Persistence and Efficacy of Spinosad Residues in Farm Stored Wheat

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ABSTRACT Degradation and insecticidal effectiveness of spinosad residues were evaluated in Kansas during November 2000 to November 2001 in farm bins holding wheat (34-metric ton capacity). About 50 kg of hard red winter wheat from each of three bins were brought to the laboratory and treated separately with 1-ml aqueous suspensions of spinosad to provide rates of 0.1, 0.5, 1, 3, 6 mg (AI)/kg of wheat. Wheat treated with distilled water served as the control treatment. Untreated and spinosad-treated wheat samples (250 g each) were placed in three plastic pouches of two different mesh sizes, and buried 2.5 cm below the grain surface. Pouches with large mesh openings were used to monitor insect infestations and kernel damage in untreated and spinosad-treated samples. Pouches with small mesh were used for extracting spinosad residues and for conducting laboratory bioassays with adults of the lesser grain borer, *Rhyzopertha dominica* (F.) and red flour beetle, *Tribolium castaneum* (Herbst) at 28°C and 65% RH. Wheat temperature and relative humidity near the pouches during the 1 yr of storage ranged from −10 to 32°C and 50 to 70%, respectively. Moisture of wheat samples varied from 12.4 to 13%. Observed spinosad residues on wheat samples were 25% less than the calculated rates of 0.1 to 6 mg/kg. However, these residues were stable during the 1 yr of storage, and killed all *R. dominica* adults exposed for 14 d in the laboratory. Mortality of *T. castaneum* adults increased with an increase in spinosad rate. The linear regression slope of LD_{50}s (0.3–2.7 mg/kg) against storage time was not significantly different from zero, indicating no loss in spinosad toxicity to *T. castaneum* adults. Insect species, insect numbers, and kernel damage over time in wheat samples inside pouches with large mesh openings were highly inconsistent, and failed to accurately characterize spinosad performance. Laboratory bioassays with *R. dominica* and *T. castaneum* adults using grain from pouches with small mesh openings accurately gauged spinosad persistence and insecticidal activity under the field conditions.

KEY WORDS stored wheat, spinosad residues, efficacy assessment, stored-grain insects

SPINOSAD IS A BROAD-SPECTRUM bacterial insecticide that is currently registered on over 100 crops in the United States (Thompson et al. 2000). Spinosad is not, however, labeled for use on stored wheat. Laboratory tests have shown spinosad to be effective against key insect pests associated with stored wheat (Fang et al. 2002, Subramanyam et al. 2003). Promising insecticides for stored-wheat protection are needed because of the uncertain future of currently registered organophosphate wheat protectants malathion and chlorpyrifos-methyl under the 1996 Food Quality Protection Act (Anonymous 1997).

Spinosad applied to field crops generally loses activity after a week (Brunner and Doerr 1996, Liu et al. 1999), because spinosad degrades on exposure to sunlight (Saunders and Bret 1997). The stability and performance of spinosad applied to farm-stored wheat is unknown. In the United States, wheat in farm bins is typically stored for 3–9 mo (Martin et al. 1997). Therefore, any potential insecticide applied to wheat needs to provide protection from insect infestations for at least 9 mo. The objectives of our research were to determine the stability of spinosad residues on hard red winter wheat stored in farm bins for 1 yr and to verify activity against insects through field and laboratory bioassays.

Materials and Methods

Spinosad. Spinosad (NAF-85; Lot No. NF16160P12) was obtained from Dow AgroSciences, Indianapolis, IN, as a solution with 48% purity. Stock solutions and spinosad dilutions for wheat treatment were made in distilled water.

Insects. Insects used in tests were the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), and red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *R. dominica* was reared on whole, hard red winter wheat, and *T. castaneum* was reared on whole wheat flour + 5% yeast

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(by weight) at 28°C, 65% RH, and a photoperiod of 14:10 (L:D) h.

Wheat Treatment and Handling. Hard red winter wheat was purchased from the Manhattan Cooperative, Manhattan, KS. About 25 metric tons was placed per bin in three separate, 34-metric ton capacity bins of 5 m diameter at the Grain Storage and Training Center, Kansas State University, Manhattan, KS. From each bin, 50 kg of wheat was taken to the laboratory and held at −13°C in a freezer for 1 wk to kill any residual insect infestations. Wheat (1 kg) from each bin was treated with 1 ml of spinosad solution to obtain rates of 0.1, 0.5, 1, 3, or 6 mg (AI) spinosad per kilogram of wheat. Wheat (1 kg) treated with 1 ml distilled water served as the control treatment. Wheat treated with spinosad solution or distilled water was mechanically tumbled for 10 min in tempering drums. After tumbling, untreated and spinosad-treated wheat were placed in separate plastic pouches that had either large (2-mm^2) or small (0.6-mm^2) mesh openings. Each pouch measured 17.8 by 22.5 cm, and held 250 g of untreated or spinosad-treated wheat. Pouches were made by heat-sealing three of the four sides before adding wheat. The fourth side was sealed after adding wheat. The large mesh openings allowed entry of insect adults and larvae, while the small mesh openings prevented insect entry. Wheat in pouches with large mesh openings was used to monitor natural infestations in bins, while wheat in pouches with small mesh openings was used to analyze spinosad residues and to conduct laboratory bioassays with *R. dominica* and *T. castaneum* adults.

