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Evaluation of methoprene alone and in combination with diatomaceous earth to control *Rhyzopertha dominica* (Coleoptera: Bostrichidae) on stored wheat[☆]

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Abstract

A series of experiments were conducted in which different formulations of the insect growth regulator methoprene were evaluated for control of *Rhyzopertha dominica* (F.), the lesser grain borer, a major internal insect pest of stored wheat. In the first test, application rates of 10-ppm *R,S*-methoprene (a racemic mixture of the *R* and *S* isomers of methoprene) and 1 and 5-ppm *S*-methoprene (*S*-isomer only) gave 100% suppression of *F*₁ adult progeny of *R. dominica* for 24 weeks. In the second test, adult *R. dominica* were exposed at 27°C and 32°C, 57% and 75% relative humidity (r.h.) on untreated wheat and wheat treated with 1- to 10-ppm *S*-methoprene dust. Survival after a 3-week exposure decreased with increasing concentration of dust, and ranged from 69% to 99%, but no *F*₁ adult progeny were produced in treated wheat. In the final test, concentrations of 0, 0.25, 0.50, 0.75, and 1.0 ppm *S*-methoprene EC were combined with concentrations of 0, 75, 150, 225, and 300 ppm of the commercial diatomaceous earth (DE) Protect-It[®]. Within each methoprene concentration, survival generally decreased with increasing concentration of DE, and was generally greater at 75% than at 57% r.h. Only the wheat treated with 0-ppm methoprene contained an appreciable number of *F*₁ adults. In summary, both the dust and EC formulations of *S*-methoprene gave 100% suppression of *F*₁ adult progeny *R. dominica* at application rates of 1 ppm, and combination treatments involving reduced rates of methoprene and DE gave effective control of *R. dominica*.

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Keywords: Methoprene; Diatomaceous earth; Combinations; *Rhyzopertha dominica*

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1. Introduction

The lesser grain borer, *Rhyzopertha dominica* (F.), is a major cosmopolitan insect pest of stored wheat. Females lay eggs on the exterior of the wheat kernel, and the first-instars hatch and bore into the kernel. The larvae feed and develop inside the kernel, and upon reaching the adult stage, bore out of the kernel and create a large exit hole. Because the majority of the development occurs inside the kernel, *R. dominica* is difficult to kill with contact insecticides applied directly to stored wheat.

Insect growth regulators (IGRs) are insecticides that mimic insect molting hormones, inhibit chitin synthesis, or are ecdysteroid agonists, and thereby disrupt the normal development of insects (Oberlander et al., 1997). They are selective for insects and have low mammalian toxicity, and are usually considered to be “reduced-risk insecticides”. With the increasing emphasis on using chemicals that pose less risk, have low mammalian toxicity, and are specific to insects, there is renewed interest in using IGRs in pest management systems for stored grain (Oberlander et al., 1997).

Methoprene is a juvenile hormone agonist that has been evaluated against a number of stored-product insect species (see list in Oberlander et al., 1997). It is generally effective against externally feeding stored-product insects such as *Tribolium castaneum* (Herbst), the red flour beetle, and *Oryzaephilus surinamensis* (L.), the sawtoothed grain beetle (Mian and Mulla, 1982a, b), but it does not give good control of *Sitophilus* species (Samson et al., 1990). It is also effective against *R. dominica* (Oberlander et al., 1997). A formulation of methoprene composed of the *R* and *S* isomers (*R,S*-methoprene, Diacon[®], 65.7% active ingredient [AI]) was registered in the United States during the 1980s for direct application to stored grain, but received little use because of its expense and the existence of other insecticides, and it was withdrawn from the market.

Another reduced-risk insecticide registered for use on stored wheat is the inert dust diatomaceous earth (DE), which is a natural product composed primarily of the remains of fossilized diatoms. Marine and freshwater deposits of DE are distributed worldwide (Korunic, 1997; Fields and Korunic, 2000), and commercial formulations are available for purchase. However, *R. dominica* is more tolerant to DE than other stored-grain beetle genera such as *Sitophilus*, *Oryzaephilus*, and *Cryptolestes* (Korunic, 1998; Fields and Korunic, 2000), and application rates for *R. dominica* are slightly higher than for other stored-grain beetles. Another potential problem is that even when used at label rates, which range from 300 to 1000 ppm depending on the specific commercial formulation, physical properties of grain such as test weight and flow rate may be affected by the DE application (Korunic et al., 1996, 1998).

A series of experiments were conducted to evaluate different formulations of methoprene to control *R. dominica* on stored wheat. The objectives of these tests were to determine: (1) efficacy of a new emulsifiable concentrate (EC) formulation which contained the *S*-isomer of methoprene (*S*-methoprene, Diacon II, 33.5% AI) compared to the older *R,S* racemic mixture (65% AI); (2) efficacy of a new *S*-methoprene dust formulation; and (3) feasibility of combination treatments of *S*-methoprene EC with DE.

