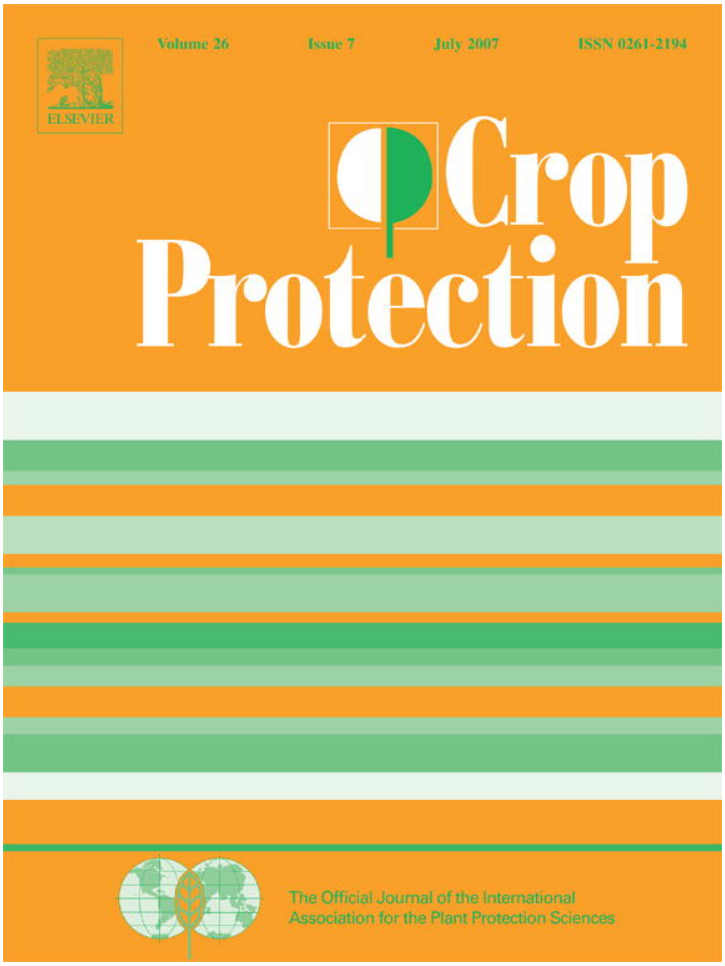

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Evaluation of spinosad as a grain protectant on three Kansas farms ☆

Bhadriraju Subramanyam[a,*], Michael D. Toews[b], Klein E. Ileleji[c], Dirk E. Maier[c], Gary D. Thompson[d], Terry J. Pitts[e]

[a]*Department of Grain Science and Industry, 201 Shellenberger Hall, Kansas State University, Manhattan 66506, Kansas, USA*
[b]*USDA-ARS Grain Marketing and Production Research Center, 1515 College Avenue, Manhattan, Kansas 66502, USA*
[c]*Department of Agricultural & Biological Engineering, 225 South University Street, Purdue University, West Lafayette, Indiana 47907, USA*
[d]*Dow AgroSciences, 9330 Zionsville Road, Indianapolis, Indiana 46268, USA*
[e]*Bayer CropScience, 1400 Preston Road, Suite 400, Plano, Texas 75093, USA*

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Abstract

The persistence and insecticidal activity of a commercial insecticide, spinosad, based on the fermentation products of the actinomycete bacterium *Saccharopolyspora spinosa* Mertz and Yao (Actinomycetales: Actinomycetaceae), was evaluated on three Kansas farms between July 2002 and January 2003. Spinosad was applied to newly harvested hard red winter wheat at 1 mg (a.i.)/kg of grain at the time of storage in round, steel bins with capacity of 60–125 metric tons. Insect populations in grain samples collected monthly from bins receiving spinosad treatment were compared with populations in bins receiving no treatment (control), bins receiving 3 mg (a.i.)/kg of chlorpyrifos-methyl, and bins receiving 1 mg (a.i.)/kg spinosad + 3 mg (a.i.)/kg chlorpyrifos-methyl treatment. The actual spinosad residue on wheat immediately after treatment was 30% less than the application rate of 1 mg (a.i.)/kg, but there was no significant degradation of these residues during the 6-month test period. None of the July samples from any of the four treatments had live adults of stored-product insects. No live adults of the lesser grain borer, *Rhyzopertha dominica* (F.), and very low densities ($\leqslant 3$ live adults/kg of sample) of the red flour beetle, *Tribolium castaneum* (Herbst); rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); and sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), were found in grain samples collected between August and January from bins treated with spinosad or spinosad + chlorpyrifos-methyl. The density of all four insect species combined in untreated grain samples steadily increased from 0.5 adults/kg in August 2002 to a high of 22.1 adults/kg in January 2003. In grain samples from bins treated with chlorpyrifos-methyl, the density of *R. dominica* adults increased from 0.4 adults/kg in September 2002 to a maximum of 10.2 adults/kg in January 2003, whereas densities of the other three species were $\leqslant 1$ adult/kg. Laboratory bioassays with monthly grain samples collected from the field applications showed that spinosad, alone or in combination with chlorpyrifos-methyl, was effective in killing adults of *R. dominica* and *C. ferrugineus*, but not *T. castaneum*. Progeny production of these three species was suppressed on spinosad-treated grain, irrespective of the sampling month. Results show that a single application of spinosad at 1 mg (a.i.)/kg is effective for managing common stored-grain insects, including *R. dominica,* for at least 6 months.

Keywords: Stored grain; Protectants; Persistence; Insects; Efficacy; Grain quality

1. Introduction

Spinosad, a commercial insecticide based on the fermentation products of the naturally occurring soil actinomycete *Saccharopolyspora spinosa* Mertz and Yao (Mertz and Yao, 1990) (Actinomycetales: Actinomycetaceae), is effective at 1 mg (a.i.)/kg of grain against several species of stored-product insects according to laboratory (Fang et al., 2002a,b; Toews and Subramanyam, 2003;

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*Corresponding author. Tel.: +1 785 532 4092; fax: +1 785 532 7010.
E-mail address: sbhadrir@ksu.edu (Bh. Subramanyam).