Pouches were placed in seven randomly chosen locations within each bin. At each location, one pouch with large mesh openings and two pouches with small mesh openings for each treatment, were placed horizontally 2.5 cm below the wheat surface. Each location had a total of 18 pouches. Pouches were placed just below the grain surface, because temperature and moisture fluctuations are greatest in this area (Jayas 1995). Two HOBO data-logging units (Onset Computer Corporation, Bourne, MA) were placed at each location to monitor wheat temperature and relative humidity of intergranular air. Pouches and HOBO units were placed in the bins in November 2000 and removed bimonthly from November 2000 through November 2001.

Insect species infesting the upper surface layers of wheat in each bin were determined by inserting 10 perforated plastic probe traps (Storgard WB II traps, Trécé, Salinas, CA) just below the wheat surface (Subramanyam et al. 1993). These unbaited traps capture insects moving randomly through the grain mass. Two traps were placed 30 cm apart near the bin center; four traps were placed near bin periphery in each cardinal direction and four were placed halfway between bin center and bin periphery. Traps were checked bimonthly between November 2000 and July 2001 and monthly between July and November 2001. Although *R. dominica* is an economically important insect pest of stored wheat (Reed et al. 1991, Vela-Coiffier et al. 1997), this species was not found in probe traps or large mesh pouches before August 2001. Therefore, the wheat surface of each bin was seeded with 2,000 laboratory-reared *R. dominica* adults weekly for 3 wk starting 24 August 2001.

Determining Spinosad Residues. Untreated or spinosad-treated wheat from one of the two small mesh pouches was sent to Dow AgroSciences, Indianapolis, IN, for residue analysis, while wheat in the other pouch was used for laboratory bioassays. At least 500 g was required for extracting spinosad residues from wheat. Therefore, for residue analysis untreated or spinosad-treated wheat samples in pouches from all three bins were pooled (750 g). Wheat samples were prepared by mixing with dry ice and grinding through an Agvise model 2001 Hammer mill (Agvise Laboratories, Northwood, ND) equipped with a 0.32-cm screen. After preparation, the samples were stored frozen in high-density polyethylene containers. Spinosyn A and spinosyn D residues were determined in all samples using the following analytical method (Hastings and Clements 2000). Samples were extracted with 80% acetonitrile/20% water. An aliquot of the extract from each sample was diluted with acetonitrile, and purified using strong cationic exchange, solid phase extraction plate (Jones Chromatography, Lakewood, CO). The spinosyns were eluted with a solution of 0.1 M ammonium acetate in acetonitrile: methanol. The analytes were then evaporated to dryness and reconstituted in acetonitrile:methanol:water (4:4:2 vol:vol:vol). Samples were analyzed by high performance liquid chromatography (HPLC). Analysis was performed using a YMC ODS AM column (YMC, Wilmington, NC) installed in a Agilent model 1100 HPLC (Agilent Technology, Wilmington, DE) coupled to a Model API 2000 mass spectrometer (Applied Biosystems, Foster City, CA) operating in the positive ion APCI ionization mode. The ions that were monitored during the analysis were m/z 732.6 and 746.6 for spinosyn A and spinosyn D, respectively. The limit of detection and limit of quantitation were 0.003 μg/g and 0.010 μg/g, respectively, for each of the analytes. The total spinosad residues, expressed as mg/kg, included residues of spinosyn A plus spinosyn D.

Evaluating Efficacy of Spinosad Residues. Natural infestations in November 2000 could not be determined in wheat from large mesh pouches because it represented time 0. Wheat from pouches sampled at bimonthly intervals between January and November 2001 were sifted over a 2.1-mm round-holed sieve to separate live and dead insects. All insects were separated by species and counted. Damage to kernels in each large mesh pouch was determined by examining 100 kernels. Kernels without germs and those with irregular or round holes in the endosperm due to feeding or adult emergence were considered damaged. The number of damaged kernels was expressed as a percentage of the total.

