Pest Manag Sci **59**:538–544 (online: 2003)
DOI: 10.1002/ps.660

Contribution of contact toxicity and wheat condition to mortality of stored-product insects exposed to spinosad†

Michael D Toews* and Bhadriraju Subramanyam
Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506-2201, USA

Abstract: Spinosad, a reduced-risk commercial insecticide derived from a bacterial fermentation product, possesses both contact and oral toxicities against insects. Contact toxicity of spinosad to adults of *Rhyzopertha dominica* (F), *Sitophilus oryzae* (L), and *Tribolium castaneum* (Herbst) was evaluated by exposure for 24 or 48 h to treated glass Petri dishes. Adults were exposed to different deposits (0.001–0.79 mg cm^{-2}) of spinosad in 24-h tests and to deposits of 0, 0.0016 and 0.016 mg cm^{-2} in 48-h tests. *Rhyzopertha dominica* was most susceptible to spinosad in 24- and 48-h tests, followed by *S oryzae*, and *T castaneum*. The 24-h LD_{50} values were 0.0004, 0.077 and 0.189 mg cm^{-2} for *R dominica, S oryzae*, and *T castaneum*, respectively. All *R dominica* adults were dead following 48 h exposure to both spinosad deposits, whereas mortality of *S oryzae* and *T castaneum* ranged from 10 to 85% and 12 to 48%, respectively. *Rhyzopertha dominica, T castaneum*, and *O surinamensis* adults were exposed for 14 days to whole wheat, cracked wheat and wheat flour treated with 0, 0.1 and 1.0 mg kg^{-1} of spinosad. *Rhyzopertha dominica* adults were highly susceptible to spinosad, followed by *O surinamensis* and *T castaneum*. Immatures (eggs and larvae) of *T castaneum* and *O surinamensis* exposed for 14 days were more susceptible on spinosad-treated whole wheat than on treated cracked wheat and wheat flour. This is the first report documenting contact activity of spinosad, and the effect of grain condition on spinosad toxicity, to stored-product insects.

Keywords: reduced-risk insecticide; stored-grain insects; exposure methods; toxicity; *Saccharopolyspora spinosa*

1 INTRODUCTION

Spinosad is a reduced-risk commercial insecticide derived from metabolites of the actinomycete bacterium *Saccharopolyspora spinosa* Mertz and Yao.[1] It is registered for use on over 100 crops or sites in 24 countries.[2] Spinosad is currently not registered for use on stored grain. However, laboratory and field tests using stored wheat have shown spinosad to be effective against several stored-product insects.[3,4] Spinosad applied to wheat at 0.1 and 1.0 mg kg^{-1} was effective in killing all adults and preventing population growth of the lesser grain borer, *Rhyzopertha dominica* (F).[3,4] A rate of 1.0 mg kg^{-1} was necessary for complete control and progeny suppression of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), flat grain beetle, *Cryptolestes pusillus* (Schönherr) and confused flour beetle, *Tribolium confusum* (Jacquelin du Val). First instars of the Indianmeal moth, *Plodia interpunctella* (Hübner) were highly susceptible to spinosad at 1.0 mg kg^{-1}. A rate of 3.0 mg kg^{-1} was necessary to control adults of the rice weevil, *Sitophilus oryzae* (L). Adult sawtoothed grain beetle, *Oryzaephilus surinamensis* (L) and red flour beetle, *Tribolium castaneum* (Herbst) were least susceptible to spinosad. Mortality of *O surinamensis* adults at 1.0 mg kg^{-1} was 60% and that of *T castaneum* adults was 25%.[4] Mortality of *T castaneum* adults was 43% at 20 mg kg^{-1}.

Reasons for the above differences in susceptibility to spinosad among species are not clear. Bret *et al*[5] reported spinosad oral toxicity to be about 5–10 times greater than contact toxicity. The relative contribution of oral and contact toxicities to overall susceptibility of stored-grain insects to spinosad is unknown, because in previous tests, insects were exposed to spinosad-treated wheat.[3,4] The increased susceptibility of *R dominica* and *S oryzae*, insects that develop internally and feed on whole kernels, compared with *T castaneum* and *O surinamensis*, insects that feed and develop externally on cracked wheat or flour, merited further investigation. Therefore, experiments were designed to evaluate contact toxicity of spinosad against