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Toews et al., 2003; Huang et al., 2004) and pilot-scale field trials (Fang et al., 2002b; Flinn et al., 2004). Spinosad has low mammalian toxicity, compared with other traditional insecticides (Thompson et al., 2000) and a single application at 1 mg (a.i.)/kg or higher persisted on stored grain for 6–12 months with minimal loss in insecticidal activity (Fang et al., 2002b; Flinn et al., 2004). The United States Environmental Protection Agency (US-EPA) granted Dow AgroSciences (Indianapolis, Indiana, USA) an experimental use permit (Experimental Use Permit no. 62719-EUP-50) on May 31, 2002, for conducting trials on farms to document the effectiveness of spinosad at 1 mg (a.i.)/kg on wheat, barley, oats, sorghum, and corn to manage stored-product insects.

There are no studies documenting the effectiveness of spinosad applied to wheat stored in bins on farms, except for the pilot-scale field trials of Fang et al. (2002b) and Flinn et al. (2004). In the present study, the field persistence and efficacy of spinosad applied to newly harvested wheat stored in commercial-scale bins on three Kansas farms was determined for a period of 6 months, starting with wheat harvest and storage in July 2002 and ending in January 2003. The study spanned 6 months because wheat on Kansas farms is typically stored for a duration of three to nine months (Martin et al., 1997), and wheat is most susceptible to stored-product insect infestation when the grain is warm (Hagstrum, 1987, 2001). The current study objectives were to (1) quantify live insects in wheat receiving 0 mg (a.i.)/kg of spinosad (control, untreated wheat), 1 mg (a.i.)/kg spinosad, 3 mg (a.i.)/kg chlorpyrifos methyl, or 1 mg (a.i.)/kg spinosad + 3 mg (a.i.)/kg chlorpyrifos methyl over the 6-month test period, (2) determine mortality and progeny production of key stored-product insects exposed monthly in the laboratory to grain samples collected from each of the four treatments, and (3) evaluate grain quality parameters (dockage, test weight, damaged kernels, foreign material, shrunken and broken kernels, total defects, and insect-damaged kernels) following federal grain-grading procedures. In addition, data were collected on spinosad residue degradation and grain temperatures during the test period.

2. Materials and methods

2.1. Bin locations and preparation

On-farm, grower-owned round, steel grain bins of 60–125 metric ton capacity were used in this study. Bin locations in Kansas were near Abilene (125 metric tons), Morganville (100 metric tons), and Brewster (125 metric tons). Abilene is 43 miles west, Morganville is 50 miles northwest, and Brewster is 283 miles west of Kansas State University, Manhattan. These farms were selected based on availability of four identical bins on each farm, heterogeneous climatic conditions, and voluntary participation of producers. Empty test bins were cleaned by producers to remove old grain and debris and any residual insect populations 2–3 weeks before commencement of the study.

2.2. Grain treatment

Grain bins were filled in 2002 during the first 2 weeks of July with newly harvested hard red winter wheat. Treatments included untreated wheat (control), 1 mg (a.i.)/kg spinosad (SpinTor 2SC (Dow AgroSciences, Indianapolis, Indiana, USA)), 3 mg (a.i.)/kg chlorpyrifos-methyl (Reldan® 4E, Gustafson LLC, Plano, Texas, USA), or 1 mg (a.i.)/kg spinosad + 3 mg (a.i.)/kg chlorpyrifos-methyl. Chlorpyrifos-methyl was used at one-half the labeled rate because Dow AgroSciences was considering potential protectants utilizing the 3 mg (a.i.)/kg chlorpyrifos-methyl in combination with another active ingredient. Since this study, Bayer CropScience received a registration and released a product (Storcide II®) utilizing 3 mg/kg chlorpyrifos-methyl in combination with 0.5 mg (a.i.)/kg deltamethrin. Insecticides were diluted with water, and applications were made with a pressure sprayer (Little Gus Applicator, Gustafson LLC, Plano, Texas, USA) calibrated to deliver the target rate of insecticide to match the speed of the grain flow. Grain at each farm site was treated while being augured into the bins by grower-owned augers. Separate sprayers were used for each chemical to prevent cross contamination. The same auger was used at each site, but bin filling was administered in the following treatment order: untreated control, spinosad, chlorpyrifos-methyl, and spinosad + chlorpyrifos-methyl. Grain in each bin was leveled to the eaves after bin filling to permit installation of cables for monitoring grain temperatures and for monthly grain sampling.

2.3. Temperature measurements

Headspace and grain bulk temperatures were monitored throughout the study. Headspace temperatures were measured using a single HOBO® data logger (HOBO TEMP, Onset Computer Corporation, Bourne, Massachusetts, USA) at the center of each bin. During monthly sampling visits grain temperatures 5 cm and 1.8 m below the grain surface were measured with thermocouple wires (Type K, Omega Engineering, Inc., Stamford, Connecticut, USA) connected to a digital thermometer (model 51, Fluke Corp., Everett, Washington, USA). Grain temperatures were recorded at the center and four cardinal directions halfway between the bin center and bin periphery. These loggers were supported on a 2.5-cm diameter wooden dowel inserted into the grain.