Untreated wheat or wheat treated with a given rate of spinosad from one small mesh pouch removed from a bin were separated into two, 100-g lots. Each lot was placed in a 150-ml plastic container with lid. A 10-

mm-diameter hole in the lid covered with a 0.6-mm^2 mesh permitted air diffusion. Wheat in containers was infested with 50 unsexed, 2- to 4-wk-old adults of *R. dominica* or *T. castaneum.* The containers were closed with lids after insect introduction and held at conditions used for insect rearing. Wheat was checked after 14 d to count live and dead adults. Mortality at each spinosad rate was calculated from the number of dead insects out of the total exposed. An additional 50 g of untreated wheat from pouches were used for determining kernel moisture using the Perten Single Kernel Characterization System (SKCS model 4100, Perten Instruments, Reno, NV) (Psotka 1999).

Data Analysis. Changes in temperature and relative humidity were similar among bins and within bin locations. Therefore, temperature or relative humidity data were pooled to show daily means throughout the year using the PROC MEANS procedure (SAS Institute 1988). Kernel moisture (mean ± SEM) provided by the SKCS instrument was based on processing 300 kernels. Wheat temperature, intergranular relative humidity, and kernel moisture data were plotted over time to show changes during the storage period. Kernel moisture changes were compared by constructing 95% CL around the means. Means with nonoverlapping 95% CL were considered significantly different ($P < 0.05$). The relationship between observed spinosad residues and expected spinosad rate for November 2000 samples was described by fitting a linear regression forced through the origin using the PROC REG procedure (SAS Institute 1988). Spinosad residues changed very little during the 1 yr of storage in bins. Therefore, residue data at each rate were fit to linear regressions. Regression slopes were tested for departure from 0 at the $\alpha = 0.05$ level (SAS Institute 1988).

Adults of stored-product insect species captured monthly in probe traps between July and November 2001 were expressed as mean ± SEM number of adults/trap/bin/30 d. Means of each insect species across all 5 mo were used to calculate insect relative abundance. Live and dead adults of each insect species recovered from large mesh pouches in July, September, or November 2001 were transformed to $\log(x + 1)$ scale and subjected to one-way analysis of variance (ANOVA) using the PROC GLM procedure (SAS Institute 1988) to determine differences among spinosad rates. Percentage of wheat kernels damaged in large mesh pouches were transformed as arcsine $(x)^{0.5}$ scale (Zar 1984) to stabilize hetereoscedastic variances. Kernel damage differences among rates in July, September, or November 2001 were determined by one-way ANOVA followed by Fisher protected least significant difference (LSD) test for separating means at the $\alpha = 0.05$ level (SAS Institute 1988).

Percent mortality of *R. dominica* or *T. castaneum* adults after 14 d of exposure to untreated and spinosad-treated wheat at each bimonthly interval were transformed to arcsine$(x)^{0.5}$ scale and subjected to one-way ANOVA using the PROC GLM procedure (SAS Institute 1988). Treatment means were separated using Fisher protected LSD test at the $\alpha = 0.05$ level. Mortality of *T. castaneum* adults at each bimonthly interval was rate dependent. Therefore, bimonthly data were corrected for mortality on untreated wheat (Abbott 1925) and subjected to probit analysis using the PROC PROBIT procedure (SAS Institute 1988) for estimating LD_{50}s. Loss in spinosad toxicity to *T. castaneum* adults during the 1 yr of storage was determined by linear regression of LD_{50}s against storage time using the PROC REG procedure (SAS Institute 1988). The regression slope was tested for departure from 0 at the $\alpha = 0.05$ level.

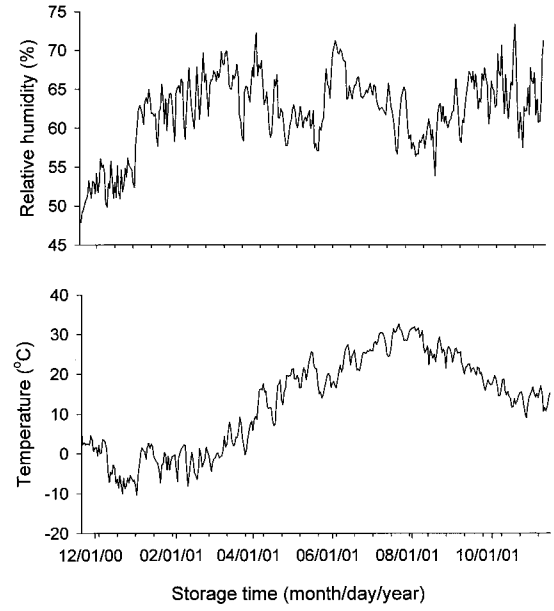

Fig. 1. Mean temperature and intergranular relative humidity near pouches buried 2.5 cm below the surface of wheat stored in farm bins from November 2000 to November 2001.

Results

Wheat Temperature, Relative Humidity, and Moisture. Temperatures 2.5 cm below the wheat surface at all sampling locations in bins ranged from –10°C (December 2000 and January 2001) to 32°C (July 2001). Relative humidity at these same locations mostly ranged from 55 to 70%. Relative humidity during December 2000 was lower (50–55%) than the other months (Fig. 1). Moisture content of wheat samples in pouches did not change significantly ($P > 0.05$) during the 1 yr of storage (Fig. 2).