2. Materials and methods

2.1. Experiment 1: residual efficacy of methoprene EC on stored wheat

The purpose of this experiment was to compare application rates of 1 and 5 ppm, the proposed label rates for the new *S*-methoprene formulation, with 10 ppm of *R–S*-methoprene. An

untreated control was included as a separate treatment (four treatments total). Individual replicates for each treatment consisted of 500 g of wheat (90% whole-kernel, 10% cracked Karl variety hard red winter wheat), and there were four replicates for each treatment. Each methoprene concentration was formulated separately and each replicate was sprayed with an individual solution at the rate of 0.35 ml of formulated spray per 500 g of wheat. This rate is proportional to 0.7 ml/kg, the labeled field spray rate for the organophosphate insecticide chlorpyrifos-methyl, which is labeled in the USA for direct application to stored wheat. The wheat was laid in a thin layer on a $0.6 \times 0.3 \text{ m}^2$ flat surface, and the wheat was misted with the insecticide solution using a Badger 100 artists' air brush (Franklin Park, IL, USA) to spray the solution directly onto the wheat. The untreated control replicates were sprayed with 0.35 ml of tap water per 500 g of wheat. After each replicate was treated, the wheat was put in a glass jar and hand-tumbled for about 30 s to ensure uniform coverage.

Experimental units consisted of 40 ml plastic vials containing 30 g of wheat. Each treated 500 g sample was subdivided into 10 individual vials containing 30 g of wheat each (two storage temperatures, 22°C and 32°C, to reflect upper and lower limits for initial storage of wheat, five samples at 6-week intervals, including an initial 0-week sample). The remainder of the wheat was discarded. The bioassays for the 0-week sample immediately after treatment were conducted by exposing twenty 1–2-week-old mixed-sex adult *R. dominica* for 3 weeks. The vials containing the wheat and the insects were held inside $26 \times 36.5 \times 15 \text{ cm}^3$ plastic boxes, with egg crate grids cut to fit the bottom, and containing an aqueous saturated NaBr solution to maintain humidity at 57% r.h., which corresponded to an approximate grain moisture content of 12.5% (Greenspan, 1977). The egg crates provided a false floor to hold the vials above the saturated salt solution. The lids of the boxes were not ventilated. The humidity boxes were put in an incubator set at 27°C, which is in the optimum temperature range for development of *R. dominica* (Howe, 1965; Fields, 1992). Temperature and relative humidity inside the boxes were monitored with HOBO recording sensors (Onset Computer, Pocasset, MA, USA). After 3 weeks, the adults were removed from the wheat, mortality was assessed, and the whole-kernel wheat, insect frass, and ground flour was returned to the vials. The vials were then put back in the humidity boxes and held in the incubators for another 8 weeks when the wheat was removed, all F_1 adults were recorded, and the wheat was discarded. Only adults were recorded because *R. dominica* is an internal feeder, and adult emergence was therefore a valid measure of insecticide efficacy.

At intervals of 6 weeks, separate sets of vials were removed from the two incubators and held for 2 d in an incubator at 27°C. Bioassays were done at 27°C as described for the 0-week sample, and the wheat for the F_1 counts was also held inside the 27°C incubator. The test was analyzed using the General Linear Models Procedure (GLM) of the Statistical Analysis System (SAS Institute, 2000), with insecticide application, storage temperature, and storage week as main effects, and percentage mortality after 3 weeks and the number of F_1 adults as the response variables. The Regression Procedure of SAS was also used to determine the significance of regressions with storage week as the independent variable.

2.2. Experiment 2: efficacy of *S*-methoprene dust to control *R. dominica*

The purpose of this test was to establish the efficacy of *S*-methoprene dust against *R. dominica*. Insecticidal treatments evaluated in this study were an untreated control and six rates of

S-methoprene dust, 1, 2, 4, 6, 8, and 10 ppm (seven concentrations). The *S*-methoprene dust was 10.5% [AI], therefore actual application rates to obtain these concentrations in 1 kg of wheat would be 9.6, 19.0, 38.0, 57.1, 76.2, and 95.2 mg. Humidity chambers of 57% r.h. were prepared, using saturated NaBr as described for Experiment 1. Chambers were also created by using saturated aqueous NaCl to maintain a relative humidity (r.h.) of 75%, to correspond to approximate grain moisture content of 14.5% (Greenspan, 1977). There were five replicates for each concentration, including the untreated control, and tests were conducted at 27°C and 32°C (and 57% and 75% r.h.).

Individual bioassay containers consisted of 30 g wheat inside the plastic vials described in Experiment 1, with 10% cracked wheat in each vial. To ensure adequate covering and mixing of dust with the wheat, 200 g of wheat were treated for each replicate at each concentration of *S*-methoprene dust. The wheat was weighed into a 0.475-l glass jar, the dust was added to the wheat, and the jar was hand-rolled for 1 min. The amount of *S*-methoprene dust needed to give concentrations of 1, 2, 4, 6, 8, and 10 ppm in 200 g of wheat was 1.9, 3.8, 7.8, 11.4, 15.2, and 19.0 mg, respectively. After each replicate was treated, the wheat was then subdivided into six vials containing 30 g each.

Twenty mixed-sex adult *R. dominica* were exposed for 3 weeks in each of the vials, then insects were removed from the wheat and mortality was assessed. The wheat, insect frass, and ground flour from feeding damage were returned to the vials, which were put back into the humidity boxes and returned to the respective temperature incubators. After 8 weeks, the vials were removed, all F_1 adults were removed from each vial and counted, and the wheat was discarded. The test was analyzed using the GLM Procedure of SAS with concentration, temperature, and relative humidity as main effects, and mortality after 3 weeks and the number of F_1 adults as the response variables. The Regression Procedure of SAS was also used to determine the significance of regressions with concentration as the independent variable, and percentage mortality and F_1 adults as the independent variables. Lack-of-fit tests were conducted using Table Curve (Version 2.0, Jandel Scientific, CA, USA) software to determine the variation that could be explained by any model fitted to the data (maximum R^2), variation explained by the given equation (R^2), and to fit linear and non-linear regression curves to the data.