2.4. Insect sampling and grain analyses

A comprehensive sampling plan was implemented to monitor natural infestations of stored-product insects in each bin. Insect infestations in farm-stored grain begin with insects entering through the eaves and colonizing the grain

surface (Hagstrum, 1989, 2001; Vela-Coiffer et al., 1997). In addition, the grain surface is subjected to greater temperature and moisture extremes and insect pressure than grain in the middle and bottom layers of the grain mass (Hagstrum, 1987). Therefore, sampling the top 1.8 m of each bin was deemed adequate for evaluating the performance of the insecticide treatments. Wheat samples were obtained in each bin immediately after filling the bins and then monthly for 6 months. Grain samples were obtained with a 1.8-m-long grain trier (Model 39C-OH, Seedburo Equipment Co., Chicago, Illinois, USA) by probing the bin center and at the four cardinal directions, halfway between the center and bin periphery. Each location in the grain was probed twice, and each probe sample yielded about 750 g of wheat (total per bin, 7.5 kg). Separate grain triers were used for each treatment. Grain samples were pooled across sampling locations by bin, stored in plastic bags, labeled, and immediately brought to the laboratory.

Grain samples were processed in the laboratory to enumerate live insects, conduct bioassays with three insect species, assess grain quality, and determine spinosad residues. Grain was first weighed and then passed over an inclined sieve (White, 1983; Hagstrum and Subramanyam, 2000) to extract live adult insects. Separate sieves were used for each treatment to prevent cross contamination. Extracted adults were identified to species and expressed as number of live insects per kg of grain. An approximate 1000-g portion of the pooled sample was shipped overnight to the Grain Inspection, Packers, and Stockyard Administration (GIPSA) center in Topeka, Kansas, for grain grading according to official techniques (GIPSA, 1997). An additional 1000-g sample from each spinosad or spinosad + chlorpyrifos-methyl bin was shipped overnight to Dow AgroSciences for spinosad residue analysis. Analytical procedures following the methods of Hastings and Clements (2000) were used to detect levels of spinosyn A and spinosyn D. The total spinosad residue, expressed as mg(a.i.)/kg of wheat, was obtained by summing the levels of spinosyns A and D. Fang et al. (2002a) provide a detailed description for extraction and quantification of spinosad residues from wheat samples. Grain quality and spinosad residue analyses were performed immediately after treatment (time 0), and at three and 6 months after treatment. The remaining sample portion (about 4.4 kg) was frozen at −20 °C to kill any live insects. After two weeks, these samples were held at room conditions before use in bioassays.

2.5. Laboratory bioassays

The residual activity of field-applied grain protectants against insects was verified in the laboratory by exposing the grain samples collected monthly to three insect species. Species included the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae); these three insect species are commonly associated with farm-stored wheat in Kansas (Reed et al., 1991). Laboratory insect colonies were established in 1999 at the Department of Grain Science and Industry (Kansas State University) from colonies at other laboratories. Insect rearing procedures for these species were previously described by Fang et al. (2002a).

From the monthly grain samples collected from each bin, two 250-g wheat lots were placed in separate 0.94-l glass jars fitted with wire-mesh screens and filter paper lids. All jars were then placed in growth chambers for 14 days at 28 °C and 65% RH to equilibrate grain moisture content to 12 ± 1%, quantified with a calibrated moisture tester (Motomco Model 919® Automatic Grain Moisture Tester, Auburn, Illinois, USA). Each jar was infested with 50, 2-week old unsexed adults of *R. dominica*, *T. castaneum*, or *C. ferrugineus*. There were a total of 6 jars for each treatment and month (2 jars per bin × 3 bins of a given treatment across the three farms). All infested jars were held at 28 °C and 65% RH in a growth chamber. After 2 weeks, three jars from each treatment by species combination were sifted over a 1.68-mm diameter sieve (Seedburo Equipment Co., Chicago, Illinois, USA) to extract insects from the grain. Adult mortality was assessed by counting the number of dead insects out of the total exposed, and insect mortality was expressed as a percentage. The remaining three jars were checked after eight weeks, following procedures explained earlier, to assess the number of adult progeny (F_1) produced. The original number of 50 adults introduced into each jar was subtracted from the number of adults found after 8 weeks to determine the actual number of progeny produced.

2.6. Statistical analyses

The field study was designed as a completely randomized block, with bin locations serving as blocks. Count data on the number of live adults per kg of grain (x) were transformed to logarithmic scale [$\log_{10}(x+1)$] (Steel and Torrie, 1980) to normalize heteroscedastic treatment variances and analyzed by using repeated measures analysis of variance (PROC MIXED, SAS Institute, 2003) with the first-order autoregressive co-variance model (Littell et al., 2002). Because the interaction term was generally significant, differences among the four treatments were determined by species and month, and a Fisher's protected least significant difference (lsd) test was used for separating treatment means.

Mortality and progeny production data were also analyzed as completely randomized block designs. Mortality data were transformed to angular values (Zar, 1984), and progeny data (x) were transformed to logarithmic scale [$\log_{10}(x+1)$] (Steel and Torrie, 1980) to normalize treatment variances before analyses with repeated measures analysis of variance with the first-order autoregressive

co-variance model. Mortality or progeny production differences among the four treatments by species and month were determined using the Fisher's lsd test.

Whole kernel spinosad residue degradation data (on untransformed scale, because variances were similar across treatments) immediately after grain treatment and at three and 6 months post-treatment for spinosad and spinosad + chlorpyrifos-methyl treatments were analyzed separately using one-way analysis of variance. Grain quality variables were analyzed with repeated measures analyses of variance as described earlier. Percentages were transformed to angular values (Zar, 1984). All comparisons were considered significant at the $\alpha = 0.05$ level. Although data were transformed for statistical analyses, means and standard errors based on untransformed data are presented in tables and figures.

3. Results

Temperature in the headspace at 5 cm and 1.8 m below the grain surface decreased over the 6-month study period. Headspace and temperatures 5 cm below the grain surface decreased to 20 °C in early October, whereas it took nearly until December for the grain 1.8 m below the surface to cool to 20 °C (Fig. 1). Likewise, headspace and grain surface temperatures decreased to 10 °C in mid-November, whereas the grain 1.8 m below the grain surface had only decreased to 13 °C when the study was terminated in January 2003.