Persistence of Spinosad Residues. Observed spinosad residues in November 2000 (beginning of the experiment) on wheat samples at the expected or calculated rates of 0.1, 0.5, 1, 3, and 6 mg/kg were 0.11, 0.49, 0.82, 2.8, and 4.17 mg/kg, respectively. The mean loss of spinosad residues across all rates was ≈25% (Fig. 3).

Observed spinosad residues at each rate changed very little during storage in farm bins (Fig. 4). The

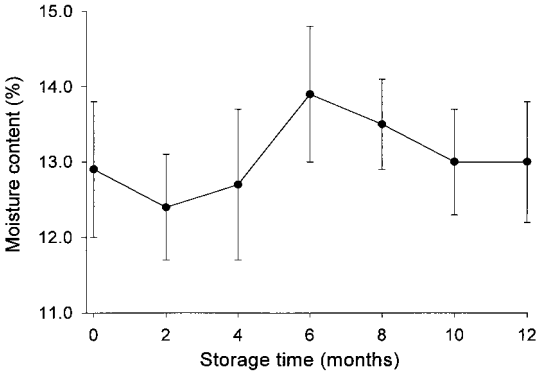

Fig. 2. Mean ± 95% CL moisture of untreated wheat samples in pouches from November 2000 to November 2001.

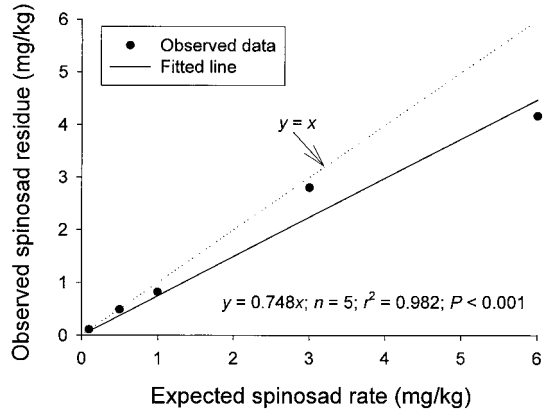

Fig. 3. Relationship between observed spinosad residues and expected spinosad rates in November 2000 wheat samples.

slope of the linear regression line for each spinosad rate against storage time was not significantly different from 0 ($P > 0.05$), indicating that the residues were stable throughout the year.

Insects in Probe Traps and Large Mesh Pouches. Stored-product insects were not found in probe traps and large mesh pouches during November 2000 to

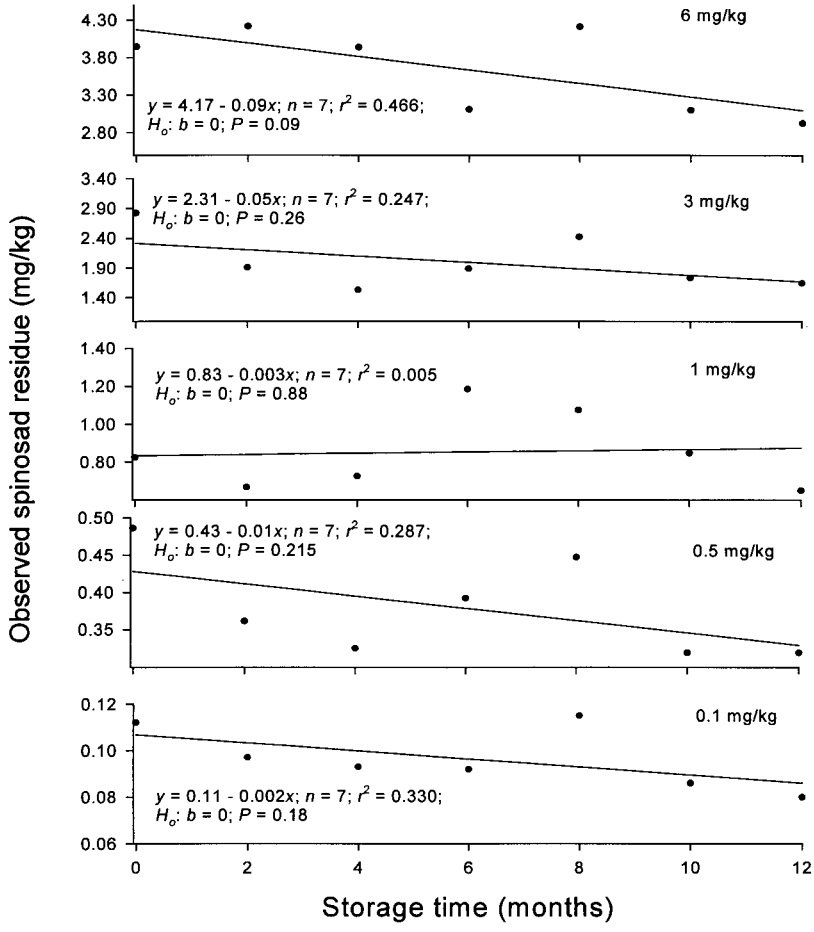

Fig. 4. Linear regressions showing stability of observed spinosad residues from November 2000 to November 2001 in farm-stored wheat. The top right corner of each graph shows the expected spinosad rate.

