

# Knockdown and Mortality of Adults of Eight Species of Stored-Product Beetles Exposed to Four Surfaces Treated with Spinosad

MICHAEL D. TOEWS,<sup>1</sup> BHADRIRAJU SUBRAMANYAM, AND JACLYN M. ROWAN

Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506

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**ABSTRACT** Contact toxicity of a commercial bacterial fermentation insecticide, spinosad, to adults of eight stored-product beetles was evaluated on four different surfaces. Aqueous spinosad suspension was sprayed with an airbrush to 30.5-cm<sup>2</sup> surfaces of concrete, galvanized steel, unwaxed floor tile, or waxed floor tile to obtain deposits of 0.05 or 0.1 mg (AI)/cm<sup>2</sup>. Control surfaces were sprayed with distilled water. Approximately 24 h after distilled water or spinosad application, 30 adult beetles were confined, by species, to each untreated and spinosad-treated surface. Insects on surfaces were exposed for 24 h to assess knockdown at 25 ± 1°C and 50 ± 10% RH, and then were held on food for an additional 24 h to assess mortality. Knockdown and mortality of each insect species on all four surfaces were significantly greater on spinosad-treated surfaces than on distilled water-treated surfaces. Knockdown and mortality of all species on all surfaces was similar at the two spinosad deposit levels. Except for *Tribolium* spp., mortality of all other species exposed to spinosad was 99–100%. *Tribolium* spp. were highly susceptible to spinosad on concrete (98–100% mortality); however, on unwaxed floor tile, steel, and waxed floor tile recovery on food after knockdown resulted in only 72–92% mortality. Our results suggest that spinosad has excellent contact activity against adults of stored-product insects, especially on concrete, and has potential for use as a general surface, spot, or crack/crevice spray to control insects in empty bins, warehouses, food-processing facilities, and retail stores.

**KEY WORDS** spinosad, surface treatments, stored-product insects, contact toxicity

SEVERAL INSECTICIDES ARE registered for application to general surfaces (walls, floors, and ceilings), spots (≤60-cm<sup>2</sup> areas), cracks, or crevices in empty bins, warehouses, retail stores, and food-processing facilities (Subramanyam et al. 1993, White and Leesch 1995). Insecticides intended for these applications can be grouped as inorganics (inert dusts and boric acid), organophosphates (chlorpyrifos and chlorpyrifos-methyl), insect growth regulators (IGRs) (S-hydroxyprone), carbamates (bendiocarb), botanicals (pyrethrins), and pyrethroids (cyfluthrin, fenvalerate, and deltamethrin) (Subramanyam et al. 1993). Application of insecticides to surfaces, spots, cracks, or crevices must leave a residue lethal enough to kill insects crawling on the treated surfaces. The susceptibility of stored-product insects exposed to insecticide residues varies with the insect species (Toews and Subramanyam 2003), type of surface treated (Burkholder and Dicke 1966, White 1982, Williams et al. 1983, Giga and Canhou 1991, Arthur 1997a), degree of insecticide coverage on the treated surface (Arthur 1999a), age of

the residue (Arthur 1997a, 1998a), temperature (Arthur 1999b), duration of exposure (Arthur 1997b; 1998a, b), insecticide and formulation used (Williams et al. 1983, Arthur 1994), availability of untreated refugia (Barson 1991, Cox et al. 1997), and accessibility to a food source during or after insecticide exposure (Arthur 2000a, b).

Spinosad, a commercially available insecticide derived from the fermentation products of the actinomycete bacterium *Saccharopolyspora spinosa* Mertz & Yao (Mertz and Yao 1990), belongs to a new class of insecticides called the naturalytes. Spinosad has low mammalian toxicity (Thompson et al. 2000). Recent studies have shown that spinosad, as a grain protectant, is highly effective against a variety of stored-product insects (Fang et al. 2002a). Spinosad degrades quickly when exposed to sunlight (UV light) (Liu et al. 1999). However, spinosad residues on treated wheat samples degraded very little during 12 mo of storage in farm bins in Kansas (Fang et al. 2002b), because the samples were not directly exposed to sunlight. Therefore, spinosad residues applied to indoor surfaces, such as those found in empty bins, warehouses, retail stores, and food-processing facilities, may persistent and exert insecticidal activity for extended periods.

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<sup>1</sup> Current address: USDA-ARS, Grain Marketing and Production Research Center, Manhattan, KS 66502.

Adán et al. (1996) reported spinosad to be toxic by ingestion and contact to the Mediterranean fruit fly, *Ceratitidis capitata* (Weidemann). Contact activity to stored product insects is unknown except for the study by Toews and Subramanyam (2003). They showed that spinosad has contact activity on treated glass surfaces against three species of stored-product beetles. The current study was designed to extend the findings of Toews and Subramanyam (2003) on the contact toxicity of spinosad applied to concrete, galvanized steel, unwaxed floor tile, and waxed floor tile against eight economically important stored-product beetles commonly encountered in grain stores, warehouses, food-processing facilities, and retail stores.