2.3. Experiment 3: efficacy of methoprene–diatomaceous earth combinations to control *R. dominica*

The purpose of this experiment was to evaluate combination applications of *S*-methoprene EC with DE. The label rate for the marine diatom formulation of the commercial DE Protect-It[®] is 300 ppm when used as a surface treatment to stored wheat. In our previous test with methoprene EC, applications at 1.0 ppm were effective, therefore the combinations were based on maximum levels of 300 ppm Protect-It[®] and 1 ppm *S*-methoprene. Five rates of *S*-methoprene (0, 0.25, 0.50, 0.75, and 1.0 ppm) and five rates of Protect-It[®] (0, 75, 150, 225, and 300 ppm) (25 treatment combinations) were combined, and humidity chambers using saturated salt solutions of NaBr and NaCl were created as described for Experiment 2. There were five replicates for each combination, done as individual blocks, and tests were conducted at 27°C, 57% and 75% r.h.

The actual spray rate for each methoprene treatment was based on the field spray rate for chlorpyrifos-methyl on stored wheat, as described for Experiment 1. Individual lots consisted of 650 g of wheat, to ensure sufficient amounts for treatment with the DE. The amount of

formulated spray needed to treat 650 g of wheat is 0.46 ml, and methoprene was applied as described for Experiment 1, using the Badger 100 artists' airbrush to mist the formulated spray directly onto individual lots of 650 g of wheat. Approximately 10% of the 650 g consisted of cracked wheat. After the methoprene was applied, the wheat was subdivided into samples of 125 g for each of the five DE treatments, and each sample was put into a 0.475-l glass jar. The appropriate amount of DE for each treatment was mixed in with the wheat, then hand-rolled for 1 min to distribute the DE into the wheat. Each of two vials was filled with 30 g of wheat (one vial for each humidity level), and the remainder of the wheat was discarded.

Twenty 1–2-week-old mixed-sex adult *R. dominica* were exposed in separate vials, which were in turn placed in plastic boxes containing either saturated NaBr or NaCl. Each replicate was treated separately following the process described previously. The humidity boxes were placed in a temperature incubator set at 27°C, the adults were exposed for 3 weeks and then removed from the vials and checked for mortality. The wheat, insect frass, and ground flour from feeding damage were returned to the vials, which were put back into the humidity boxes and the temperature incubator. After 8 weeks, the wheat was removed, emerged F_1 adults were tabulated, and the wheat was discarded.

The test was analyzed using the GLM Procedure of SAS, and analyses were conducted within each level of methoprene concentration, with concentration of DE and relative humidity as main effects, and mortality after 3 weeks and the number of emerged F_1 adults as the response variables. The Regression Procedure of SAS was used to determine the significance of regressions, with concentration as the independent variable and percentage mortality and F_1 adults as the independent variables. Linear and non-linear regression curves were fitted to the data and R^2 values were calculated as described for Experiment 2.

3. Results

3.1. Experiment 1: residual efficacy of methoprene EC on stored wheat

Although the overall GLM procedure showed a significant effect for storage temperature, when each of the data points was compared for storage week and insecticide treatment (24 total comparisons), only one was significant, which indicated random variation rather than a specific temperature effect. When linear and quadratic regressions were run for storage week as the independent variable for each of the four treatments, no regressions were significant ($P \geq 0.05$), which showed there was no specific trend with respect to week. Therefore, data for survival were compared by storage week within each insecticide treatment and temperature. During the 24-week study, survival of *R. dominica* after the 3-week incubation period ranged from 85.8% to 100% in untreated wheat and in wheat treated with the three methoprene treatments, and in three of the four treatments, survival was lowest at week 0 compared to the remaining weeks (Table 1).

The number of F_1 adult *R. dominica* produced from the 3-week exposures on untreated wheat and in wheat treated with the three methoprene concentrations was significant with respect to the main effects storage week ($F = 18.1$, d.f. = 4, 120, $P < 0.01$) and insecticide treatment ($F = 991.7$, d.f. = 1, 120, $P < 0.01$), but not storage temperature ($F = 3.2$, d.f. = 3, 120, $P = 0.07$). Storage week \times treatment and temperature \times treatment were the only significant interactions ($P < 0.05$).

Table 1

Initial survival (%; mean \pm SE) of 20 *Rhyzopertha dominica* adults after exposure for 3 weeks on 30 g of untreated wheat and 30 g of wheat treated with 10 ppm *R-S*-methoprene (*R-S*), 1 and 5 ppm *S*-methoprene (*S*), and the number of F_1 progeny (mean \pm SE) produced by these adults. Tests were conducted at 6-week intervals after the wheat was stored^a