Table 1. Insects (mean ± SEM) captured by perforated probe traps in farm bins during 2001

Species	No. adults/trap/30 d						% of total
	Jul	Aug	Sept	Oct	Nov	Total	
T. stercorea	65.3 ± 27.4	1433.6 ± 617.6	266.0 ± 66.5	659.3 ± 320.1	302.9 ± 199.3	2727.0 ± 1094.8	60.3
P. interpunctella[a]	36.9 ± 13.9	398.9 ± 165.1	270.6 ± 134.1	36.8 ± 18.3	12.9 ± 8.6	756.0 ± 292.4	16.7
A. advena	103.8 ± 83.3	310.0 ± 30.2	156.0 ± 29.0	92.1 ± 39.1	58.6 ± 8.6	720.5 ± 107.7	15.9
O. surinamensis	23.8 ± 12.5	51.4 ± 20.5	22.0 ± 14.3	9.3 ± 7.7	12.4 ± 9.6	118.8 ± 36.8	2.6
C. ferrugineus	13.4 ± 4.5	35.4 ± 9.6	7.4 ± 1.5	15.7 ± 9.8	22.4 ± 15.2	94.3 ± 19.5	2.1
R. dominica	0.0 ± 0.0	12.9 ± 12.9	36.0 ± 36.0	18.6 ± 18.0	25.2 ± 25.2	92.7 ± 27.3	2.1
T. castaneum	2.5 ± 1.4	7.9 ± 4.0	0.9 ± 0.5	0.0 ± 0.0	0.5 ± 0.5	11.7 ± 2.6	0.3
Total	245.6 ± 103.5	2250.0 ± 475.8	758.9 ± 69.4	831.8 ± 315.2	434.8 ± 171.3	4521	

Each mean is based on three replications. Insects were not captured in probe traps between Nov 2000 and May 2001.
[a] Larvae only.

May 2001. Adults of seven stored-product insect species were captured in probe traps, whereas five insect species were found in pouches with large mesh openings from July to November 2001. A total of 4,521 insects was captured in probe traps. The hairy fungus beetle, *Typhaea stercorea* (L.), was the predominant species, followed by Indianmeal moth, *Plodia interpunctella* (Hübner), and foreign grain beetle, *Ahasverus advena* (Waltl) (Table 1). The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), *T. castaneum*, *R. dominica* and sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) constituted <3% of the total insects captured in traps. Trap captures in August 2001 were 3–9 times higher than captures in July, September, October, and November (Table 1). *R. dominica* adults were not captured in traps before August 2001. Seeding the grain surface with adults during August to September 2001 resulted in small numbers being captured in traps (13–93 adults/trap/30 d). Unlike probe traps, very few insects were found in untreated and spinosad-treated wheat samples enclosed in large mesh pouches (Table 2). Both live and dead insects were recovered from pouches, and differences in insect numbers on untreated and spinosad-treated wheat samples were not significant ($P > 0.05$). Insect damage to kernels was evident in July, September, and November 2001 wheat samples (Table 3). However, kernel damage among treatments was significantly different ($F = 3.68$; df = 5, 12; $P = 0.03$) only in July samples. For example, kernel damage at 0.5 to 6 mg/kg was significantly lower ($P < 0.05$) than damage at 0 mg/kg. About 8–20% of kernels from the September and November 2001 pouch samples were damaged.

Insect Mortality in Laboratory Bioassays. In laboratory bioassays, mortality of *R. dominica* adults on untreated wheat samples was 2–9%, but in March and November 2001 mortality was 43% and 21%, respectively, for reasons unknown. *R. dominica* mortality at bimonthly intervals was consistently 99–100% on spinosad-treated wheat (Table 4). *T. castaneum* mortality

Table 2. Insects (mean ± SEM) recovered from pouches with large mesh openings during 2001