### Materials and Methods

**Insects.** Insects used in tests were obtained from colonies established in 1999 in the Department of Grain Science and Industry (Kansas State University, Manhattan, KS). Cultures were reared in 0.95-liter glass jars at 30°C, 50 ± 10% RH, and a photoperiod of 16:8 (L:D) h. Internal infesting species tested included the lesser grain borer, *Rhyzopertha dominica* (F.), and rice weevil, *Sitophilus oryzae* (L.); these species were reared on whole, hard red winter wheat. External infesting species tested included the red flour beetle, *Tribolium castaneum* (Herbst); confused flour beetle, *Tribolium confusum* Jacquelin du Val; rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); merchant grain beetle, *Oryzaephilus mercator* (Fauvel); sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); and warehouse beetle, *Trogoderma variabile* Ballion. *Tribolium* spp. were reared on whole-wheat flour + 5% (by weight) brewer's yeast. Cultures of *C. ferrugineus* and *Oryzaephilus* spp. were reared on rolled oats + 5% (by weight) brewer's yeast, whereas *T. variabile* was reared on poultry feed + 10% (by weight) dry dog chow.

**Spinosad.** The spinosad formulation used was Spin-Tor 2 SC (Dow AgroSciences, Indianapolis, IN), containing 240 mg (AI)/ml. Spinosad was diluted in distilled water for treatment of the various surfaces. This formulation is not registered in the United States for use on stored grain or surfaces, but an experimental use permit for use on grain was approved by the U.S. Environmental Protection Agency on 31 May 2002 (EPA Experimental Use Permit No. 62719-EUP-50).

**Surfaces.** Individual surfaces (30.5 by 30.5 cm) of concrete, galvanized steel, unwaxed floor tile, and waxed floor tile were fabricated in the laboratory. The unwaxed floor tiles (Tarkett Inc., Vails Falls, NY) were purchased at a local hardware store and cleaned with an industrial floor cleaner (All Clean, Industrial Maintenance Supply, Wichita, KS), according to the manufacturer's directions. Waxed floor tile surfaces were prepared by applying three coats of industrial floor wax (Reflections, Industrial Maintenance Supply, Wichita, KS) to unwaxed floor tiles. Galvanized steel surfaces (0.91 mm in thickness or 20 gauge) were procured and cut by a local machine shop. The metal surface was also cleaned using the industrial floor

cleaner. Dry concrete mix (Bildcrete, Western Bag Products, Harrisonville, MO) was mixed with three parts water and poured into 2.5-cm-deep plywood frames, lined with 0.1-mm (4-mil)-thick polyethylene sheeting. The concrete was allowed to dry for 48 h, at which time the frames and polyethylene were removed and the surfaces were left to cure for 3 mo before use in tests. Trials with the concrete surfaces were conducted on the smooth side that contacted the polyethylene while the concrete cured. Surfaces were cleaned gently with a broom before being treated with water or spinosad solution.

**Treatment of Surfaces and Insect Exposure.** Each replication of the four surface types was treated with 4 ml of distilled water (control treatment) or an aqueous suspension (4 ml) of spinosad to provide deposits of 0.05 or 0.1 mg (AI)/cm<sup>2</sup>. Surfaces were sprayed with distilled water and spinosad solutions by using an artist's airbrush (model 150-M, Badger Air-Brush Co., Franklin Park, IL) connected to an air compressor providing 103.4-kPa pressure. Separate airbrushes were used for distilled water and spinosad treatments. After treatment, surfaces were allowed to dry for 24 h in a walk-in growth chamber maintained at 25 ± 1°C and 50 ± 10% RH.

After water or spinosad application, each individual surface was subdivided into nine equal quadrats with a dimension of 10.2 by 10.2 cm. Thirty adults of mixed age and sex of each of the eight insect species (one species per arena) were introduced into separate quadrats on each surface. Insects in each quadrat were confined in a 7.3-cm-diameter arena made from 0.8-mm thick Tygon tubing (Saint-Gobain Performance Plastics Corporation, Akron, OH). Insects were prevented from escaping the arena by placing a glass petri dish (9 cm in diameter) on top of the tubing. Insects on surfaces were observed after 24 h and scored as either moribund (knocked down) or mobile (live). The 24-h exposure was used, because several researchers (Adán et al. 1996, Foster et al. 1996, Scott 1998, Wanner et al. 2000, Fang et al. 2000a) have reported spinosad to be slow acting on insects with mortality increasing as a function of exposure time. Knocked down and mobile adults of each species were transferred to separate glass petri dishes containing ≈3 g of the respective diet used for rearing the species. All dishes were placed in the growth chamber for an additional 24 h to allow insect recovery before assessing final mortality. Knockdown was based on the number of moribund insects out of the total number exposed. Mortality was based on the number of dead insects out of the total number exposed.