Treatment	Week	% survival		F_1 adults	
		22°C	32°C	22°C	32°C
Untreated	0	81.9 \pm 3.3b	84.6 \pm 6.3b	440.3 \pm 22.3a	431.5 \pm 70.8a
	6	100 \pm 0.0a	100 \pm 0.0a	303.2 \pm 5.1b	344.3 \pm 15.9ab
	12	100 \pm 0.0a	100 \pm 0.0a	187.5 \pm 18.8c	283.8 \pm 14.8bc
	18	100 \pm 0.0a	100 \pm 0.0a	187.5 \pm 7.4c	215.8 \pm 17.9c
	24	100 \pm 0.0a	97.5 \pm 1.4a	313.5 \pm 14.8b	326.0 \pm 33.2ab
10 ppm <i>R-S</i>	0	86.3 \pm 6.3a	93.6 \pm 1.2b	0.5 \pm 0.3a	0.0 \pm 0.0a
	6	97.5 \pm 2.5a	98.8 \pm 1.3a	0.0 \pm 0.0a	0.0 \pm 0.0a
	12	100 \pm 0.0a	100 \pm 0.0a	0.0 \pm 0.0a	0.0 \pm 0.0a
	18	96.3 \pm 3.8a	100 \pm 0.0a	0.0 \pm 0.0a	0.0 \pm 0.0a
	24	100 \pm 0.0a	98.8 \pm 1.3a	0.0 \pm 0.0a	0.0 \pm 0.0a
5 ppm <i>S</i>	0	89.9 \pm 3.5b	91.1 \pm 2.3b	0.0 \pm 0.0a	0.5 \pm 0.5a
	6	100 \pm 0.0a	100 \pm 0.0a	0.0 \pm 0.0a	0.0 \pm 0.0a
	12	100 \pm 0.0a	100 \pm 0.0a	0.0 \pm 0.0a	0.0 \pm 0.0a
	18	98.8 \pm 1.3a	100 \pm 0.0a	0.0 \pm 0.0a	0.0 \pm 0.0a
	24	100 \pm 0.0a	100 \pm 0.0a	0.0 \pm 0.0a	0.0 \pm 0.0a
1 ppm <i>S</i>	0	85.8 \pm 9.5a	96.1 \pm 2.5a	0.8 \pm 0.5a	0.5 \pm 0.5a
	6	88.4 \pm 4.3a	100 \pm 0.0a	0.0 \pm 0.0a	1.8 \pm 1.0a
	12	88.4 \pm 4.3a	100 \pm 0.0a	0.0 \pm 0.0a	1.0 \pm 1.0a
	18	96.3 \pm 4.3a	100 \pm 0.0a	0.0 \pm 0.0a	1.0 \pm 1.0a
	24	100 \pm 0.0a	98.8 \pm 1.3a	0.0 \pm 0.0a	0.0 \pm 0.0a

^a Means within columns for each treatment followed by different letters are significantly different ($P < 0.05$, Waller–Duncan k -ratio t -test, SAS Institute).

The number of F_1 adults in untreated controls ranged from 187.5 to 440.3, and while storage week was significant in the ANOVA, regressions with storage week as the dependent variable were not significant with respect to the number of F_1 adults in the untreated controls at either of the two storage temperatures ($P \geq 0.05$). Even though survival in the untreated controls was lowest at week 0, these survivors produced a large number of F_1 adults although the number of F_1 adults in the untreated control wheat varied throughout the study. The average number of F_1 adults in wheat treated with methoprene was usually < 1 at both storage temperatures.

3.2. Experiment 2: efficacy of *S*-methoprene dust to control *R. dominica*

Survival of *R. dominica* after the 3-week exposure period on wheat treated with *S*-methoprene dust was significant with respect to main effects relative humidity ($F = 19.5$, d.f. = 1, 108, $P < 0.01$), temperature ($F = 8.6$, d.f. = 1, 108, $P < 0.01$) and ppm concentration ($F = 22.2$, d.f. = 6, 108, $P < 0.01$). The relative humidity \times concentration and the temperature \times concentration

interactions were the only significant interactions ($P < 0.05$). Regression analyses for survival of *R. dominica* at each of the four relative humidity and temperature combinations were significant with respect to concentration as the independent variable ($P < 0.05$), and linear equations were fitted to the data (Table 2).

At each relative humidity and temperature, survival of *R. dominica* on untreated wheat was generally 100%, but as the concentration of methoprene increased there was a corresponding decrease in survival (Fig. 1A–D). The methoprene dust apparently exhibited some toxicity toward *R. dominica*. At each temperature, linear regression slopes indicated that survival was lower at 57% than at 75% r.h., and at each relative humidity survival was lower at 27°C compared to 32°C. The number of F_1 adults on untreated wheat was significantly different at each temperature and at each relative humidity ($P < 0.05$). At 27°C, 57% and 75% r.h., 207.8 ± 20.9 and 280.8 ± 12.4 adults, respectively, were collected from the untreated wheat, at 32°C, 57% and 75% r.h., 321.2 ± 70.0 and 496.6 ± 28.9 adults, respectively, were collected. No F_1 adult *R. dominica* were produced from the 3-week exposures on any of the methoprene concentrations.

3.3. Experiment 3: efficacy of methoprene–diatomaceous earth combinations to control *R. dominica*

Survival of *R. dominica* after the 3-week exposure period was always highly significant with respect to the main effect, concentration of DE, and in all treatments containing methoprene, survival was also significant for the main effect, relative humidity (Table 3). Regressions for concentration of DE as the independent variable within each of the five treatment levels of methoprene were also significant ($P < 0.05$), and linear and non-linear equations were fitted to the data (Table 4, Fig. 2A–E). Within each methoprene concentration, survival generally decreased with increasing concentration of DE, and was generally greater at 75% than at 57% r.h. (Fig. 3A–E).