* Correspondence to: Michael D Toews, Department of Grain Science and Industry, 201 Shellenberger Hall, Kansas State University, Manhattan, KS 66506-2201, USA
E-mail: mtoews@wheat.ksu.edu
† This paper reports research results only. Mention of a proprietary product name does not constitute an endorsement for its use by Kansas State University
Contract/grant sponsor: USDA/CSREES (RAMP); contract/grant number: 00-51101-9674
(*Received 16 October 2002; accepted 1 November 2002*)

R dominica, *S oryzae*, and *T castaneum* adults to determine whether the trends in species susceptibility are similar to those observed on stored wheat. In addition, adults of *R dominica*, *T castaneum*, and *O surinamensis*, and immatures of *T castaneum* and *O surinamensis*, were exposed to treated whole wheat, cracked wheat and wheat flour to determine the effect of grain condition on susceptibility to spinosad.

2 EXPERIMENTAL METHODS

2.1 Insects

Rhyzopertha dominica and *S oryzae* cultures were reared on whole, hard red winter wheat. *Tribolium castaneum* and *O surinamensis* were reared on whole wheat flour and rolled oats, respectively. Wheat flour and oats were fortified with 5% brewer's yeast by weight. Insects were reared at 30 °C and 60% RH with a 14:10 h light:dark cycle. A completely randomized design was used for all experiments. Experiments were conducted in environmental chambers with a 14:10 h light:dark cycle at 29 (±1) °C and 45 (±5)% RH.

2.2 Contact toxicity tests: rate/response bioassays

An aqueous suspension of technical spinosad (480 mg AI ml^{-1}) was obtained from Dow AgroSciences (Indianapolis, Indiana, USA). Different rates of spinosad were made by diluting this in acetone. Control dishes were treated with acetone only. Inside surfaces of 9-cm diameter glass Petri dishes (bottoms and lids) were treated with different rates of spinosad suspended in acetone to obtain nominal deposits of 0.001–0.79 mg cm^{-2}. The inside surface of each dish bottom and lid was treated with 1.0 ml each of acetone or spinosad solution. Treating the inside surface of dish lids was necessary to ensure exposure of adults such as *S oryzae* that could walk upside down on glass surfaces. Acetone was evaporated by placing dishes under the laboratory fume hood for 1 h. Forty one-week-old unsexed adults of *R dominica*, *S oryzae* or *T castaneum* were separated from cultures and introduced into each untreated or treated glass dish (replicate). Each deposit level was replicated three times. After insect introduction, Petri dish lids were held in place with 2.5 × 30 cm^2 sections of Parafilm (American National Can, Neenah, Wisconsin, USA) stretched around the edges to prevent insect escape. Insects were exposed to treated dishes for 24 h and then placed in clean dishes with 10 g of whole wheat (*R dominica* and *S oryzae*) or whole-wheat flour plus yeast (*T castaneum*) for an additional 24 h to allow for recovery before assessing mortality.

Mortality of adults in spinosad treatments was corrected for control mortality,[6] before subjecting data to probit analyses[7] using the complementary log–log (CLL) model.[8] Goodness-of-fit of data to the model was assessed using the chi-squared test at the $\alpha = 0.05$ level.[9]

2.3 Contact toxicity tests: time/response bioassays

Time-mortality responses of *R dominica*, *S oryzae* and *T castaneum* adults were determined by exposing 40 adult insects each to glass Petri dishes treated with acetone only or spinosad (0.0016 and 0.016 mg cm^{-2}). Each deposit level was replicated three times. Dishes were treated with acetone or spinosad solutions as described above. After 48 h of exposure, adults were transferred to clean dishes with food for an additional 24 h before evaluating mortality.

Mortality (proportion killed, x) of insects in spinosad treatments was corrected for control mortality and then transformed to arcsine $(x)^{0.5}$ to normalize heteroscedastic variances.[10] Statistical comparisons were made using two-way analysis of variance and pairwise comparisons of least squares means (LSMEANS) at the $\alpha = 0.05$ level.

2.4 Effects of spinosad on *Rhyzopertha dominica* egg laying

Many eggs of *R dominica* were found in spinosad-treated dishes in preliminary experiments. Therefore, we exposed 40 (<two-week-old) *R dominica* adults to spinosad deposits of 0, 0.00016, 0.0016, 0.016 or 0.16 mg cm^{-2} on glass Petri dishes. There were three replicates at each spinosad deposit level. After 24 h, all adults were collected and the eggs in each dish were counted. Insect mortality due to contact exposure to spinosad was 0% in the acetone-only treated dishes, 34 (±11)% at 0.00016 mg cm^{-2}, 79 (±5)% at 0.0016 mg cm^{-2}, 98 (±1)% at 0.016 mg cm^{-2}, and 100% at 0.16 mg cm^{-2}. All live and dead *R dominica* adults were sexed by examining the internal genitalia.[11] The difference in egg production per female between the control treatment and among spinosad treatments was determined by linear contrast, whereas the rate of decrease in egg production as a function of spinosad rate was described by a two-parameter non-linear regression model.