Month	Spinosad rate (mg/kg)	No. adults/pouch/61 d[a]						
		P. interpunctella[b]		*R. dominica*		*C. ferrugineus*[c]	*S. oryzae*[d]	*T. stercorea*[c]
		Live	Dead	Live	Dead	Live	Dead	Live
Jul	0.0	6.1 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.1	1.3 ± 1.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.5	0.6 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0
	1.0	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	3.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	6.0	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0
Sept	0.0	1.3 ± 1.3	4.8 ± 3.1	1.9 ± 1.9	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
	0.1	1.9 ± 1.9	9.4 ± 6.7	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.5	6.5 ± 5.1	3.9 ± 3.9	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	3.9 ± 3.4
	1.0	0.3 ± 0.3	7.4 ± 3.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	3.0	1.9 ± 1.9	2.6 ± 1.7	0.0 ± 0.0	0.3 ± 0.3	0.6 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
	6.0	1.9 ± 1.9	4.5 ± 4.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Nov	0.0	0.0 ± 0.0	4.1 ± 2.5	0.0 ± 0.0	0.4 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.1	3.7 ± 3.1	7.1 ± 7.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.5	0.0 ± 0.0	0.8 ± 0.8	0.0 ± 0.0	1.2 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	1.0	0.4 ± 0.4	1.7 ± 1.7	0.0 ± 0.0	3.7 ± 3.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	3.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.1 ± 3.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	6.0	0.4 ± 0.4	3.7 ± 3.1	1.2 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Each mean is based on three replications. For each month and species, live or dead insects among rates were not significant (F among species = 0.47-2.10; df = 5, 36; $P = 0.795$-0.088; one-way ANOVA).
[a] Insects were not found in pouches between November 2000 and May 2001.
[b] Larvae only.
[c] Dead insects were not found in pouches.
[d] Live insects were not found in pouches.

Table 3. Kernel damage (mean ± SEM) to wheat samples in pouches with large mesh openings during 2001

Spinosad rate (mg/kg)	Kernel damage/61 d (%)[a]		
	Jul	Sept[b]	Nov[c]
0.0	6.0 ± 2.1a	16.3 ± 5.6	20.3 ± 15.3
0.1	3.0 ± 1.5ab	18.3 ± 9.2	11.7 ± 10.2
0.5	1.7 ± 1.7b	19.3 ± 9.9	18.3 ± 11.3
1.0	0.0 ± 0.0b	7.7 ± 6.2	11.7 ± 9.2
3.0	0.3 ± 0.3b	11.0 ± 5.5	15.7 ± 13.2
6.0	0.0 ± 0.0b	9.0 ± 4.6	12.0 ± 9.6

Each mean is based on three replications. For Jul; means among rates followed by different letters are significantly different ($P < 0.05$; Fisher's protected LSD).

[a] Damage to wheat kernels was not evident in samples removed before July 2001.

[b] Kernel damage among rates was not significant ($F = 0.36$; df = 5, 12; $P = 0.868$; one-way ANOVA).

[c] Kernel damage among rates was not significant ($F = 0.14$; df = 5, 12; $P = 0.978$; one-way ANOVA).

on untreated wheat was ≤4% (Table 4). Mortality of *T. castaneum* adults at 1–6 mg/kg was significantly higher ($P < 0.05$) than at 0 and 0.1 mg/kg. Mortality was 75–100% at ≥3 mg/kg. On spinosad-treated wheat, *T. castaneum* mortality increased with the spinosad rate. The LD_{50}S ranged from 0.3 to 1.3 mg/kg during most months, but was twice as high (2.7 mg/kg) in July 2001 bioassays (Table 5). Probit regression intercepts ranged from –3 to 1.8 and the slopes from 2.4 to 3.2. The chi-square values for January, March, and July 2001 were not significant ($P > 0.2$), indicating adequate fit of data to the probit model. The significant chi-square values ($P < 0.05$) for the remaining four probit regressions indicated heterogeneous responses of *T. castaneum* on spinosad-treated wheat samples. The use of unsexed adults of mixed ages (2–4 wk) perhaps contributed to this heterogeneity. Despite a slight increase in LD_{50} with storage time, there was no significant loss in spinosad toxicity to *T. castaneum* adults during the 1 yr of storage (Fig. 5), as evidenced by a nonsignificant slope of the linear regression of LD_{50}S against storage time ($P > 0.05$).

Table 4. Mortality (mean ± SEM) of *R. dominica* and *T. castaneum* adults exposed for 14 d to untreated and spinosad-treated wheat samples removed from farm bins at bimonthly intervals from November 2000 to November 2001