Only the 0-ppm methoprene treatment contained an appreciable number of F_1 adult *R. dominica*, and while the number of adults was significant with respect to concentration of DE, relative humidity was not significant (Table 5). The number of adults ranged from 36.4 ± 8.7 to 116.2 ± 18.1 at 57% r.h. and 30.4 ± 9.5 to 135.1 ± 19.9 at 75% r.h., and generally decreased with increasing concentration of DE. Regressions were significant for this treatment (Table 5 footnotes), and linear regression lines were fitted to the data (Fig. 3A). Few adults were found in any of the other methoprene treatments (Fig. 3B–E), and regressions were not significant ($P \geq 0.05$).

Table 2

Equation parameters (mean \pm SE) for linear equations of the form $y = a - bx$, where y = percentage survival of *Rhyzopertha dominica* after exposure for 3 weeks on wheat treated with 0, 1, 2, 4, 6, 8, and 10 ppm *S*-methoprene dust (data pooled), maximum R^2 (max R^2) for any equation fitted to the data, R^2 values of the linear equations, and R^2 of each equation as a % of the maximum (% R^2), at two temperatures and relative humidities (r.h.)

Temperature (°C)	% r.h.	a	b	Max R^2	R^2	% R^2
27	57	97.4 ± 2.6	2.84 ± 0.46	0.57	0.54	94.7
	75	96.0 ± 2.2	1.04 ± 0.40	0.27	0.18	66.7
32	57	97.7 ± 4.4	5.45 ± 0.78	0.63	0.60	95.2
	75	98.2 ± 2.9	3.24 ± 0.52	0.62	0.54	87.1

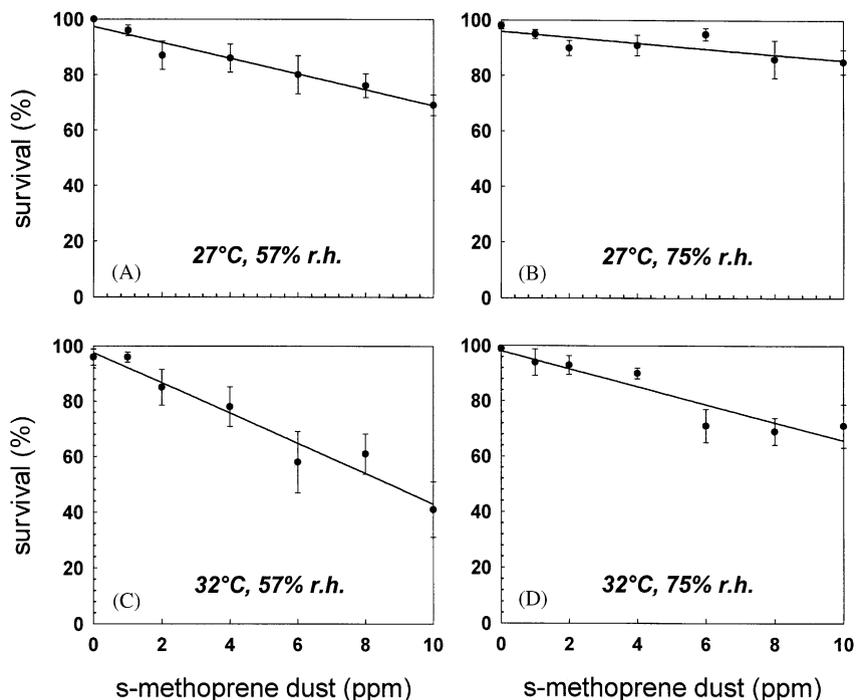


Fig. 1. (A–D) Percentage survival (mean \pm SE) of *Rhyzopertha dominica* exposed on wheat treated with 0, 1, 2, 4, 6, 8, and 10 ppm *S*-methoprene dust and held at 27° and 32°C, 57% and 75% r.h. Curve-fit regression lines are from equations in Table 2.

Table 3

F and *P* values for the analysis of survival (%) of *Rhyzopertha dominica* with respect to the main effects concentration of diatomaceous earth (DE) (0, 75, 150, 225, and 300 ppm), and relative humidity (r.h., 57% or 75%) at each level of methoprene, and the r.h. \times DE interaction (d.f. are 1, 32 for all effects). Wheat was held at 27°C

Methoprene (ppm)	r.h.		DE		r.h. \times DE	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
0.0	1.9	0.18	32.8	<0.01	0.1	0.91
0.25	23.7	<0.01	110.8	<0.01	3.3	0.07
0.50	13.3	<0.01	79.2	<0.01	1.6	0.20
0.75	34.1	<0.01	123.8	<0.01	8.2	<0.01
1.0	8.2	<0.01	32.3	<0.01	2.7	0.10

4. Discussion

Several published reports document the effectiveness of methoprene in suppressing progeny production by *R. dominica*, and these tests often involve a range of doses or tests with different formulations (Oberlander et al., 1997). However, toxicity may vary depending on the source of the methoprene, and the minimum effective dose needed to suppress progeny production of *R.*

Table 4

Equation parameters (mean \pm SE) for linear equations of the form $y = a - bx$ and non-linear equations of the form $y = a - b(x)^{0.5}$, where y = percentage survival of *Rhizopertha dominica* adults after exposure on wheat treated with 0, 75, 150, 225, and 300 ppm of DE at each concentration of *S*-methoprene EC, and held at 27°C, 57% and 75% r.h. The maximum R^2 (max R^2) for any equation fitted to the data, R^2 values of the linear and non-linear equations, and R^2 of each equation as a % of the maximum (% R^2) are also given for each equation