2.5 Effect of wheat condition on spinosad toxicity

Hard red winter wheat was purchased in August 2001 from a local farmer's cooperative elevator. Whole-wheat kernels were held at −17 °C for 10 days to kill any residual insect infestation. Wheat was cleaned by passing it twice over an inclined sieve with sieve openings of 2.54 mm,[12] to remove dockage, cracked wheat and dead insects. A portion of the cleaned wheat was cracked in a laboratory mill (Allis Chalmers Manufacturing Co, Milwaukee, Wisconsin, USA) with LaPage cut rollers having 6.3 corrugations cm^{-1}. The cracked wheat was sifted using standard testing sieves (Seedburo Equipment Company, Chicago, Illinois, USA). Cracked wheat used in tests consisted of wheat fractions that were retained on top of a sieve screen with 841-μm openings after passing through a sieve with 2000-μm openings. Whole-wheat

flour was obtained by grinding wheat kernels for 1 min in a Stein Laboratory Mill (Model M-1, Fred Stein Laboratories, Inc, Atchison, Kansas, USA). Whole-wheat flour consisted of particles of ground wheat that passed through a sieve with 250-μm openings.

Whole wheat, cracked wheat, or wheat flour were equilibrated to 13 (±0.5)% moisture by spreading samples in 2.5-cm-thick layers on steel plates, and placing them in separate sealed plastic boxes over a glycerol and water solution mixture (specific gravity, 1.167) for 4 week.[13] Equilibrated samples (200 g each) of whole wheat, cracked wheat or wheat flour were placed in 0.47-liter glass jars and treated with 0.4 ml of acetone or spinosad diluted in acetone to obtain rates of 0, 0.1 or 1.0 mg kg^{-1}. Jars were closed with lids fitted with 7-cm diameter filter papers and wire mesh screens. Immediately following acetone or spinosad application, jars were harnessed in a 208-liter cardboard drum using Velcro® strips and tumbled for 20 min on a ball-mill roller (Model 200VS, Morse Manufacturing Co, Inc, East Syracuse, New York, USA), to ensure uniform coverage of acetone or spinosad on whole wheat and wheat fractions. Each 200 g of acetone-treated and spinosad-treated samples were further divided into three 25-g samples, and two 9-g samples. The 25-g samples were placed in separate 150-ml plastic containers for bioassays with adults of three insect species. The 9-g samples were placed in 30-ml plastic condiment cups for bioassays with immatures of two insect species.

Twenty (<seven-day-old) adults of *R dominica*, *T castaneum* or *O surinamensis* were introduced into separate plastic containers holding untreated and spinosad-treated whole wheat or wheat fractions. Each species by treatment combination was replicated five times. Containers were examined after 14 days to determine adult mortality.

Twenty eggs of *T castaneum* or *O surinamensis* were introduced into each plastic condiment cup. *Rhyzopertha dominica* was not used in these tests, because these insects require whole kernels for proper development. Each species by treatment combination was replicated five times. Immature mortality (egg plus larval mortality) in each cup was assessed after 14 days.

Mortality data (proportion killed, x) of adults or immatures of each insect species on spinosad-treated whole wheat, cracked wheat and wheat flour were corrected for mortality in the corresponding control.[6] Corrected mortality data were transformed to arcsine $(x)^{0.5}$ and subjected to two-way analysis of variance for determining significant interactions or main effects. Significant interactions were further analyzed using the slice option of the LSMEANS statement in the general linear models procedure.[9] Means among wheat conditions for adults or immatures were separated using interaction LSMEANS at the $\alpha = 0.05$ level.

3 RESULTS

3.1 Contact toxicity tests: rate/response bioassays

Mortality of each insect species on dishes treated with acetone (control) was ≤5%. Adults of *R dominica* were highly susceptible to spinosad, followed in diminishing order by *S oryzae*, and *T castaneum* (Fig 1). The CLL regression model intercepts for *T castaneum, R dominica* and *S oryzae* were 0.48, 4.22 and 1.64, respectively. The corresponding regression slopes were 1.15, 1.35 and 1.80, respectively. Predicted 24 h LD_{50} (95% CL) values were 0.0004 (0.0003–0.0006), 0.077 (0.067–0.088), and 0.185 (0.108–0.321) mg cm^{-2}, respectively. Comparison of the LD_{50} values indicated that *R dominica* adults were 192 and 462 times more susceptible to spinosad than *S oryzae* and *T castaneum*, respectively. Furthermore, *S oryzae* adults were twice as susceptible as *T castaneum* to spinosad.