There were obvious trends in the number of live adult insects recovered from grain samples in the untreated and insecticide-treated bins. There were no live adults in grain samples immediately after bin filling (July 2002), so these data were not included in statistical analyses. Insects increased throughout the study in the untreated and chlorpyrifos-methyl-treated bins, whereas very few live insects were recovered in the bins treated with spinosad and spinosad + chlorpyrifos-methyl (Table 1). There were treatment by month interactive effects for *C. ferrugineus* ($F = 2.4$; $df = 15, 46$; $P = 0.01$) and *T. castaneum* ($F = 2.8$; $df = 15, 46$; $P < 0.01$), but not for *R. dominica* ($F = 1.6$; $df = 15, 46$; $P = 0.10$) and *Oryzaephilus surinamensis* ($F = 0.8$; $df = 15, 46$; $P = 0.68$). A significant insecticide-treatment effect was observed for *C. ferrugineus* ($F = 19.7$; $df = 3, 46$; $P < 0.01$) and *T. castaneum* ($F = 8.7$; $df = 3, 46$; $P < 0.01$), but not for *R. dominica* ($F = 2.3$; $df = 3, 46$; $P = 0.09$) and *O. surinamensis* ($F = 1.5$; $df = 3, 46$; $P = 0.23$). Longer storage time significantly increased the number of insects recovered for all species, including *C. ferrugineus* ($F = 3.0$; $df = 5, 46$; $P = 0.01$), *T. castaneum* ($F = 2.7$; $df = 5, 46$; $P = 0.03$), *R. dominica* ($F = 2.4$; $df = 5, 46$; $P = 0.05$), and *O. surinamensis* ($F = 4.6$; $df = 5, 46$; $P < 0.01$).

Comparisons by month and species (Table 1) showed no significant differences ($P > 0.05$) among treatments in *R. dominica* or *O. surinamensis* numbers for any of the months, but significant differences ($P < 0.05$) among treatments were observed in numbers of *C. ferrugineus* for October, November, December, and January and in numbers of *T. castaneum* for December. When comparisons among treatments were based on the total numbers of all beetles; however, significant differences among treatments were observed for each of the months between October 2002 and January 2003. Although treatment differences in the total number of insects were observed by month, these differences arose as a result of the overall comparisons that included the untreated control, and the procedure for separating means did not show any significant differences among treatments receiving an insecticide application.

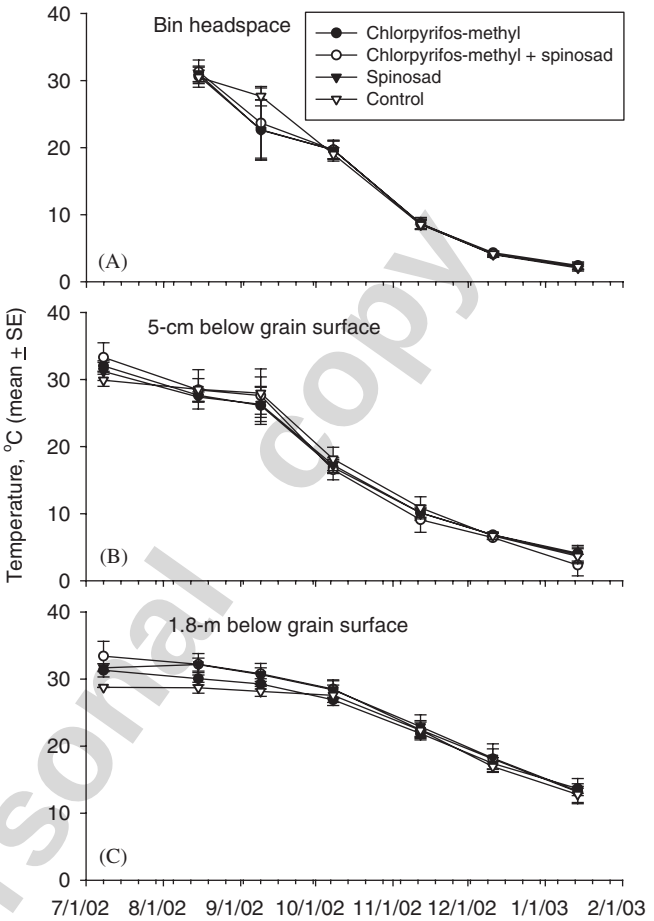

Fig. 1. Headspace temperature (A) and grain temperature 5 cm (B) and 1.8 m below the grain surface (C), measured monthly from each of the 12 bins between July 2002 and January 2003. Each mean is based on $n = 3$ bins.

Despite careful calibration before and during applications, actual spinosad residues immediately after application at 1 mg (a.i.)/kg in both the spinosad and spinosad + chlorpyrifos-methyl treatments was approximately 0.68 to 0.70 mg (a.i.)/kg (Table 2). Observed spinosad residues in the bins receiving spinosad alone or spinosad + chlorpyrifos-methyl decreased by about 9–11% over 6 months, except in the spinosad + chlorpyrifos-methyl treatment at 3 months in which a 34% decrease was observed. Nevertheless, residue decrease over time was not significant

Table 1
Live adults (mean ± SE) per kilogram of wheat of four insect species recovered from untreated wheat (control) or insecticide-treated wheat samples collected monthly from farm bins in Kansas between August 2002 and January 2003[a]