**Experimental Design and Data Analysis.** The experiment was designed as a split plot, with surface type and spinosad deposit level (laid out in a randomized complete block) serving as the main plots, and insect species randomly assigned to a surface type by deposit level treatment as the subplot. The entire experiment was replicated four times. The response variables were percentage of the exposed adults that was knocked down after 24 h and percentage that was dead after the recovery period. The percentage of adults that recov-

**Table 1.** Percentage of knockdown (mean  $\pm$  SE) of adults of eight stored-product beetles after exposure to four surfaces treated with spinosad at 0, 0.05, and 0.1 mg/cm<sup>2</sup>

Species	Deposit (mg/cm <sup>2</sup> )	Surface type			
		Concrete	Unwaxed floor tile	Galvanized steel	Waxed floor tile
<i>T. confusum</i>	0	30.9 $\pm$ 17.0	12.5 $\pm$ 5.7	29.2 $\pm$ 23.0	12.5 $\pm$ 3.4
	0.05	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	99.2 $\pm$ 0.8	99.1 $\pm$ 0.9
	0.1	99.1 $\pm$ 0.9	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	97.5 $\pm$ 2.5
<i>R. dominica</i>	0	71.9 $\pm$ 14.0	23.5 $\pm$ 4.7	38.2 $\pm$ 15.3	47.4 $\pm$ 6.6
	0.05	98.4 $\pm$ 1.6	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
	0.1	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
<i>O. mercator</i>	0	46.7 $\pm$ 14.7	16.7 $\pm$ 11.8	27.5 $\pm$ 24.3	27.5 $\pm$ 18.7
	0.05	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
	0.1	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
<i>T. castaneum</i>	0	2.5 $\pm$ 2.5	0.8 $\pm$ 0.8	5.0 $\pm$ 4.0	1.7 $\pm$ 1.7
	0.05	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	99.1 $\pm$ 0.9	98.3 $\pm$ 1.7
	0.1	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	99.2 $\pm$ 0.8
<i>C. ferrugineus</i>	0	58.6 $\pm$ 16.0	27.6 $\pm$ 4.7	27.3 $\pm$ 11.4	34.9 $\pm$ 18.5
	0.05	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
	0.1	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
<i>S. oryzae</i>	0	0.9 $\pm$ 0.9	0.8 $\pm$ 0.8	0.8 $\pm$ 0.8	0.0 $\pm$ 0.0
	0.05	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	99.2 $\pm$ 0.8	96.4 $\pm$ 1.6
	0.1	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	98.3 $\pm$ 1.7
<i>O. surinamensis</i>	0	1.7 $\pm$ 1.0	2.2 $\pm$ 2.2	5.7 $\pm$ 5.7	0.9 $\pm$ 0.9
	0.05	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	97.5 $\pm$ 1.6	100.0 $\pm$ 0.0
	0.1	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	99.2 $\pm$ 0.8	100.0 $\pm$ 0.0
<i>T. variable</i>	0	15.8 $\pm$ 9.5	22.5 $\pm$ 2.8	11.8 $\pm$ 5.1	22.5 $\pm$ 5.3
	0.05	99.2 $\pm$ 0.8	88.8 $\pm$ 11.2	100.0 $\pm$ 0.0	97.6 $\pm$ 1.5
	0.1	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0

Mean knockdown among deposit levels for each species and surface type combination was significant ( $F$ , range = 5.01–1103.66;  $df$  = 2, 9;  $P$  < 0.001; one-way ANOVA). For each species and surface type combination, mean knockdown on spinosad-treated surface was significantly greater ( $P$  < 0.05; LSMEANS test) than knockdown on untreated surface, whereas mean knockdown was not significantly different ( $P$  > 0.05; LSMEANS test) between the spinosad deposit levels.

ered was calculated as  $100 * ((\text{number knocked down} - \text{number dead}) / \text{total exposed})$ . All percentages ( $x$ ) were corrected for knockdown, mortality, or recovery on control surfaces (Abbott 1925) and transformed to arcsine ( $x$ )<sup>0.5</sup> (Zar 1984) to normalize heteroscedastic treatment variances. Statistical inferences were made using the MIXED procedure (SAS Institute 1999), with degree of freedom adjustments for the variance components made following the methods of Kenward and Roger (1997). Significant interactions were further analyzed using the slice option of the LSMEANS statement in the mixed procedure. All treatment means were compared using LSMEANS at the  $\alpha$  = 0.05 level.