3.2 Contact toxicity tests: time/response bioassays

Mortality of *T castaneum, R dominica* and *S oryzae* adults in the control treatment was <1%. The

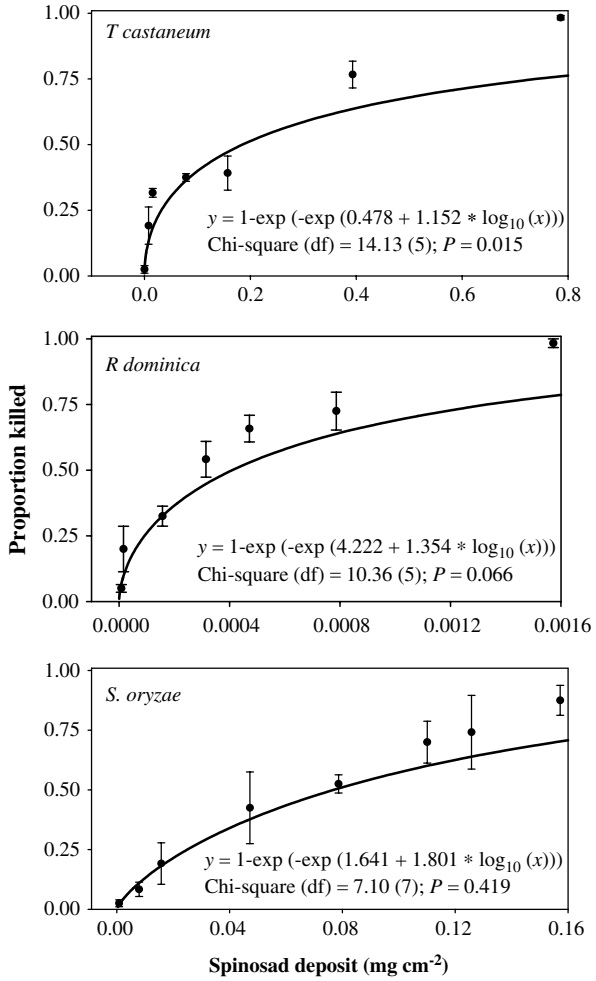

Figure 1. Back-transformed probit-mortality curves describing responses of three insect species exposed for 24 h to spinosad-treated glass Petri dishes. Means and standard errors are actual data. Note: the *x*-axis scale is different among species.

Table 1. Mortality of *Tribolium castaneum, Rhyzopertha dominica*, and *Sitophilus oryzae* adults exposed for 48 h to spinosad deposits on glass Petri dishes

Insect species	Spinosad deposit (mg cm^{-2})	Corrected mortality (% (±SE))[a]
T castaneum	0.0016	12.5 (±5.2) d
T castaneum	0.016	48.3 (±4.6) c
R dominica	0.0016	100.0 a
R dominica	0.016	100.0 a
S oryzae	0.0016	10.8 (±5.8) d
S oryzae	0.016	85.0 (±8.7) b

[a] Means followed by different letters are significantly different ($P < 0.05$).

insect species by spinosad rate interaction was highly significant ($F = 14.25$; $df = 2, 12$; $P < 0.001$). Therefore, means were separated using the interaction LSMEANS (Table 1). All *R dominica* were killed within 48 h. Mortality of *S oryzae* and *T castaneum* was greater at 0.016 than at 0.0016 mg cm^{-2}. *Tribolium castaneum* adults were least susceptible to spinosad, and <50% of the exposed adults were killed at either of the two spinosad deposit levels.

3.3 Effects of spinosad on *Rhyzopertha dominica* egg laying

Rhyzopertha dominica laid very few eggs (0.31 (±0.24) eggs per female per dish) in dishes treated with acetone only, whereas 3.9–7.5 eggs were laid in dishes treated with spinosad. Despite significant mortality after the 24 h exposure, adults exposed to spinosad-treated dishes laid 15–24 times more eggs than those exposed to control dishes ($F = 153.89$; $df = 1, 10$; $P < 0.001$). The mean number of females among spinosad treatments was similar (range = 19.0–22.3 females per dish; control = 20.3 females per dish). Number of eggs laid per female in spinosad treatments decreased with an increase in spinosad deposit level (Fig 2). Furthermore, many dead females had extruded ovipositors.