Month, Year	Treatment	*R. dominica*	*C. ferrugineus*	*T. castaneum*	*O. surinamensis*	Total
August, 2002	Control	0.04±0.04[b]	0.4±0.2[b]	0.04±0.04[b]	0.0	0.5±0.3[b]
	C-methyl[c]	0.0	0.0	0.0	0.0	0.0
	Spinosad	0.0	0.3±0.3	0.0	0.0	0.3±0.3
	Spinosad + C-methyl	0.0	0.0	0.0	0.0	0.0
September	Control	2.6±1.6[b]	1.0±0.5[b]	0.4±0.3[b]	0.0	4.1±2.4[b]
	C-methyl	0.4±0.4	0.0	0.0	0.2±0.2[b]	0.6±0.4
	Spinosad	0.0	0.0	0.0	0.0	0.0
	Spinosad + C-methyl	0.0	0.0	0.0	0.0	0.0
October	Control	3.9±2.2[b]	2.5±1.2[d]	4.4±3.0[b]	0.9±0.5[b]	11.7±4.6a
	C-methyl	0.7±0.4	0.0	0.0	1.1±1.1	1.9±1.2b
	Spinosad	0.0	0.0	0.0	0.2±0.2	0.2±0.2b
	Spinosad + C-methyl	0.0	0.0	0.0	0.1±0.1	0.1±0.1b
November	Control	4.6±2.3[b]	2.8±1.5a	4.7±3.9[b]	1.0±0.8[b]	13.1±7.7a
	C-methyl	1.2±0.7	0.04±0.04b	0.0±0.0	0.8±0.8	2.0±1.5b
	Spinosad	0.0	0.0b	0.04±0.04	0.1±0.1	0.2±0.2b
	Spinosad + C-methyl	0.0	0.0b	0.04±0.04	0.04±0.04	0.08±0.08b
December	Control	4.6±2.4[b]	3.4±0.5a	9.2±4.3[e]	0.6±0.4[b]	17.7±2.8a
	C-methyl	3.4±2.7	0.04±0.04b	0.0±0.0	0.7±0.7	4.1±3.4b
	Spinosad	0.0	0.0b	0.0±0.0	0.1±0.1	0.1±0.1b
	Spinosad + C-methyl	0.0	0.04±0.04b	0.0±0.0	0.1±0.1	0.1±0.1b
January, 2003	Control	2.6±1.7[b]	17.1±13.3a	1.7±1.2[b]	0.8±0.4[b]	22.1±12.7a
	C-methyl	10.2±9.5	0.6±0.6b	0.05±0.05	0.7±0.7	11.4±10.8ab
	Spinosad	0.0	0.0b	0.0	0.3±0.3	0.3±0.3b
	Spinosad + C-methyl	0.0	0.0b	0.0	0.1±.01a	0.1±0.1b

Each mean is based on $n = 3$ bins. Within months and species, means among treatments followed by different letters are significantly different ($P < 0.05$; Fisher's protected lsd test).
[a]Live adults were not found in grain samples taken immediately (July, 2002) after filling the bins.
[b]For the indicated months and species, insect numbers among treatments were not significantly different (F, range = 0.56–3.93; $df = 3, 8$; P, range = 0.05–0.66; one-way analysis of variance).
[c]C-methyl = chlorpyrifos-methyl.
[d]$F = 10.44$; $df = 3, 8$; $P < 0.01$; one-way analysis of variance.
[e]$F = 10.06$; $df = 3, 8$; $P < 0.01$; one-way analysis of variance.

Table 2
Spinosad residues (mean ± SE) on wheat samples collected from bins receiving spinosad or spinosad + chlorpyrifos-methyl

Month, Year	Spinosad (mg (a.i.)/kg)[a]	Spinosad + chlorpyrifos-methyl (mg (a.i.)/kg)[b]
July, 2002	0.70±0.03	0.68±0.11
October, 2002	0.62±0.02	0.45±0.10
January, 2003	0.62±0.07	0.62±0.07

Each mean is based on $n = 3$ bins.
[a]$F = 1.71$; $df = 2, 6$; $P = 0.26$; one-way analysis of variance.
[b]$F = 2.24$; $df = 2, 6$; $P = 0.19$; one-way analysis of variance.

($P > 0.05$) in bins treated with either spinosad or spinosad+chlorpyrifos-methyl.

In laboratory bioassays, adult mortality after 14 days showed that *T. castaneum* was less susceptible to spinosad-treated grain than were *R. dominica* and *C. ferrugineus* (Table 3). Complete mortality of *R. dominica* and *C. ferrugineus* generally was observed in grain treated with spinosad or spinosad+chlorpyrifos-methyl, regardless of the month, whereas mortality in the untreated grain ranged from 3% to 44% (Table 3). Mortality of *T. castaneum* adults in spinosad-treated grain ranged from 49% to 82%. Grain treated with chlorpyrifos-methyl resulted in high mortality of *C. ferrugineus* and *T. castaneum* throughout the study, but survivorship of *R. dominica* increased after one month. There was a significant insecticide treatment by month interaction ($F = 438$; $df = 18, 54$; $P < 0.01$), insecticide effect ($F = 487.3$; $df = 3, 54$; $P < 0.01$), and month-of-study effect ($F = 11.4$; $df = 3, 54$; $P < 0.01$) for *R. dominica*. Similar findings were observed with *C. ferrugineus* (interaction $F = 2.0$; $df = 18, 54$; $P = 0.02$; treatment effect $F = 123.8$; $df = 3, 54$; $P < 0.01$; month effect $F = 2.6$; $df = 6, 54$; $P = 0.03$) and *T. castaneum* (interaction $F = 1.9$; $df = 18, 54$; $P = 0.02$; treatment effect $F = 161.1$; $df = 3, 54$; $P < 0.01$; month effect $F = 5.7$; $df = 6, 54$; $P < 0.01$).