Month	Spinosad rate (mg/kg)	Mortality (%)	
		R. dominica	*T. castaneum*
Nov 2000	0.0	9.0 ± 1.0b	0.0 ± 0.0f
	0.1	100.0 ± 0.0a	5.4 ± 1.8e
	0.5	100.0 ± 0.0a	29.8 ± 0.2d
	1.0	100.0 ± 0.0a	79.5 ± 5.6c
	3.0	100.0 ± 0.0a	94.0 ± 3.5b
	6.0	100.0 ± 0.0a	100.0 ± 0.0a
Jan 2001	0.0	2.0 ± 2.0b	4.0 ± 3.1d
	0.1	100.0 ± 0.0a	6.6 ± 1.8d
	0.5	100.0 ± 0.0a	43.8 ± 2.5c
	1.0	100.0 ± 0.0a	84.7 ± 2.4b
	3.0	100.0 ± 0.0a	99.3 ± 0.7a
	6.0	100.0 ± 0.0a	100.0 ± 0.0a
Mar 2001	0.0	43.3 ± 8.8b	3.3 ± 1.8c
	0.1	100.0 ± 0.0a	10.0 ± 8.1c
	0.5	100.0 ± 0.0a	82.7 ± 95.3b
	1.0	100.0 ± 0.0a	95.3 ± 0.7ab
	3.0	100.0 ± 0.0a	100.0 ± 0.0a
	6.0	100.0 ± 0.0a	100.0 ± 0.0a
May 2001	0.0	3.3 ± 1.8b	0.7 ± 0.7d
	0.1	100.0 ± 0.0a	0.7 ± 0.7d
	0.5	100.0 ± 0.0a	28.7 ± 12.8c
	1.0	100.0 ± 0.0a	87.3 ± 2.4b
	3.0	100.0 ± 0.0a	92.0 ± 2.3b
	6.0	100.0 ± 0.0a	100.0 ± 0.0a
Jul 2001	0.0	2.2 ± 1.3b	0.0 ± 0.0d
	0.1	99.3 ± 0.7a	0.0 ± 0.0d
	0.5	100.0 ± 0.0a	2.7 ± 1.8cd
	1.0	100.0 ± 0.0a	18.4 ± 10.5c
	3.0	100.0 ± 0.0a	50.1 ± 10.0b
	6.0	100.0 ± 0.0a	80.3 ± 11.3a
Sept 2001	0.0	0.7 ± 0.7b	0.7 ± 0.7d
	0.1	100.0 ± 0.0a	0.7 ± 0.7d
	0.5	100.0 ± 0.0a	10.1 ± 3.1c
	1.0	100.0 ± 0.0a	59.7 ± 6.6b
	3.0	99.4 ± 0.6a	75.3 ± 8.6b
	6.0	100.0 ± 0.0a	98.0 ± 1.2a
Nov 2001	0.0	20.9 ± 4.0b	2.0 ± 1.2d
	0.1	100.0 ± 0.0a	1.3 ± 1.3d
	0.5	100.0 ± 0.0a	8.5 ± 3.8d
	1.0	100.0 ± 0.0a	51.5 ± 5.7c
	3.0	100.0 ± 0.0a	79.5 ± 6.1b
	6.0	100.0 ± 0.0a	98.6 ± 1.4a

Each mean is based on three replications. For each month and species, means among rates followed by different letters are significantly different ($P < 0.05$; Fisher's protected LSD).

Discussion

Spinosad residues did not degrade significantly under Kansas farm conditions, based on the residue analysis and their consistent performance against *R. dominica* and *T. castaneum* in laboratory bioassays. Less than 25% deposition of spinosad on wheat samples is similar to that found when applying organophosphate protectants to stored grain. Arthur et al. (1992) reported 27% less chlorpyrifos-methyl on stored wheat treated at the calculated rate of 6 mg/kg. Bengston et al. (1983) found 25% less residue of chlorpyrifos-methyl 1 wk after treating wheat at a rate of 10 mg/kg. Errors in preparing spinosad dilutions, application, mixing, coverage on kernels, and recovery during extraction may account for the loss observed.

Both the grain temperature and intergranular relative humidity varied throughout the storage yr, but moisture of wheat samples in pouches was very stable. There were no significant loss of spinosad residues despite wide variation in temperature (−10–32°C) and relative humidity (50–70%). Spinosad was reported to degrade quickly under sunlight (Brunner and Doerr 1996, Saunders and Bret 1997, Liu et al. 1999). Our wheat samples in mesh pouches were buried under the grain surface, and therefore, not directly exposed to sunlight. This explains lack of significant degradation of spinosad residues. Laboratory bioassays against *R. dominica* and *T. castaneum* adults showed that there was also no significant loss of efficacy during 1 yr of storage.