Methoprene (ppm)	% r.h.	a	b	Max R^2	R^2	% R^2
0	57	95.4 \pm 7.7	0.15 \pm 0.042	0.36	0.37	97.3
	75	102.4 \pm 7.0	0.15 \pm 0.038	0.40	0.49	81.6
0.25	57	84.2 \pm 6.8	0.29 \pm 0.036	0.73	0.80	91.3
	75	97.0 \pm 6.4	0.35 \pm 5.811	0.59	0.61	96.7
0.50	57	92.9 \pm 8.2	4.86 \pm 0.673 ^a	0.69	0.73	94.5
	75	93.6 \pm 2.9	0.21 \pm 0.031	0.66	0.68	97.1
0.75	57	94.3 \pm 4.8	5.40 \pm 0.395 ^a	0.89	0.91	97.8
	75	97.5 \pm 6.3	3.20 \pm 0.518 ^a	0.62	0.64	96.9
1.0	57	91.2 \pm 8.6	0.25 \pm 0.047	0.55	0.59	93.2
	75	95.0 \pm 8.9	0.14 \pm 0.048	0.26	0.29	89.6

^a Non-linear equations $y = a - b(x)^{0.5}$, other equations are linear $y = a - b(x)$.

dominica could be dependent on the source of methoprene or the specific formulation used in the studies. In studies in which technical grade methoprene was diluted and applied to wheat, McGregor and Kramer (1975) reported 100% suppression of *R. dominica* at 2 ppm, which was the lowest dose tested, Mian and Mulla (1982a) reported 98–100% suppression at 1–10 ppm, and Amos and Williams (1977) reported 99% suppression at 1 ppm. In a test involving Altosid[®], a trade formulation of methoprene, 100% suppression was also achieved at 5 ppm (Strong and Diekman, 1973). In residual tests in which formulations of *S*-methoprene and the *R, S* racemic mixture were applied to maize and paddy rice, effective rates cited for control of *R. dominica* on maize were 2 ppm for the mixture and 1 ppm for *S*-methoprene (Samson et al., 1990). In the present Experiment 1, *S*-methoprene was as effective as 10 ppm of *R-S*-methoprene. The *R, S* mixture was the formulation of methoprene with the trade name Diacon[®], the commercial product sold in the USA in the mid-80s, and was a 65.7% [AI] EC formulation. The new commercial product Diacon II[®], which was approved by the US Environmental Protection Agency in early 2002 (EPA registration # 2724-427), is a 33.6% [AI] EC formulation of the *S*-isomer only. Efficacy of *S*-methoprene was maintained for 6 months of grain storage, which is consistent with residual stability reported for other isomers and formulations of methoprene (Mian and Mulla, 1982b, 1983; Edwards and Short, 1984; Arthur et al., 1990; Samson et al., 1990).

Methoprene is effective against *R. dominica* even though this species spends the majority of its life cycle inside the wheat kernel. The eggs, which are laid outside the kernels, could be exposed to methoprene residues, or the larvae could come into contact with the residues before entering the kernel. Methoprene does have contact toxicity toward eggs of some stored-grain beetles, including