3.4 Effect of wheat condition on spinosad toxicity

Control mortality was low (0–7%) in adult bioassays. *Tribolium castaneum* mortality at 0.1 mg kg^{-1} was ≤1%, regardless of wheat condition (Fig 3). Mortality at 1.0 mg kg^{-1} was similar among whole wheat, cracked wheat and wheat flour treatments. Mortality of *O surinamensis* exposed to spinosad was 3.9–5.5 times greater in whole wheat than cracked wheat or wheat flour. However, mean mortality of this species was <20% in all spinosad treatments. The wheat condition by spinosad rate interaction was not significant for *T castaneum* ($F = 1.65$; $df = 2, 24$; $P = 0.214$) or *O surinamensis* ($F = 0.01$; $df = 2, 24$; $P = 0.985$). *Tribolium castaneum* mortality was similar in whole wheat and wheat fractions at 0.1 or 1.0 mg kg^{-1} ($F = 0.50$; $df = 2, 24$; $P = 0.614$), but varied between the two rates ($F = 14.27$; $df = 1, 24$; $P = 0.001$). Wheat

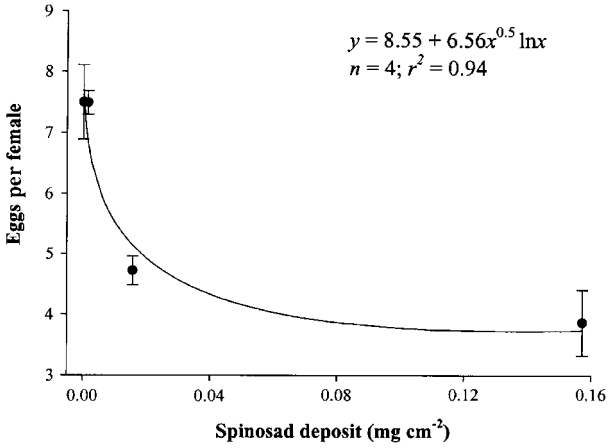

Figure 2. Relationship between mean (±SE) number of eggs per female laid by *Rhyzopertha dominica* and spinosad deposit level. In control (acetone-treated) dishes, *Rhyzopertha dominica* laid 0.31 (±0.24) eggs per female.

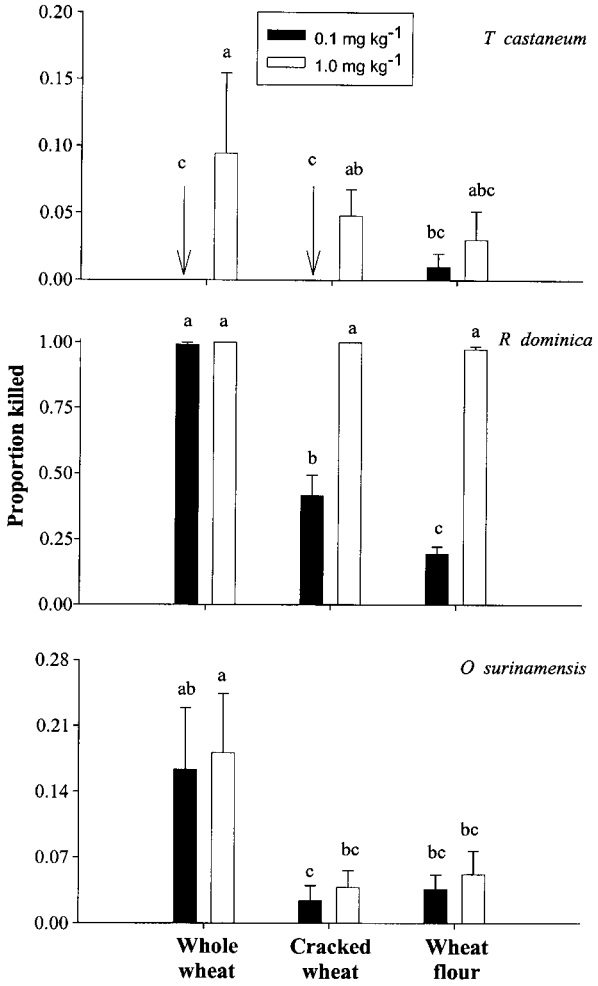

Figure 3. Corrected mortality of *Tribolium castaneum, Rhyzopertha dominica*, and *Oryzaephilus surinamensis* adults exposed for 14 days to whole wheat, cracked wheat and wheat flour treated with spinosad at 0.1 and 1.0 mg kg^{-1}. For each species, different letters above bars indicate significant differences ($P < 0.05$) among treatments. Note: the *y*-axis scale is different among species.

condition influenced *O surinamensis* mortality ($F = 6.55$; $df = 2, 24$; $P = 0.005$). However, mortality was similar between the two spinosad rates ($F = 0.46$; $df = 1, 24$; $P = 0.504$).