## Results

When treated with distilled water only, knockdown of *R. dominica* adults on unwaxed floor tiles, galvanized steel, and waxed floor tiles was  $\leq$ 47%, whereas on concrete it was 72% (Table 1). Knockdown of *O. mercator* and *C. ferrugineus* on concrete surfaces treated with distilled water was 47 and 59%; for all other species knockdown on distilled water-treated surfaces was  $\leq$ 38%. The mortality of *R. dominica*, *C. ferrugineus*, *O. mercator*, and *T. variable* on concrete surfaces treated with distilled water was 58–84%. However, mortality of the remaining species on the control surfaces ranged from 1 to 51% (Table 2). The knockdown and mortality of insect species on spinosad-treated surfaces was always significantly greater

( $P$  < 0.05) than on control surfaces. There were no differences in knockdown or mortality between the spinosad deposits levels ( $P$  > 0.20). Knockdown on spinosad treated surfaces, regardless of species, ranged from 89 to 100% but was generally >98%. Mortality of all insect species, except *T. castaneum* and *T. confusum*, was 100% on spinosad-treated surfaces. *Tribolium* spp. experienced higher mortality on spinosad-treated concrete surfaces (99.5%) than on unwaxed floor tile (87%), steel (84%), or waxed floor tile (80%).

Data from the control treatment were analyzed to measure the effects of insect exposure to the surfaces without interacting with the spinosad treatments. The surface type by insect species interaction for knockdown of the eight species was not significant ( $F$  = 0.94;  $df$  = 21, 82.1;  $P$  = 0.539). In addition, differences were not detected among the four surface types ( $F$  = 0.85;  $df$  = 3, 8.93;  $P$  = 0.501). However, knockdown varied among species (Fig. 1). The order of knockdown was *R. dominica* > *C. ferrugineus* > *O. Mercator* > *T. confusum* > *T. variable* > *T. castaneum* > *O. surinamensis* > *S. oryzae*. The surface type by insect species interaction for mortality of insects after the recovery period was not significant ( $F$  = 1.46;  $df$  = 21, 82.2;  $P$  = 0.116). Mortality of the insect species varied among the surfaces (Fig. 2) with higher mortality observed after exposure to concrete than to unwaxed floor tile, steel, or waxed floor tile. Additionally, there were differences in mortality among species (Fig. 3). The trend in mortality generally followed that observed for

Table 2. Percentage of mortality (mean  $\pm$  SE) of adults of eight stored-product beetles after exposure to four surfaces treated with spinosad at 0, 0.05, and 0.1 mg/cm<sup>2</sup>

Species	Deposit (mg/cm <sup>2</sup> )	Surface type			
		Concrete	Unwaxed floor tile	Galvanized steel	Waxed floor tile
<i>T. confusum</i>	0	15.3 $\pm$ 7.0	4.2 $\pm$ 1.6	4.2 $\pm$ 3.2	13.3 $\pm$ 7.2
	0.05	100.0 $\pm$ 0.0	88.3 $\pm$ 7.4	92.4 $\pm$ 5.4	87.4 $\pm$ 8.4
	0.1	100.0 $\pm$ 0.0	81.4 $\pm$ 6.4	92.1 $\pm$ 5.9	83.8 $\pm$ 4.6
<i>R. dominica</i>	0	83.7 $\pm$ 6.6	44.4 $\pm$ 5.3	53.5 $\pm$ 7.5	48.3 $\pm$ 11.3
	0.05	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
	0.1	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
<i>O. mercator</i>	0	64.9 $\pm$ 20.0	18.3 $\pm$ 8.0	23.6 $\pm$ 13.8	15.0 $\pm$ 5.0
	0.05	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
	0.1	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
<i>T. castaneum</i>	0	1.7 $\pm$ 1.0	2.5 $\pm$ 1.6	4.2 $\pm$ 2.1	0.8 $\pm$ 0.8
	0.05	100.0 $\pm$ 0.0	91.4 $\pm$ 2.8	75.3 $\pm$ 7.2	72.0 $\pm$ 8.6
	0.1	98.3 $\pm$ 1.0	87.9 $\pm$ 3.3	78.3 $\pm$ 9.5	77.1 $\pm$ 5.3
<i>C. ferrugineus</i>	0	66.4 $\pm$ 12.7	35.4 $\pm$ 7.9	26.6 $\pm$ 13.6	42.9 $\pm$ 21.2
	0.05	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
	0.1	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
<i>S. oryzae</i>	0	20.9 $\pm$ 8.4	5.0 $\pm$ 5.0	10.0 $\pm$ 5.3	3.3 $\pm$ 3.3
	0.05	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
	0.1	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
<i>O. surinamensis</i>	0	4.6 $\pm$ 1.8	2.2 $\pm$ 2.2	8.5 $\pm$ 3.0	3.6 $\pm$ 3.6
	0.05	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
	0.1	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
<i>T. variable</i>	0	57.7 $\pm$ 5.9	50.8 $\pm$ 11.2	20.1 $\pm$ 2.3	40.0 $\pm$ 4.9
	0.05	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	99.0 $\pm$ 1.0	99.2 $\pm$ 0.8
	0.1	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	99.2 $\pm$ 0.8	100.0 $\pm$ 0.0