Adult progeny of the three species after eight weeks in laboratory bioassays also followed trends similar to those observed for insects recovered in bins. Across all months,

Table 3
Mortality (%mean ± SE) of adults of three stored-product insect species exposed for 14 days to untreated (control) wheat or insecticide-treated wheat samples collected monthly from farm bins between July 2002 and January 2003

Month, Year	Treatment	Species		
		R. dominica	*C. ferrugineus*	*T. castaneum*
July, 2002	Control	43.7±2.7c	22.9±12.3b	2.6±1.7c
	C-methyl[a]	94.5±1.4b	99.3±0.7a	100.0a
	Spinosad	100.0a	100.0a	77.9±10.2b
	Spinosad + C-methyl	100.0a	100.0a	100.0a
August	Control	27.0±2.9c	8.2±3.3b	4.7±2.4c
	C-methyl	47.0±0.6b	100.0a	97.4±2.6a
	Spinosad	100.0a	100.0a	81.5±8.5b
	Spinosad + C-methyl	100.0a	99.3±0.7a	97.9±2.1a
September	Control	2.9±1.5c	13.8±1.6b	12.1±1.1b
	C-methyl	14.0±1.8b	96.6±1.8a	92.5±2.7a
	Spinosad	100.0a	100.0a	75.3±7.8a
	Spinosad + C-methyl	100.0a	98.0±2.0a	91.0±4.7a
October	Control	3.5±1.5c	20.2±13.6b	14.1±3.0b
	C-methyl	9.1±3.1b	93.9±3.1a	90.5±5.9a
	Spinosad	100.0a	99.4±0.6a	82.0±4.4a
	Spinosad + C-methyl	100.0a	90.1±9.9a	94.6±2.7a
November	Control	11.3±5.6b	28.3±5.3b	6.0±2.3c
	C-methyl	43.7±26.4b	95.2±3.0a	90.1±4.3a
	Spinosad	100.0a	100.0a	58.9±3.1b
	Spinosad + C-methyl	100.0a	100.0a	75.8±11.8ab
December	Control	9.9±2.0c	40.4±4.7c	6.7±4.1b
	C-methyl	65.3±22.8b	92.2±4.0b	92.1±4.1a
	Spinosad	100.0a	100.0a	70.3±3.3a
	Spinosad + C-methyl	100.0a	100.0a	75.8±11.2a
January, 2003	Control	12.0±4.7c	24.4±5.1b	7.5±1.8c
	C-methyl	33.0±6.3b	98.1±1.9a	85.9±8.4a
	Spinosad	100.0a	61.2±25.4ab	49.4±4.0b
	Spinosad + C-methyl	100.0a	90.1±7.9a	73.0±13.4ab

Each mean is based on $n = 3$ bins. Within months and species, means among treatments followed by different letters are significantly different ($P < 0.05$; Fisher's protected lsd test).
[a]C-methyl = chlorpyrifos-methyl.

there were as many as 1431 *R. dominica*, 260 *C. ferrugineus*, and 513 *T. castaneum* adults in untreated grain. Less than two progeny, regardless of species, were observed in most of the jars containing grain treated with spinosad or spinosad + chlorpyrifos-methyl (Table 4). The sole exception occurred during the January 2003 bioassay when 7.7 *C. ferrugineus* adults were observed in the spinosad + -chlorpyrifos-methyl treatment. Chlorpyrifos-methyl provided good protection against progeny development of *C. ferrugineus* and *T. castaneum*, but progeny of *R. dominica* were observed in samples collected 3 months after storage. There were significant insecticide-treatment by month-of-storage interactions for *R. dominica* ($F = 6.2$; $df = 18, 54$; $P < 0.01$), *C. ferrugineus* ($F = 1.9$; $df = 18, 54$; $P = 0.03$), and *T. castaneum* ($F = 4.3$; $df = 18, 54$; $P < 0.01$). There were significant independent insecticide-treatment effects for all species (*R. dominica* $F = 935.7$; $df = 3, 54$; $P < 0.01$; *C. ferrugineus* $F = 56.9$; $df = 3, 54$; $P < 0.01$; *T. castaneum* $F = 826.8$; $df = 3, 54$; $P < 0.01$). Month-of-study effects were significant for *C. ferrugineus* ($F = 2.9$; $df = 6, 54$; $P = 0.02$) and *T. castaneum* ($F = 3.8$; $df = 6, 54$; $P = 0.01$), but not for *R. dominica* ($F = 1.4$; $df = 6, 54$; $P = 0.23$).

Each of the grain quality parameters was not statistically significant ($P > 0.05$) over time (statistical data not shown). Therefore, the zero-, three-, and 6-month grain quality data for each of the parameters were pooled across months (Table 5). The maximum count of insect-damaged kernels from chlorpyrifos-methyl treated bins was much greater than the remaining treatments, but heterogeneity among these counts resulted in lack of statistical differences in final grain quality. However, qualitative differences, especially with respect to insect-damaged kernels, were observed among treatments. For example, grain samples collected in January 2003 from an untreated bin and a bin treated with chlorpyrifos-methyl were officially labeled 'sample grade' (unfit for processing as human food) as a result of musty odors. The latter bin also had >32 insect-damaged kernels per 100 g. The United States Food and Drug Administration's Defect Action Level for

Table 4
Adult progeny (mean ± SE) of three stored-product insect species produced after 8 weeks on untreated (control) wheat or insecticide-treated wheat samples collected monthly from farm bins between July 2002 and January 2003