More than 97% of *R dominica* adults were killed in all treatments, except at 0.1 mg kg^{-1} in cracked wheat (42%) and flour (19%). The wheat condition by spinosad rate interaction for *R dominica* was significant ($F = 63.57$; $df = 2, 24$; $P < 0.001$). Examination of this interaction, while controlling for differences among wheat conditions, yielded significant differences in insect mortality between spinosad rates for cracked wheat ($F = 181.14$; $df = 1, 24$; $P = 0.001$) and flour ($F = 231.86$; $df = 1, 24$; $P = 0.001$). *Rhyzopertha dominica* mortality among wheat conditions at 1.0 mg kg^{-1} was similar ($F = 2.46$; $df = 2, 24$; $P = 0.106$) after the variation in mortality at 0.1 mg kg^{-1} was removed. However, significant differences were found among wheat conditions at 0.1 mg kg^{-1} ($F = 150.60$; $df = 2, 24$; $P < 0.001$).

Mortality of *O surinamensis* and *T castaneum* immatures in untreated replicates was 6.5–12.7 times greater than in adult bioassays. Control mortality of immature *T castaneum* was 3% in flour, 13% in cracked wheat and 25% in whole wheat. *Oryzaephilus surinamensis* control mortality was 52% in flour, 56% in cracked wheat and 59% in whole wheat. *Tribolium castaneum* and *O surinamensis* mortality were greater in whole wheat treated with spinosad at 1.0 mg kg^{-1} than in the remaining treatments (Fig 4). There was a significant wheat condition by spinosad rate interaction for *T castaneum* ($F = 7.32$; $df = 2, 23$; $P = 0.004$) and *O surinamensis* ($F = 9.50$; $df = 2, 22$; $P = 0.001$). These interactions were further analyzed. Mortality of *T castaneum* immatures increased with spinosad rate in whole wheat ($F = 59.74$; $df = 1, 23$; $P < 0.001$), cracked wheat ($F = 13.01$; $df = 1, 23$; $P = 0.001$) and flour ($F = 6.30$; $df = 1, 23$; $P = 0.020$). The same trend was observed for *O surinamensis* only in whole wheat ($F = 35.34$; $df = 1, 22$; $P = 0.001$).

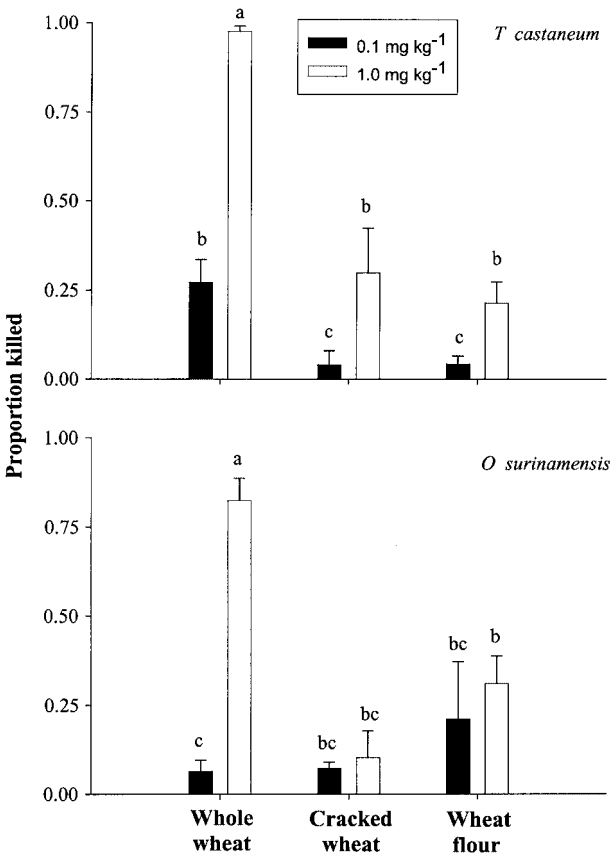

Figure 4. Corrected mortality of *Tribolium castaneum* and *Oryzaephilus surinamensis* immatures exposed as eggs for 14 days to whole wheat, cracked wheat and wheat flour treated with spinosad at 0.1 and 1.0 mg kg^{-1}. For each species, different letters above bars indicate significant differences ($P < 0.05$) among treatments.