In our study, spinosad-treated wheat samples of 12–13% moisture were exposed to temperatures ≤32°C. Under these conditions, spinosad appears to be more stable on grain than does chlorpyrifos-methyl. The half-life of chlorpyrifos-methyl on 11.9% moisture

Table 5. Probit regression estimates for *T. castaneum* exposed for 14 d to spinosad-treated wheat samples removed bimonthly from November 2000 to November 2001

Month	Storage time (months)	Slope ± SEM	Intercept ± SEM	LD_{50} (95% CL) (mg/kg)	χ^2 (df)	*P*
Nov 2000	0	2.4 ± 0.4	0.5 ± 0.2	0.6 (0.3–1.0)	13.9 (3)	0.003*
Jan 2001	2	3.1 ± 0.3	0.9 ± 0.1	0.5 (0.5–0.6)	3.7 (3)	0.290
Mar 2001	4	3.2 ± 0.3	1.8 ± 0.2	0.3 (0.2–0.3)	1.0 (3)	0.800
May 2001	6	3.0 ± 0.8	0.5 ± 0.3	0.7 (0.1–1.6)	30.0 (3)	0.000*
Jul 2001	8	2.4 ± 0.2	−1.0 ± 0.1	2.7 (2.4–3.2)	2.0 (3)	0.567
Sept 2001	10	2.5 ± 0.6	−0.2 ± 0.2	1.2 (0.4–2.6)	23.8 (3)	0.000*
Nov 2001	12	2.9 ± 0.5	−3.0 ± 0.2	1.3 (0.7–2.1)	13.7 (3)	0.003*

Mortality of adults on spinosad-treated wheat was corrected for mortality on untreated wheat (0 to 4%). Probit regression estimates for each bimonthly bioassay were based on five spinosad rates and a total of 750 *T. castaneum* adults. * Chi-square values for goodness-of-fit of data to the probit model were significant ($P < 0.05$).

wheat stored at 30°C was 19 wk (Desmarchelier and Bengston 1979). Arthur et al. (1992) reported the half-life of chlorpyrifos-methyl applied to wheat stored at 30°C and 11.2, 12.1 and 13.7% moisture to be 8.9, 12.1, and 6.7 wk, respectively. Desmarchelier and Bengston (1979) and Arthur et al. (1992) also found an increase in degradation of chlorpyrifos-methyl with an increase in grain temperature. Further work is needed to determine the stability of spinosad residues on >13% moisture wheat at temperatures >32°C.

Temperatures from November 2000 to May 2001 (<10°C) were too low for insect activity. Therefore, insects were not captured in traps during this period. Several insect species were found in traps from May through November 2001. However, very few insect species and numbers were found in large mesh pouches. The recovery of very few insects in large mesh pouches is not surprising. The large mesh openings allow insects to freely move in and out of the pouches. In addition, spinosad-treated grain may have been avoided by the insects. Kernel damage among rates (including the control treatment) was not significant in September and November 2001 samples, but was significant for the July 2001 samples. All damaged kernels were devoid of germs. This type of damage is generally caused by *P. interpunctella* larvae (Madrid and Sinha 1982), which were found in greater numbers than other insects in large mesh pouches.

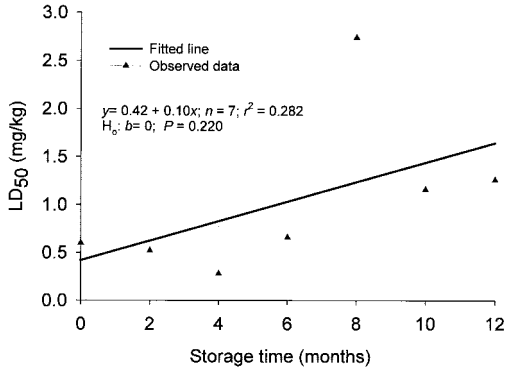

Fig. 5. Linear regression showing insignificant loss of spinosad toxicity to *T. castaneum* adults as a function of storage time (November 2000 to November 2001).

Spinosad is effective against *P. interpunctella* larvae at 1 mg/kg (Fang et al. 2002). However, spinosad does not kill insects rapidly (Adán et al. 1996, Brunner and Doerr 1996, Wanner et al. 2000), and therefore, damage was caused probably by *P. interpunctella* larvae before they died. The lack of significant numbers of live or dead insects in pouches, and failure to detect consistent differences in insect numbers or kernel damage among treatments, suggest that the use of pouches is undesirable for determining the protective effect of spinosad in farm-stored grain.

The performance of spinosad was consistent against both beetle species tested in the laboratory during the 1 yr of storage. However, spinosad is more effective against *R. dominica* than *T. castaneum* (Fang et al. 2002, Subramanyam et al. 2003). Spinosad killed all exposed *R. dominica* within 7 d at 0.1 mg/kg (Fang et al. 2002). In our study, similar results were observed. *T. castaneum* is more tolerant to spinosad and high mortality was generally achieved at 3–6 mg/kg (Fang et al. 2002, Subramanyam et al. 2003).

In summary, spinosad residues were stable and the efficacy against *R. dominica* and *T. castaneum* adults was consistent over a period of 1 yr when stored in farm bins under Kansas conditions. Effectiveness of spinosad against *R. dominica* and other key stored-product insects (Fang et al. 2002, Subramanyam et al. 2003) and its stability in farm-stored wheat make it a promising insecticide for stored-product protection.

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