Month, Year	Treatment	Species		
		R. dominica	*C. ferrugineus*	*T. castaneum*
July, 2002	Control	1182.3 ± 22.8a	147.0 ± 6.0a	109.7 ± 11.5a
	C-methyl[a]	45.7 ± 30.8b	0.7 ± 0.7b	0.3 ± 0.3b
	Spinosad	0.3 ± 0.3c	1.0 ± 1.0b	2.7 ± 2.7b
	Spinosad + C-methyl	1.0 ± 1.0c	0.0b	0.0b
August	Control	1431.0 ± 205.4a	106.7 ± 26.8a	107.3 ± 19.0a
	C-methyl	85.0 ± 19.9b	0.3 ± 0.3b	1.0 ± 0.6b
	Spinosad	0.0c	1.7 ± 1.2b	0.0b
	Spinosad + C-methyl	0.0c	0.3 ± 0.3b	0.3 ± 0.3b
September	Control	293.3 ± 50.6a	93.0 ± 13.9a	64.3 ± 20.4[b]
	C-methyl	127.3 ± 33.0b	2.0 ± 1.5b	0.0
	Spinosad	1.0 ± 0.6c	1.3 ± 0.9b	0.0
	Spinosad + C-methyl	0.7 ± 0.3c	1.3 ± 0.9b	0.0
October	Control	141.0 ± 25.7a	39.3 ± 17.1a	62.0 ± 7.4a
	C-methyl	141.0 ± 27.9a	1.0 ± 0.6b	0.3 ± 0.3b
	Spinosad	0.0b	0.0b	0.0b
	Spinosad + C-methyl	0.3 ± 0.3b	1.3 ± 0.9b	1.3 ± 1.3b
November	Control	430.7 ± 67.1a	315.7 ± 148.5a	513.0 ± 67.2[c]
	C-methyl	282.3 ± 34.6a	14.3 ± 6.5b	0.0
	Spinosad	0.3 ± 0.3b	0.3 ± 0.3c	0.0
	Spinosad + C-methyl	0.3 ± 0.3b	0.0c	0.0
December	Control	807.3 ± 208.0a	210.3 ± 38.4a	326.0 ± 65.4a
	C-methyl	191.7 ± 118.9b	19.3 ± 13.5b	0.3 ± 0.3b
	Spinosad	0.0c	0.3 ± 0.3c	2.3 ± 1.9b
	Spinosad + C-methyl	0.3 ± 0.3c	0.0c	0.0b
January, 2003	Control	185.7 ± 21.6a	260.0 ± 108.3a	204.7 ± 52.4[d]
	C-methyl	137.3 ± 60.9a	18.7 ± 18.2b	0.0
	Spinosad	2.7 ± 0.3b	2.0 ± 1.5b	0.0
	Spinosad + C-methyl	1.7 ± 1.2b	7.7 ± 4.1b	0.0

Each mean is based on $n = 3$ bins. Within a single month and species, means among treatments followed by different letters are significantly different ($P < 0.05$; Fisher's protected lsd test).

[a] C-methyl = chlorpyrifos-methyl.

[b] $F = 153.69$; $df = 3, 8$; $P < 0.01$; one-way analysis of variance.

[c] $F = 2499.63$; $df = 3, 8$; $P < 0.01$; one-way analysis of variance.

[d] $F = 465.93$; $df = 3, 8$; $P < 0.01$; one-way analysis of variance.

Table 5
Individual wheat quality parameters (mean ± SE) for data pooled from samples collected immediately after grain treatment and after three and 6 months posttreatment

Quality factor	Treatment			
	Control	Chlorpyrifos-methyl	Spinosad	Spinosad + Chlorpyrifos-methyl
Dockage (%)	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.03
Test weight (kg/hl)	78.8 ± 0.5	77.8 ± 1.0	78.4 ± 0.8	78.4 ± 0.4
Moisture content (%)	11.2 ± 0.4	10.5 ± 0.3	11.1 ± 0.4	10.4 ± 0.1
Damaged kernels (%)	0.3 ± 0.1	0.9 ± 0.7	0.2 ± 0.1	0.1 ± 0.1
Foreign material (%)	0.01 ± 0.01	0.0 ± 0.0	0.0 ± 0.0	0.02 ± 0.01
Shrunken and broken kernels (%)	0.8 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	1.0 ± 1.0
Total defects (%)	1.1 ± 0.2	1.8 ± 0.6	1.0 ± 0.1	1.1 ± 0.1
Insect-damaged kernels (IDK)[a]	4.6 ± 1.9	23.6 ± 22.2	0.4 ± 0.2	0.6 ± 0.2
Grade[b]	1.4 ± 0.4	1.7 ± 0.4	1.3 ± 0.2	1.1 ± 0.1

Each mean is based on $n = 3$ bins.

[a] IDK are expressed as number per 100 g of wheat.

[b] Wheat in the United States is assigned a 1–5 grade, and wheat not meeting standards for grades 1–5 is considered sample grade.

insect-damaged kernels in wheat is 32 per 100 g. Wheat with more than 32 insect-damaged kernels will receive the 'sample grade' designation. None of the spinosad and spinosad + chlorpyrifos-methyl treated bins exceeded the Defect Action Level for insect-damaged kernels.

4. Discussion

At the application rate of 1 mg (a.i.)/kg, the actual residue on wheat receiving spinosad or spinosad + chlorpyrifos-methyl treatment was about 30% less, and one of the residues was 55% less than the application rate. A similar reduction in spinosad deposits was also observed by several researchers. For example, Flinn et al. (2004) reported spinosad residues in hard red winter wheat immediately after treatment at 1 mg/kg to be 12–60% less than the application rate of 1 mg/kg. Daglish and Nayak (2006), in a laboratory study, have shown 12% to 36% loss of spinosad immediately after treatment with 1 mg/kg. Plausible reasons for less than the intended deposition of spinosad on kernels could be attributed to uneven flow rate of the grain through the augers, variation in kernel-to-kernel transfer of applied insecticide during auguring, and variation in insecticide deposition due to differences in shape and size of individual kernels. The 55% decrease, or a residue of 0.45 mg (a.i.)/kg, observed in samples at three months posttreatment in spinosad + chlorpyrifos-methyl treatment could be a sampling artifact, because the spinosad residue at 6 months posttreatment from the same treatment was 0.62 mg (a.i.)/kg.