Mean mortality among deposit levels for each species and surface type combination was significant ( $F$ , range = 6.62–740.59;  $df$  = 2, 9;  $P$  < 0.001; one-way ANOVA). For each species and surface type combination, mean mortality on spinosad-treated surface was significantly greater ( $P$  < 0.05; LSMEANS test) than mortality on untreated surface, whereas mean mortality was not significantly different ( $P$  > 0.05; LSMEANS test) between the spinosad deposit levels.

knockdown, and the order of susceptibility was *R. dominica* > *C. ferrugineus* > *T. variable* > *O. mercator* > *S. oryzae* > *T. confusum* > *O. surinamensis* > *T. castaneum*. Mortality of each species (Fig. 3) was slightly greater than the corresponding knockdown (Fig. 1).

Test insects exposed to spinosad-treated surfaces were nearly always knocked down and killed. Analysis of the knockdown data, corrected for mortality in untreated replicates, showed that there were no significant two- or three-way interactions and no signif-

icant effects attributed to surface type ( $F$  = 0.9;  $df$  = 3, 95.6;  $P$  = 0.445) or insect species ( $F$  = 1.20;  $df$  = 7, 95.5;  $P$  = 0.311). The mean knockdown at the 0.05-mg/cm<sup>2</sup> rate was 98.1  $\pm$  0.9%, whereas the mean at the 0.1 mg/cm<sup>2</sup> rate was 99.9  $\pm$  0.1%. Statistically, there was a difference in knockdown between these spinosad deposit levels ( $F$  = 4.62;  $df$  = 1, 96.5;  $P$  = 0.034). The actual mortality, corrected for mortality on control replicates, showed a significant surface by species interaction ( $F$  = 3.68;  $df$  = 21, 92.5;  $P$  = 0.001). Examining this interaction for differences among spe-

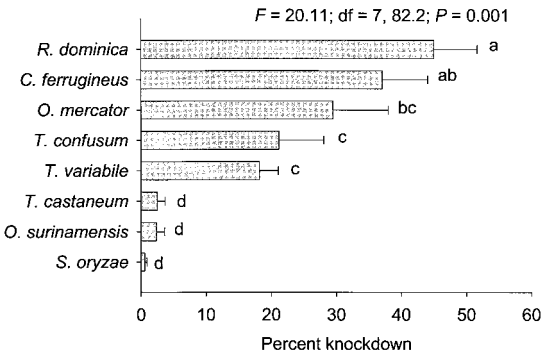


Fig. 1. Percentage of knockdown (mean  $\pm$  SE) of eight stored-product beetles after 24-h exposure to surfaces treated with distilled water (control treatment). Means followed by different letters are significantly different ( $P$  < 0.05; LSMEANS test).

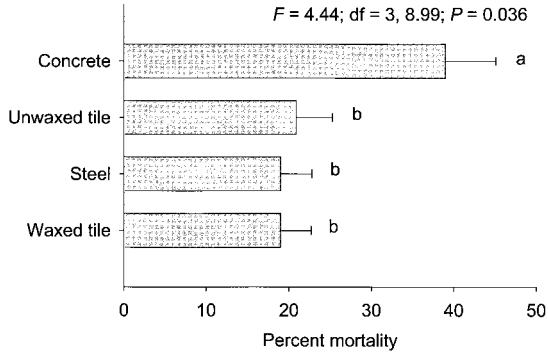


Fig. 2. Percentage of mortality (mean  $\pm$  SE) of insects after 24-h exposure to the four surface types treated with distilled water (control treatment) followed by 24-h recovery period on food. Means followed by different letters are significantly different ( $P$  < 0.05; LSMEANS test).

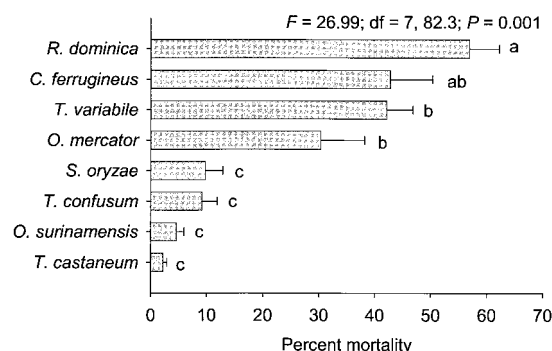


Fig. 3. Percentage of mortality (mean  $\pm$  SE) of eight stored-product beetles after 24-h exposure to surfaces treated with distilled water (control treatment) followed by 24-h recovery period on food. Means followed by different letters are significantly different ( $P < 0.05$ ; LSMEANS test).

cies, while controlling for the surface types, showed that the mortality of *T. castaneum* ( $F = 19.71$ ;  $df = 3, 92$ ;  $P = 0.001$ ) and *T. confusum* ( $F = 13.92$ ;  $df = 3, 92$ ;  $P = 0.001$ ) was highly significant, whereas that of the remaining species were highly nonsignificant ( $P$  values ranged from 0.827 to 0.999). These values strongly suggest that the responses of *Tribolium* spp. to spinosad-treated surfaces were quite different from that of the remaining species. Data show that *R. dominica*, *S. oryzae*, *T. variabile*, *C. ferrugineus*, *O. surinamensis*, and *O. mercator* experienced 100% mortality, whereas a significant number of *Tribolium* spp. survived exposure to spinosad. To fully investigate these differences, we analyzed the *Tribolium* spp. data separately.