4 DISCUSSION

Our data demonstrated contact activity of spinosad through rate/response and time/response bioassays against *T castaneum, R dominica* and *S oryzae* adults. The trend in species susceptibility to spinosad was similar to that observed on grain.[3,4] The increased susceptibility of *R dominica* adults compared with *T castaneum* and *S oryzae* may not be related to increased spinosad pickup from treated surfaces, because *R dominica* is relatively less active than the other species.[14] Other reasons for increased susceptibility of *R dominica* may include faster penetration through the cuticle and/or tarsal route, greater target site sensitivity, or decreased metabolic detoxification.

Pesticides that are efficacious by contact and have low mammalian toxicity should be investigated for treating indoor surfaces, cracks and crevices, or empty bins to control stored-product insects. Spinosad breaks down quickly when exposed to ultra-violet light; however, it may be persistent under indoor conditions such as those found in grain bins, food-processing facilities, warehouses or retail stores. Recent data show that spinosad rates of 0.1–6.0 mg kg^{-1} applied to wheat samples and stored in farm grain bins under Kansas conditions were stable during the 12-month study.[15]

The increased egg laying by *R dominica* in spinosad-treated dishes was probably a result of spinosad poisoning. Spinosad binds to nicotinic acetylcholine and gamma-aminobutyric acid receptor sites of nerve cells.[16] Poisoning symptoms in insects include leg extension, tremors, wing beating, prostration and air swallowing. Eventually insects become paralyzed from neuromuscular fatigue. The combination of abdominal bloating, caused by air swallowing, and involuntary muscular contractions might have caused female *R dominica* to protrude the ovipositor and expel the eggs. Houseflies exposed to spinosad by contact also exhibited protrusion of the ovipositor.[17] We do not know whether the eggs were fully developed, fertilized or viable. Additional work is needed to answer these questions.

Spinosad toxicity to adults and immatures was influenced by rate and wheat condition. In general,

insect mortality was greater in whole wheat than in cracked wheat or wheat flour. The reduced effectiveness of spinosad in cracked wheat or flour may be due to greater absorption of residues onto the smaller food particles that have increased surface area, resulting in less than lethal amounts being available for insect pickup. Anderegg and Madisen,[18] using the organophosphate grain protectant malathion, showed increased accumulation of residues with an increase in levels of cracked wheat and flour (dockage). Another study inferred greater performance of malathion on clean wheat than that containing damaged kernels and fine material against the granary weevil, *Sitophilus granarius* (L), *S oryzae*, and *T confusum*.[19]

In our experiments with immatures, mortality in untreated replicates was higher than expected in whole wheat, but not in cracked wheat or flour. Insects such as *T castaneum* prefer broken kernels and fines, and develop much more effectively on these grain factions than on clean, whole grains.[20] The removal of broken grains and fines in the whole-wheat treatment may have affected survival of first instars hatching from eggs. A similar explanation is tenable for the increased mortality of *O surinamensis* first instars in untreated whole wheat. However, <50% survival of *O surinamensis* first instars in cracked wheat and flour is difficult to explain. Regardless of the actual reason for mortality in untreated replications with *O surinamensis* and *T castaneum*, the data were corrected for control mortality. Therefore, immature mortality values were not inflated. In our tests, immature mortality potentially included egg and larval mortality, because we infested untreated and spinosad-treated wheat and wheat fractions with eggs of *T castaneum* and *O surinamensis*. Adán *et al*[21] reported that spinosad did not exhibit any ovicidal activity against *Ceratitis capitata* (Wiedemann) after 48-h exposure to dosages ranging from 1 to 1000 mg litre^{-1}; however, all neonate larvae reared on spinosad-treated diet at a rate of 0.22 mg kg^{-1} died before reaching the pupal stage. Further study is needed to determine if spinosad is more active against eggs or larvae of stored-product insects.

