# Method of Exposure and Grain Condition on Mortality Responses of Stored-Product Insects



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#### OBJECTIVES

Spinosad is a reduced-risk pesticide derived from a bacterial fermentation product. Spinosad has been evaluated on eight stored-grain insects on wheat and corn. Lesser grain borer is the most susceptible to spinosad, whereas red flour beetle is least susceptible. The registrant recently submitted an experimental use permit petition to US-EPA for treating stored grains with spinosad. We hypothesized that method of spinosad exposure (contact vs. ingestion) and insect feeding habits contribute to susceptibility among species.

#### METHODS AND MATERIALS

# I. Contact Toxicity

Spinosad (480 mg/ml) was formulated in acetone and applied in 1 ml to 9-cm glass Petri dishes to obtain deposits of 0.001-0.79 mg (A1)/cm². Control dishes received 1 ml of acetone. Forty (<7-d old) unsexed adults of lesser grain borer (LGB), rice weevil (RW), or red flour beetle (RFB) were introduced into spinosad-treated and control dishes. Each rate was replicated three times. Insects were removed from the treated dishes after 24 h and placed in clean dishes with 10 g of whole wheat (LGB and RW) or flour (RFB) for another 24 h to account for recovery before mortality assessments. Treatment mortality data were corrected for control mortality (<5%), and subjected to probit analyses using the complementary log-log (CLL) model distribution. For time-mortality responses, insects were exposed to 0, 0.0016 and 0.016 mg (A1)/cm² deposits for 48 h. Mortality was determined as described above. Each rate was replicated three times. Treatment mortality data were corrected for control mortality (<1%), and subjected to two-way ANOVA to determine differences among species and rates. All tests were conducted at 30°C and 60% RH.

#### II. Grain Condition

Spinosad diluted in acetone was applied to five individual replicates of cleaned whole (hard red winter) wheat, cracked wheat (broken kernels), and wholewheat flour to obtain deposits of 0, 0.1, or 1.0 mg (AI)/kg. All treatments were equilibrated to 13% moisture. Twenty (<7-d old) adults of LGB, RFB, and sawtoothed grain beetle (STGB) were introduced into independent 150-ml plastic containers with 20 g of untreated or treated media. In a parallel study, twenty eggs of RFB and STGB were introduced into independent 30-ml plastic condiment cups, each filled with 9 g of the same grain fractions used above. Containers and cups were randomized and held at 28°C and 60% RH for 14 d. For each species, corrected mortality data were transformed as arcsine  $\langle n^{0.5}\rangle$  to correct for heteroscedastic variances, and subjected to two-way ANOVA and Fisher's Protected LSD at  $\alpha$  = 0.05. Adult mortality in untreated control replicates ranged from 0-7%, while larval mortality in untreated control replicates ranged from 3-59% depending on the species and medium.

# RESULTS AND DISCUSSION

# I. Contact Toxicity

Both dose-response and time-response tests indicated LGB to be most susceptible to spinosad, followed by RW, and RFB. Predicted LD $_{50}$  (95% CL) values were LGB = 0.0004 (0.0003-0.0006), RW = 0.0768, (0.0670-0.0879) and RFB = 0.1848 (0.1075-0.3212) mg/cm² (Fig. 1). All LGB were killed within 48 h at both rates (Table 1).

# II. Grain Condition

Significant interactions between grain condition and spinosad rate were present in 4 of the 5 tests, thus interaction means were examined. At 1 mg/kg, RFB adult mortality was greater on whole wheat than other treatment combinations (Fig. 2). RFB mortality was 0% at 0.1 mg/kg regardless of grain condition. LGB adult mortality exceeded 97% on media treated with 1 mg/kg. Mortality was lower on broken kernels and flour at 0.1 mg/kg. Grain condition affected STGB adult mortality. STGB adults on whole wheat experienced greater mortality than those on broken kernels or flour. RFB larval mortality was greater on whole wheat at 1 mg/kg than other treatment combinations (Fig 3). STGB larval mortality followed the same trend.

LGB was 192 and 462 times more susceptible to spinosad by contact than RW and RFB, respectively. RW was twice as susceptible as RFB. In grain condition experiments, larval mortality was generally greater than adult mortality for each species. LGB adults and RFB and STGB adults/larvae were more susceptible to spinosad on whole wheat than on broken kernels or flour. We believe that broken kernels and flour may have absorbed more of the pesticide making less available for insects. This is the first report showing spinosad toxicity and by contact on different grain fractions to economically important stored-grain insects.



Whole grain feeding species





Red Flour Beetle Sawtoothed Grain Beet

Broken kernel and flour feeding species

Table 1. Mortality of insect species at two spinosad rates after 48 h.

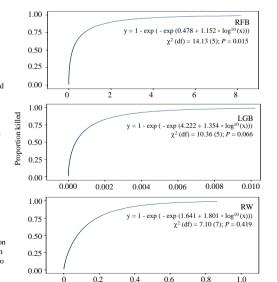
Species x rate (mg (AI)/cm <sup>2</sup> )	% Mortality (Mean $\pm$ SE)
Red Flour Beetle at 0.0016	12.5 ± 5.2d
Red Flour Beetle at 0.016	48.3 <u>+</u> 4.6c
Lesser Grain Borer at 0.0016	100 <u>+</u> 0a
Lesser Grain Borer at 0.016	$100 \pm 0a$
Rice Weevil at 0.0016	10.8 <u>+</u> 5.8d
Rice Weevil at 0.016	85.0 ± 8.7b

Species x Rate interaction: F = 14.25; df = 2, 12; P < 0.001.





Petri dish with insects



Rate, mg (AI)/cm<sup>2</sup>

Fig. 1. Back transformed probit-mortality curves describing 24 h contact toxicity of spinosad.

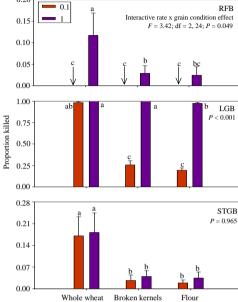
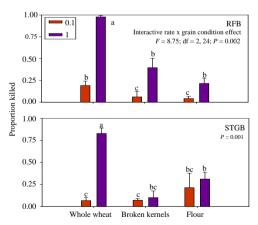


Fig. 2. Mortality of adults following 14-d exposure to spinosad-treated whole wheat and wheat fractions at 0.1 or 1 mg/kg. NOTE: The y-axis scale is different among species.



**Fig. 3.** Mortality of larvae following 14-d exposure to spinosad-treated whole wheat and wheat fractions at 0.1 or 1 mg/kg.

# CONCLUSIONS

Spinosad exhibited contact and ingestion toxicity against adults and larvae of key stored grain insect pests. Applying spinosad to clean wheat will improve its efficacy against these stored-grain insects. Excellent contact activity against some species suggests that spinosad could be used for treating surfaces (empty bins, floors, cracks, and crevices) to manage insects.

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