Results from the knockdown study for *Tribolium* spp. showed no interactions or significant treatment effects attributed to the two insect species ( $F = 0.03$ ;  $df = 1, 21.4$ ;  $P = 0.869$ ), spinosad deposit level ( $F = 0.01$ ;  $df = 1, 23.1$ ;  $P = 0.909$ ), or surface type ( $F = 1.84$ ;  $df = 3, 21.1$ ;  $P = 0.171$ ). Results from the final mortality study revealed that there were no interactions or significant differences between the two species ( $F = 1.78$ ;  $df = 1, 20.1$ ;  $P = 0.198$ ) or between spinosad rates ( $F = 2.0$ ;  $df = 1, 22.5$ ;  $P = 0.170$ ). However, the mortalities of *T. castaneum* and *T. confusum* were greater on concrete treated with spinosad than on treated unwaxed floor tile, galvanized steel, or waxed floor tile (Fig. 4). The greater mortality observed on concrete for *Tribolium* spp. was due to reduced recovery on this substrate compared with recovery on the other surfaces. Recovery on concrete was only 0.2%, whereas on the other surfaces it ranged from 13 to 20%. Statistically, these observed differences in recovery were significantly different among surface types ( $F = 4.27$ ;  $df = 3, 20.2$ ;  $P = 0.017$ ), but not between the two *Tribolium* spp. ( $F = 1.16$ ;  $df = 1, 20.3$ ;  $P = 0.295$ ), or for any two- or three-way interactions.

### Discussion

The most logical explanation for the high knockdown and mortality of some insect species on control

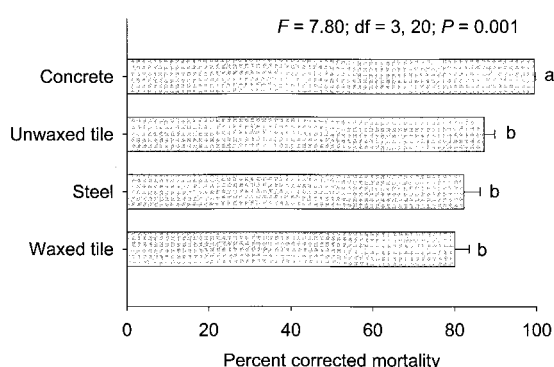


Fig. 4. Percentage of corrected mortality (mean  $\pm$  SE) of *Tribolium* spp. after 24-h exposure to spinosad at 0.05 and 0.1 mg/cm<sup>2</sup> followed by 24-h recovery on food. Means followed by different letters are significantly different ( $P < 0.05$ ; LSMEANS test).

surfaces may be attributed to lack of food or desiccation (because of 50% RH) under the test conditions, and this effect was exacerbated on concrete surfaces. The humidity level used in our test conditions is typical of humidity levels observed in mills (27–49%) (Mahroof et al. 2003) and retail stores (24–53%) (R. Roesli and Bh. Subramanyam, unpublished data). Food was not provided to insects during spinosad exposure to simulate the impact of sanitation on pesticide efficacy. Differences in knockdown and mortality of the insects on control surfaces reflects physiological differences among the species in their ability to withstand starvation or desiccation. The adverse effects of starvation and desiccation were irreversible and delayed, because the mortality of insect species was slightly greater than the corresponding knockdown. Howe (1965) reported that many stored-product insect species are dependent on high relative humidity for development and reproduction. At 30–32°C, survival of *C. ferrugineus*, *O. surinamensis*, *R. dominica*, and *T. castaneum* adults in grain was lower at 45% than at 70% RH (Evans 1983). For example, 99% mortality of these four species at 45 and 70% RH took 29–41 and 31–60 wk, respectively. The combined effect of starvation and low humidity increases mortality of adults (Khan 1983, Le Patourel 1986). The presence of food increases survival because it provides water through metabolic pathways (O'Donnell and Machin 1991).

The rough, dusty surface of concrete may have aided in removal of the waterproofing epicuticular lipid layers of the integument (Hadley 1994), resulting in rapid desiccation and death of the insects on these surfaces compared with the other surfaces. Furthermore, using newly emerged rather than mixed age adults may have improved survival under our test conditions. In our tests, insects were exposed to surfaces for 24 h. Exposing insects for <24 h (Barson 1991, Arthur 1998a) may have prevented the high mortality on water only treated surfaces observed in our tests.

