to the grain surface. In laboratory tests (Bh. Subramanyam, unpublished data), adults of the six insect species used in the field test were completely killed within 48 hours when exposed to plywood treated with 5–10 g of Insecto®/m^2. In Insecto® treatments, the grain surface received 20.0–152.6 g of Insecto® per m^2, and these high Insecto® deposits should have been lethal to insects. We believe that insects added to the grain surface were exposed only briefly to the top-dressed Insecto® before moving to the lower grain layers, where they reproduced and increased in numbers. The capture of insects in traps placed at the barrel bottom, especially during the first two sampling occasions, supports this view. The survival of insects on Insecto®-treated wheat may depend on the rate at which Insecto® particles or particle aggregates are picked up from treated grain and the rate at which the particle aggregates on the insect cuticle are dislodged (see le Patourel et al. 1988). The activity of insects and surface texture of the insect cuticle may be two important factors that regulate the rate of exchange of Insecto® particles to and from the grain.

In the laboratory, an Insecto® rate similar to that used in the field test (0.5 g/kg of grain) caused 100% mortality of *R. dominica* and *S. oryzae* adults within 7 days. The reduced effectiveness of Insecto® on these two species in the field test may be due to uneven coverage of Insecto® on the kernels resulting in exposure of insects to a rate lower than 0.5 g/kg of grain. During grain treatment, the mechanical agitation of the seed-treating device dislodged considerable amount of Insecto® which accumulated at the bottom of the hopper. Therefore, it was necessary to pass the grain and Insecto® through the device twice to minimise loss of Insecto® during treatment. Additional loss of Insecto® during grain treatment occured in the form of atmospheric dust. Therefore, for practical purposes, to compensate for handling losses, the grain must be treated with 1.0 g of Insecto®/kg of grain.

In spite of high control mortality of certain insect species on untreated wheat (wheat trial I and barley trial), our laboratory tests showed that an Insecto® dose of 1.0 g/kg of grain was effective in producing 100% mortality of at least seven insect species within 7–14 days. We do not know the reasons for the high insect mortality on untreated wheat, but such high mortality of insects on untreated grain is common (see Carlson and Ball 1962). An Insecto® dose of 0.5 g/kg of wheat or barley produced 94–100% mortality of the exposed insects. However, to compensate for loss of the silica dust during application, and loss in insecticidal activity due to adsorption of atmospheric moisture (e.g., le Patourel 1986), using an Insecto® rate of 1.0 g/kg of grain may be desirable.

Unlike older diatomaceous earth formulations, Insecto® is effective against several stored-grain beetles when used at a low rate of 0.5 –1.0 g/kg of grain. The physical characteristics (particle size distribution, percent retention on 325 mesh, and surface area) of Insecto® and older diatomaceous earth formulations (Strong and Sbur 1963; White et al. 1975) are essentially similar. Therefore, these physical characteristics fail to explain the greater effectiveness of Insecto® on insects at low rates when compared with older diatomaceous earth formulations. The toxicity of inert dusts is related to their oil adsorption capability (Ebeling 1971; le Patourel and Singh 1984), which is expressed as percent of (Risella) oil adsorbed by weight. We could not find the oil adsorption values for Perma-Guard and Kenite. For Dicalite IG 3, the oil adsorption value is between 145 and160% (Strong and Sbur 1963). Insecto® has an oil adsorption value of 175%. The higher oil-adsorbing capability of Insecto® may partially explain its superior performance over Dicalite IG 3.

Several features of Insecto® make it a potential grain protectant. For instance, Insecto® has low toxicity to vertebrates (rat oral LD_{50} [mg/kg of body weight] is > 5000), is effective on insects at low rates, its residues on grain do not breakdown, it does not leave toxic residues on treated grain, and it is relatively safe to use with minimum protective gear (dust mask). To date, resistance in stored-grain insects to diatomaceous earth dusts has not been documented. Like most silicas (le Patourel 1986), the activity of Insecto® may be reduced on high moisture grain (Bh. Subramanyam, unpublished data) or on grain stored under humid conditions. Currently, we are in the process of evaluating the long-term effectiveness of Insecto® in suppressing insects associated with farm-stored maize and wheat. These field tests are essential to assess Insecto®'s potential as a grain protectant.

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