Although the actual spinosad residue in our study was less than the target rate of 1 mg (a.i.)/kg, the residues were stable on the grain and provided effective protection against insect infestation in farm bins for at least 6 months. Daglish and Nayak (2006) reported that spinosad residues were stable for 9 months on wheat, without loss of insecticidal activity against *R. dominica*—a devastating pest of stored wheat worldwide. Fang et al. (2002b) also showed that spinosad residues were persistent on wheat, without loss of insecticidal activity against *R. dominica* and *T. castaneum*, for 12 months. The total number of live adults in bins treated with spinosad or spinosad + chlorpyrifos-methyl never exceeded 0.5 insects/kg throughout the study, whereas the total numbers in the untreated wheat ranged from 0.5 to 22.1 insects/kg. The small numbers of insects in the spinosad + chlorpyrifos-methyl treatment is due to spinosad and not chlorpyrifos-methyl, because total live insect numbers in bins treated with chlorpyrifos-methyl started to increase 2 months after treatment from 0.6 insects/kg to a maximum of 11.4 insects/kg by the end of the study. Only half the labeled rate of chlorpyrifos-methyl was used, and this could also explain the presence of live insects in this treatment. The non-uniform infestation pressure across farm sites resulted in lack of significant differences among treatments, despite the small numbers of insects found in bins receiving spinosad, compared with the greater numbers found in untreated bins and bins receiving chlorpyrifos-methyl. Although there were no significant differences among insecticide treatments in the numbers of live insects, the presence of live insects in untreated bins and chlorpyrifos-methyl treated bins is unacceptable because at the time of sale, growers delivering grain with live insects may receive a substantial price discount (Reed et al., 1989; Kenkel et al., 1994).

Unlike our field study in which we relied on natural infestations, Flinn et al. (2004) artificially infested 30.7-metric ton bins (that were designed to prevent insect emigration and immigration) with three insect species, and reported essentially no live *R. dominica*, *T. castaneum* and *C. ferrugineus* during their 6-month field study. They showed significant differences in insect numbers among spinosad, aerated, and untreated bins. Controlled comparisons of treatment effectiveness in our study were addressed through laboratory bioassays, in which there were significant differences among grain treatments in 14-day mortality and 8-week progeny-production data of the three insect species tested. Regardless of month, the 14-day adult mortality bioassays showed *R. dominica* and *C. ferrugineus* adults to be highly susceptible to spinosad, relative to the other treatments, whereas *T. castaneum* was moderately susceptible. These findings are consistent with laboratory bioassay results reported on these species by Fang et al. (2002a), Fang and Subramanyam (2003), Toews and Subramanyam (2003), Huang et al. (2004), Flinn et al. (2004), and Nayak et al. (2005). Spinosad combined with chlorpyrifos-methyl did not produce additive mortality in any of the three species tested. Chlorpyrifos-methyl was consistently better than spinosad against *T. castaneum*. The reduced activity of chlorpyrifos-methyl against *R. dominica* and *T. castaneum* adults over time can be attributed to residue degradation as reported by Arthur et al. (1992) and Fleurat-Lessard et al., (1998). Despite degradation, chlorpyrifos-methyl and spinosad activity against *C. ferrugineus* adults over time was unaffected.

Progeny suppression of *R. dominica* and *C. ferrugineus* on grain treated with spinosad alone or spinosad + chlorpyrifos-methyl was 99–100%, irrespective of the month, and this could be attributed to susceptibility of adults and first instars because eggs of both species are laid outside kernels. Although adult *T. castaneum* were less susceptible to spinosad, the high susceptibility of first instars (Toews and Subramanyam, 2003) resulted in 98%–100% progeny suppression during the 6-month study. In general, progeny suppression of *C. ferrugineus* and *T. castaneum* on grain treated with chlorpyrifos-methyl was similar to that observed on grain treated with spinosad or spinosad + chlorpyrifos-methyl. The lack of effective progeny suppression of *R. dominica* on grain treated with chlorpyrifos-methyl, and an increase in progeny production over time, is related to reduced mortality of the adults observed in our 14-day mortality study. Reduced mortality of *R. dominica* on grain treated with chlorpyrifos-methyl also could be attributed to

resistance (Zettler and Cuperus, 1990; Arthur, 1992; Guedes et al., 1996).

Flinn et al. (2004) did not find significant differences in wheat quality parameters, except for insect-damaged kernels, which were significantly high in untreated grain, compared with spinosad-treated or aerated grain. In our study, none of the grain quality parameters was statistically significant among treatments and over time. We attribute this to non-uniform infestation pressure in our naturally infested grain bins. However, we did observe an increase in insect-damaged kernels in untreated grain and grain treated with chlorpyrifos-methyl (5 and 24 kernels per 100 g, respectively), in contrast to less than 1 kernel per 100 g in grain treated with spinosad alone or spinosad + chlorpyrifos-methyl. Despite being well below the official grading threshold for sample-grade wheat of 32 insect-damaged kernels per 100 g, wheat with >7 insect-damaged kernels will not be accepted by domestic flour mills (Kenkel et al., 1994).

In summary, our field and laboratory tests have shown that a single application of spinosad at 1 mg/kg to newly harvested wheat at the time of storage prevented insect infestation, especially that of *R. dominica*, for a period of 6 months. In January 2005, spinosad at 1 mg/kg was approved by the US-EPA for treatment of stored grain and seeds of corn, barley, millets (foxtail, proso, and pearl), oats, rice, sorghum (milo), triticale, wheat, birdseed, flower seeds, ornamental seeds, and grass seeds (Federal Register 2005, vol. 70, 1349–1357); however, commercial formulations of spinosad for grain use will not become available until international tolerances are in place. Spinosad is effective against *R. dominica* populations that are resistant to traditionally used grain protectants (Nayak et al., 2005). The recent approval of spinosad as a grain protectant in the US and proposed international tolerance at 1 mg/kg should provide farmers in the United States in general, and Kansas in particular, another tool for effective management of insects in their farm-stored wheat.

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