There was high knockdown and mortality of the tested insect species on all four spinosad-treated sur-



faces. About 13–20% of knocked down *Tribolium* spp. individuals exposed to treated unwaxed floor tile, steel, and waxed floor tile recovered after placing them on food. A previous study (Toews and Subramanyam 2003) also found that *T. castaneum* was not particularly susceptible to spinosad poisoning by contact on treated glass petri dishes. Insect recovery when placed on food after insecticide poisoning has been previously reported for *T. castaneum* exposed to cyfluthrin residues on concrete (Arthur 2000b). The tiny (120- $\mu$ m) flour particles may have picked-up spinosad residues from the surface of the beetles before the pesticide could enter the insect's cuticle. The lack of recovery with *Tribolium* spp. after exposure to treated concrete could be due to abrasion of the cuticle by the rough concrete surface that enhanced penetration of spinosad into the insect's body. The activity of pesticides on insects is enhanced when applied in combination with inert dusts that abrade or adsorb the epicuticular lipid layers (Subramanyam and Roesli 2000).

In summary, our laboratory tests showed that spinosad at 0.05 and 0.1 mg/cm<sup>2</sup> gave excellent control by contact of six of the eight insect species tested on four surfaces commonly encountered in grain storage facilities, warehouses, food processing plants, and retail stores. Further studies should characterize the persistence and activity of a single application of spinosad at these two deposit levels under laboratory and field conditions.

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### References Cited

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265–267.
- Adán, A., P. D. Estal, F. Budia, M. González, and E. Vinuela. 1996. Laboratory evaluation of the novel naturally derived compound spinosad against *Ceratitis capitata*. *Pestic. Sci.* 48: 261–268.
- Arthur, F. H. 1994. Residual efficacy of cyfluthrin emulsifiable concentrate and wettable powder formulations on porous concrete and on concrete sealed with commercial products prior to insecticide application. *J. Stored Prod. Res.* 30: 79–86.
- Arthur, F. H. 1997a. Differential effectiveness of deltamethrin dust on plywood, concrete, and tile surfaces against three stored-product beetles. *J. Stored Prod. Res.* 33: 167–173.
- Arthur, F. H. 1997b. Residual susceptibility of *Plodia interpunctella* to deltamethrin dust: effects of concentration and time of exposure. *J. Stored Prod. Res.* 33: 313–319.
- Arthur, F. H. 1998a. Residual studies with cyfluthrin wettable powder: toxicity toward red flour beetles (Coleoptera: Tenebrionidae) exposed for short intervals on treated concrete. *J. Econ. Entomol.* 91: 309–319.
- Arthur, F. H. 1998b. Residual toxicity of cyfluthrin wettable powder against *Tribolium confusum* (Coleoptera: Tenebrionidae) exposed for short time intervals on concrete. *J. Stored Prod. Res.* 34: 19–25.
- Arthur, F. H. 1999a. Survival of red flour beetles (Coleoptera: Tenebrionidae) on concrete partially treated with cyfluthrin. *J. Econ. Entomol.* 92: 981–987.
- Arthur, F. H. 1999b. Effect of temperature on residual toxicity of cyfluthrin wettable powder. *J. Econ. Entomol.* 92: 695–699.
- Arthur, F. H. 2000a. Impact of food source on survival of red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae) exposed to diatomaceous earth. *J. Econ. Entomol.* 93: 1347–1356.
- Arthur, F. H. 2000b. Impact of accumulated food on survival of *Tribolium castaneum* on concrete treated with cyfluthrin wettable powder. *J. Stored Prod. Res.* 36: 15–23.
- Barson, G. 1991. Laboratory assessment of the residual toxicity of commercial formulations of insecticides to adult *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) exposed for short time intervals. *J. Stored Prod. Res.* 27: 205–211.
- Burkholder, W. E., and R. J. Dicke. 1966. The toxicity of malathion and fenthion to dermestid larvae as influenced by various surfaces. *J. Econ. Entomol.* 59: 253–254.
- Cox, P. D., D. A. Fleming, J. E. Atkinson, K. L. Bannon, and J. M. Whitfield. 1997. The effect of behaviour on the survival of *Cryptolestes ferrugineus* in an insecticide treated laboratory environment. *J. Stored Prod. Res.* 33: 257–269.
- Evans, D. E. 1983. The influence of relative humidity and thermal acclimation on the survival of adult grain beetles in cooled grain. *J. Stored Prod. Res.* 19: 173–180.
- Fang, L., Bh. Subramanyam, and F. H. Arthur. 2002a. Effectiveness of spinosad on four classes of wheat against five stored-product insects. *J. Econ. Entomol.* 95: 640–650.
- Fang, L., Bh. Subramanyam, and S. Dolder. 2002b. Persistence and efficacy of spinosad residues in farm stored wheat. *J. Econ. Entomol.* 95: 1102–1109.
- Foster, R. N., K. C. Reuter, L. Black, E. Kabla, B. Fuller, and E. A. Flora. 1996. Laboratory evaluation of dose and manner of pickup of spinosad on the migratory grasshopper, 1995. *Arthropod Manage. Tests* 21: 416–417.
- Giga, D. P., and J. Canhou, Sr. 1991. Relative toxicity and persistence of pyrethroid deposits on different surfaces for the control of *Prostephanus truncatus* (Horn) and *Sitophilus zeamais* (Motsch.). *J. Stored Prod. Res.* 27: 153–160.
- Hadley, N. F. 1994. Water relations of terrestrial arthropods. Academic, San Diego, CA.
- Howe, R. W. 1965. A summary of estimates of optimal and minimal conditions for population increase of some stored-product insects. *J. Stored Prod. Res.* 1: 177–184.
- Kenward, M. G., and J. H. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 12: 309–310.
- Khan, M. A. 1983. Untersuchungen über die Invasion von Eilaven von vorratsschädlichen Insekten durch verschieden grosse Poren des Verpackungsmaterials. Anzeiger für Schädlingkunde, Pflanzenschutz, Umweltschutz 56: 65–67.