5 CONCLUSIONS

This is the first report documenting spinosad toxicity by contact to economically important stored-product insects. Excellent contact activity against some species suggests that spinosad should be investigated for treating surfaces (cracks/crevices or floors of empty bins, food-processing facilities and warehouses) to manage insects. Future studies should focus on the stability and efficacy of surface treatments. Successful trials could have a significant impact in the grain and food industry, as the margin of safety with spinosad is better than many synthetic pesticides.[5,22] Increased insect mortality in spinosad treated-whole grain compared with cracked wheat or flour indicates that application of spinosad to clean grain will improve its efficacy in the field.

ACKNOWLEDGEMENTS

We thank Jackie Rowan, Lisa McGavran, Zeb Larson and Sara Velasquez for their excellent technical assistance. Dow AgroSciences donated the technical spinosad used in this study. We are grateful to Kun Yan Zhu and Frank Arthur for reviewing the manuscript. This project was funded by USDA/CSREES (RAMP) under Agreement No 00-51101-9674. This paper is Contribution No 02-381-J of the Kansas Agricultural Experiment Station.

REFERENCES

1 Mertz PP and Yao RC, *Saccharopolyspora spinosa* sp nov isolated from soil collected in a sugar rum still. *Internat J Sust Bacteriol* **40**:34–39 (1990).
2 Thompson GD, Dutton R and Sparks TC, Spinosad—a case study: an example from a natural products discovery programme. *Pest Manag Sci* **56**:696–702 (2000).
3 Fang L, Subramanyam Bh and Arthur FH, Effectiveness of spinosad on four classes of wheat against five stored-product insects. *J Econ Entomol* **95**:640–650 (2002).
4 Subramanyam Bh, Nelson JJ and Fang L, Effectiveness of spinosad against eight stored-product insect species on three classes of wheat. *J Stored Prod Res* (*in press*).
5 Bret BL, Larson LL, Schoonover JR, Sparks TC and Thompson GD, Biological properties of spinosad. *Down to Earth* **52**:6–13 (1997).
6 Abbott WS, A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).
7 Finney DJ, *Probit Analysis*, 3rd edn, Cambridge University Press, London (1971).
8 Robertson JL and Priesler HK, *Pesticide bioassays with arthropods*, CRC Press, Boca Raton, FL, USA (1992).
9 SAS Institute Inc, *SAS/STAT User's Guide, version 8*, SAS Institute Inc, Cary, NC, USA (1999).
10 Zar JH, *Biostatistical analysis*, Prentice-Hall Inc, Englewood Cliffs, New Jersey, USA (1984).
11 Potter C, The biology and distribution of *Rhyzopertha dominica* (Fab). *Trans Roy Entomol Soc London* **83**:449–482 (1935).
12 White GG, A modified inclined sieve for separation of insects from wheat. *J Stored Prod Res* **19**:89–91 (1983).
13 Braun JV and Braun JD, A simplified method of preparing solutions of glycerol and water for humidity control. *Corrosion* **14**:117–118 (1958).
14 Surtees G, Laboratory studies on dispersion behaviour of adult beetles in grain. IV—the lesser grain borer, *Rhyzopertha dominica* (F) (Coleoptera, Bostrychidae). *Bull Entomol Res* **54**:715–722 (1964).
15 Fang L, Laboratory and field evaluation of spinosad on wheat against stored-grain insects, *MS thesis*, Department of Grain Science and Industry, Kansas State University, USA (2002).
16 Salgado VL, Studies on the mode of action of spinosad: insect symptoms and physiological correlates. *Pestic Biochem Physiol* **60**:91–102 (1998).
17 Scott JG, Toxicity of spinosad to susceptible and resistant strains of house flies, *Musca domestica*. *Pestic Sci* **54**:131–133 (1998).
18 Anderegg BN and Madisen LJ, Effect of dockage on the degradation of [^{14}C]malathion in stored wheat. *J Agric Food Chem* **31**:700–704 (1983).
19 Strong RG and Sbur DE, Influence of grain moisture and storage temperatures on the effectiveness of malathion as a grain protectant. *J Econ Entomol* **53**:341–349 (1960).

20 Arbogast T, Beetles: Coleoptera, in *Ecology and management of food industry pests*, ed by Gorham RJ, Association of Official Analytical Chemists, Arlington, VA, USA, pp 131–176 (1991).
21 Adán A, Del Estal P, Budia F, González M and Viñuela E, Laboratory evaluation of the novel naturally derived compound spinosad against *Ceratitis capitata*. *Pestic Sci* **48**:261–268 (1996).
22 Schoonover JR and Larson LL, Laboratory activity of spinosad on non-target beneficial arthropods. *Arthropod Manag Tests* **20**:357 (1995).