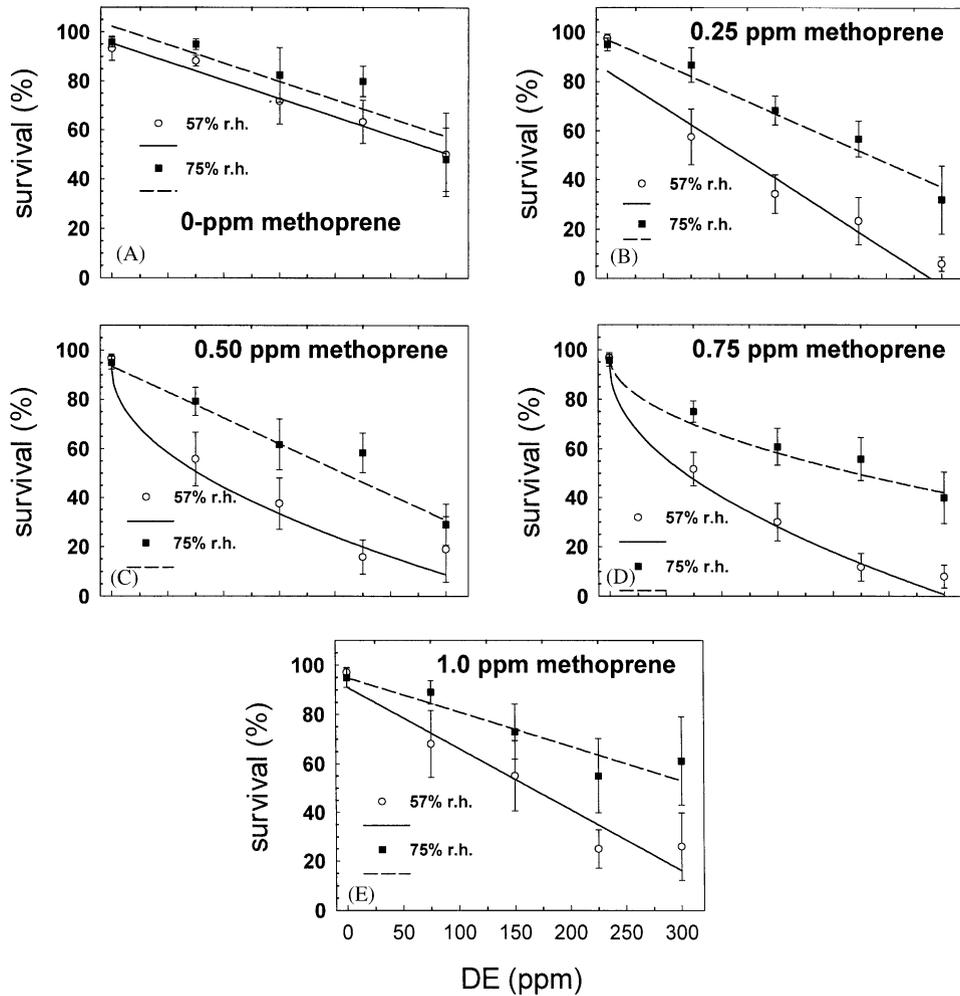


Fig. 2. (A–E) Percentage survival (mean \pm SE) of *Rhyzopertha dominica* exposed on wheat treated with 0, 75, 150, 225, and 300 ppm of DE at each level of five levels of methoprene, and held at 27°C, 57% (open circle) and 75% (solid square) r.h. Curve-fit regression lines for 57% (solid line) and 75% (dashed line) r.h. are from equations in Table 4.

those of *R. dominica* (Mian and Mulla, 1982a). All of these factors combined could account for the suppression of *R. dominica*. In contrast, mixed results have been reported for studies involving *Sitophilus* species, which oviposit directly into grain kernels. Strong and Diekman (1973) report no progeny suppression of *Sitophilus oryzae* (L.), the rice weevil, on wheat treated with 5 ppm Altosid[®] and 7% and 92% suppression at 10 and 50 ppm, respectively. In studies involving dilutions made from technical methoprene, Amos and Williams (1977) achieved 54% and 20% suppression of *S. oryzae* progeny at 10 and 20 ppm, respectively, McGregor and Kramer (1975) reported only 13% suppression at 10 ppm methoprene, and Loschiavo (1977) cites 10% and 61% suppression at 10 and 20 ppm. However, Mian and Mulla (1982a) achieved 80.7–93.1% suppression of *S. oryzae* progeny in wheat treated with 10 ppm of methoprene. Several references

Table 5

F and P values for number of F_1 adult *Rhyzopertha dominica* produced from exposure of 20 parent adults for 3 weeks on 30 g of treated wheat, for main effects relative humidity (r.h.), concentration of diatomaceous earth (DE) (0, 75, 150, 225, and 300 ppm), at each level of methoprene, and the r.h. \times DE interaction (d.f. are 1, 32 for all effects). Wheat was held at 27°C, 57% and 75% r.h.

Methoprene (ppm)	r.h.		DE		r.h. \times DE	
	F	P	F	P	F	P
0.0 ^a	4.0	0.06	21.3	<0.01	0.2	0.93
0.25	0.01	0.98	1.8	0.14	0.1	0.97
0.50	0.6	0.48	32.0	0.02	4.7	<0.01
0.75	4.4	0.04	1.0	0.42	0.7	0.58
1.0	2.0	0.16	0.8	0.56	0.8	0.56

^a Linear regression $y = a - b(x)$ significant for both 57% and 75% r.h. ($P < 0.05$), no other regressions were significant ($P \geq 0.05$). Equation for 57% r.h., $y = 112.6 \pm 8.7$, $b = 0.28 \pm 0.048$, maximum R^2 that could be calculated for any equation = 0.63, R^2 for the fitted equation = 0.60. Equation for 75% r.h., $y = 146.1 \pm 9.3$, $b = 0.37 \pm 0.051$, maximum R^2 that could be calculated for any equation = 0.71, R^2 for the fitted equation = 0.70.

mention the ineffectiveness of methoprene against *Sitophilus* (Samson et al., 1990; Oberlander et al., 1997), and in Australia methoprene was combined with organophosphates that give control of *S. oryzae* in wheat (Samson et al., 1990).

This is also the first report in which *S*-methoprene dust has been evaluated against a stored-product insect. The dust effectively eliminated F_1 adults, and in this respect was comparable to the EC formulation, but the dust also produced some slight mortality of exposed parent adults. Although the dust was not composed of abrasive or lipid-absorbing particles, mortality seemed to have been caused by the dust itself, and not by the methoprene, because there was no mortality of adult *R. dominica* exposed to grain treated with methoprene EC.

During the last 30–40 years there have been many published reports in which inert dusts, including DE, have been evaluated against stored-product insects (Golob, 1997; Korunic, 1998; Subramanyam and Roesli, 2000). Newer formulations of DE are more effective than older products and require less material to achieve control (Subramanyam and Roesli, 2000), but any dust material added to grain could affect the physical properties of grain. Application rates of these commercial DE products range from 300 to 1000 ppm, depending on the specific commodity and the target insect. Also, *R. dominica* is less susceptible to DE than most other stored-grain beetle species, including *S. oryzae* (Fields and Korunic, 2000), and can require higher rates or longer exposure intervals for control (Arthur, 2002). The *S*-methoprene dust formulation evaluated in the present Experiment 2 also suppressed progeny production of *R. dominica* at an application rate of 1 ppm. The dust was 10.5% [AI], so the actual amount of dust applied to obtain this concentration in 1 kg of wheat would be 9.6 mg, which is far lower than the label rates of 400–1000 ppm for commercial formulations of DE.