- Le Patourel, G.N.J. 1986. The effect of grain moisture content on the toxicity of a sorptive silica dust to four species of grain beetles. *J. Stored Prod. Res.* 22: 63–69.
- Liu, T. X., A. N. Sparks, Jr., W. H. Hendrix 3rd, and B. Yue. 1999. Effects of SpinTor (Spinosad) on cabbage looper (Lepidoptera: Noctuidae): toxicity and persistence of leaf residue on cabbage under field and laboratory conditions. *J. Econ. Entomol.* 92: 1266–1273.
- Mahroof, R., Bh. Subramanyam, and D. Eustace. 2003. Temperature and relative humidity profiles during heat treatment of mills and its efficacy against *Tribolium castaneum* (Herbst) life stages. *J. Stored Prod. Res.* 39: 555–569.
- Mertz, P. P., and R. C. Yao. 1990. *Saccharopolyspora spinosa* sp. nov. isolated from soil collected in a sugar rum still. *Int. J. Syst. Bacteriol.* 40: 34–39.
- O'Donnell, M. J., and J. Machin. 1991. Ion activities and electrochemical gradients in the mealworm rectal complex. *J. Exp. Biol.* 155: 375–402.
- SAS Institute. 1999. SAS/STAT user's guide, version 8, SAS Institute, Cary, NC.
- Scott, J. G. 1998. Toxicity of spinosad to susceptible and resistant strain of house flies, *Musca domestica*. *Pestic. Sci.* 54: 131–133.
- Subramanyam, Bh., and R. Roesli. 2000. Inert dusts, pp. 321–380. In Bh. Subramanyam and D. W. Hagstrum [eds.], *Alternatives to pesticides in stored-product IPM*. Kluwer Academic Publishers, Boston, MA.
- Subramanyam, Bh., J. Heaps, and P. Harein. 1993. Insecticide recommendations for food-processing plants. University of Minnesota Extension Service FS-3921-A.
- Thompson, G. D., R. Dutton, and T. C. Sparks. 2000. Spinosad - a case study: an example from a natural products discovery programme. *Pest Manage. Sci.* 56: 696–702.
- Toews, M. D., and Bh. Subramanyam. 2003. Contribution of contact toxicity and wheat condition to mortality of stored-product insects exposed to spinosad. *Pest Manage. Sci.* 59: 538–544.
- Wanner, K. W., B. V. Helson, and B. J. Harris. 2000. Laboratory and field evaluation of spinosad against the gypsy moth, *Lymantria dispar*. *Pest Manage. Sci.* 56: 855–860.
- White, N.D.G. 1982. Effectiveness of malathion and pirimiphos-methyl applied to plywood and concrete to control *Prostephanus truncatus* (Coleoptera: Bostrichidae). *Proc. Entomol. Soc. Ont.* 113: 65–69.
- White, N.D.G., and J. G. Leesch. 1995. Chemical control, pp. 287–330. In Bh. Subramanyam and D. W. Hagstrum [eds.], *Integrated management of insects in stored products*. Marcel Dekker, New York.
- Williams, P., R. L. Semple, and T. G. Amos. 1983. Relative toxicity of three pyrethroid insecticides on concrete, wood, and iron surfaces for the control of grain insects. *Gen. Appl. Entomol.* 15: 7–10.
- Zar, J. H. 1984. *Biostatistical analysis*. Prentice-Hall, Englewood Cliffs, NJ.

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