Efficacy of most DE products tends to decline with increases in grain moisture content or relative humidity (Golob, 1997; Korunic, 1998). In the present Experiments 1 and 2 and in other tests, variations in humidity have had little effect on efficacy of methoprene (Samson et al., 1990). Combining *S*-methoprene EC with DE (Experiment 3) produced an additive effect and reduced the concentrations of both materials required to suppress progeny of *R. dominica*, compared with

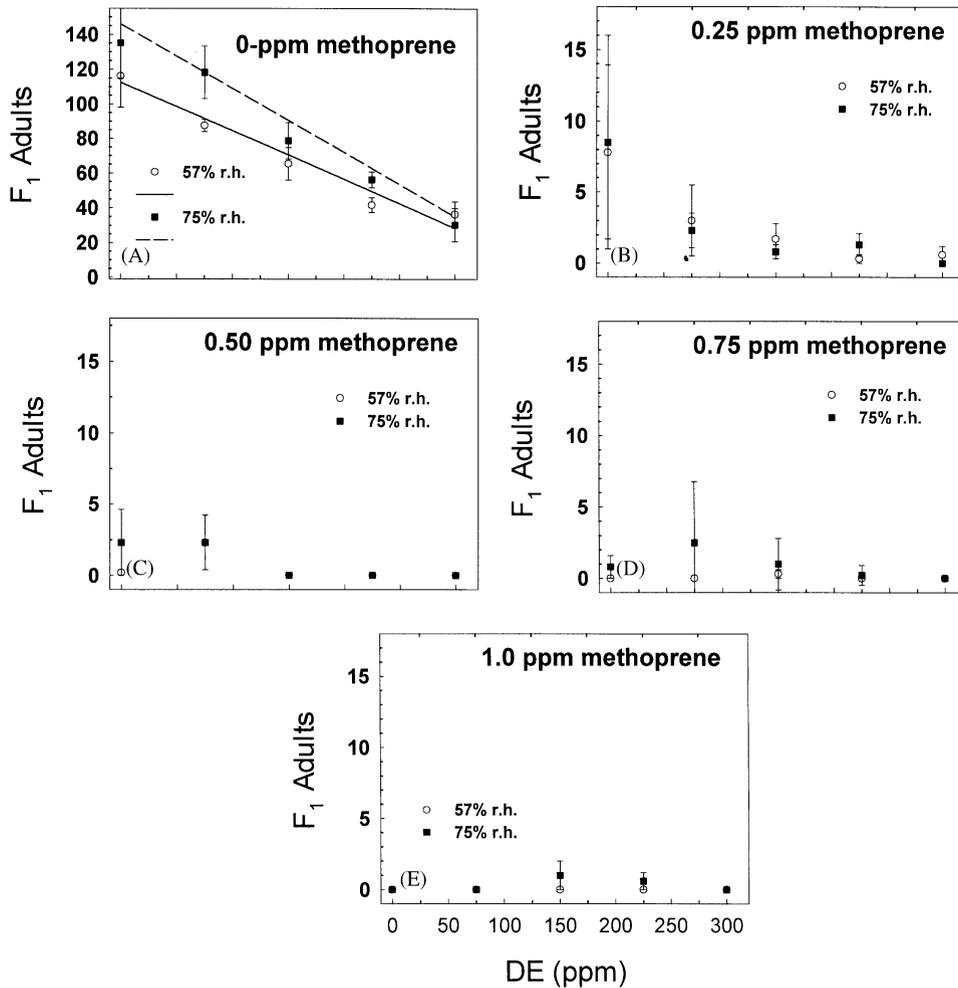


Fig. 3. (A–E) Number of *F*₁ adult *Rhizopertha dominica* (mean ± SE) produced from 20 parent adults exposed for 3 weeks on 30 g of wheat treated with 0, 75, 150, 225, and 300 ppm of DE at each level of five levels of methoprene, and held at 27°C, 57% (open circle) and 75% (solid square) r.h. Curve-fit regression lines for 57% (solid line) and 75% (dashed line) r.h. for 0-ppm methoprene are from the footnotes in Table 5.

each insecticide separately. A combination treatment involving methoprene EC and DE could be a viable control strategy. Reducing the amount of DE needed to control *R. dominica* could alleviate some of the effects of DE on physical properties of grain because less material would be used in insecticide applications. The addition of methoprene could also help reduce some of the effects of humidity on the efficacy of DE. Methoprene could potentially be combined with DE to achieve control of *S. oryzae*. However, *S. oryzae* is more susceptible to DE than *R. dominica*, and therefore a combination treatment to control this species may not be as practical as a combination treatment to control *R. dominica*. However, there seem to be potential additive effects of DE that warrant further investigation. Similar additive effects have been reported for combinations of DE with the fungal pathogen *Beauveria bassiana* (Balsamo) Vuillemin (Lord, 2001).

Currently, there are few insecticides registered in the United States to control *R. dominica*. The organophosphate insecticide chlorpyrifos-methyl (Reldan®) is labeled for direct application to stored wheat but *R. dominica* is not listed on the insecticide label. Therefore, the product does not imply control of this particular species. There are also some isolated reports of resistance to chlorpyrifos-methyl in some populations of *R. dominica* within the USA (Zettler and Cuperus, 1990; Guedes et al., 1996). Perhaps more important, under the terms of the 1996 Food Quality Protection Act (FQPA), all organophosphates are being reviewed (Peltier, 1998), and the continued registration of chlorpyrifos-methyl is uncertain. Methoprene alone or in combination with DE could be especially beneficial to control *R. dominica* in stored wheat.

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