Comparison of Aeration and Spinosad for Suppressing Insects in Stored Wheat

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J. Econ. Entomol. 97(4): 1465–1473 (2004)

ABSTRACT Field studies were conducted from July 2002 to January 2003 for evaluating the effects of controlled aeration and a commercial biological insecticide, spinosad, in suppressing insect populations in stored wheat. Six cylindrical steel bins were filled with newly harvested (2002 crop year) hard red winter wheat on 9 and 10 July 2002. Each bin contained 30.7 metric tons (1,100 bu) of wheat. Wheat in two bins was left untreated (control), whereas wheat in two bins was treated with spinosad, and in another two bins was subjected to aeration by using aeration controllers. Spinosad was applied to wheat at the time of bin filling to obtain a rate of 1 mg ([AI])/kg. Aeration controllers were set to run the fans when ambient air temperature fell below 23.9, 18.3, and 7.2°C for the first, second, and third cooling cycles, respectively. We added 400 adults each of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); lesser grain borer, *Rhyzopertha dominica* (F.); and red flour beetle, *Tribolium castaneum* (Herbst), to the grain at monthly intervals between July and October 2002. Insect density in the bins was estimated monthly by taking 3-kg grain samples from 21 locations within each bin by using a pneumatic grain sampler. No live *T. castaneum* or *C. ferrugineus* and very low densities of *R. dominica* (<0.008 adults per kilogram) were found in wheat treated with spinosad during the 6-mo sampling period. Density of *C. ferrugineus* and *T. castaneum* in aerated bins did not exceed two adults per kilogram (the Federal Grain Inspection Service standard for infested wheat), whereas *R. dominica* increased to 12 adults per kilogram in November 2002, which subsequently decreased to three adults per kilogram in January 2003. In the untreated (control) bins, *R. dominica* density increased faster than that of *C. ferrugineus* or *T. castaneum*. Density of *R. dominica* peaked at 58 adults per kilogram in October 2002 and decreased subsequently, whereas *T. castaneum* density was 10 adults per kilogram in October 2002 but increased to 78 adults per kilogram in January 2003. Density of *C. ferrugineus* increased steadily during the 6-mo study period and was highest (six adults per kilogram) in January 2003. This is the first report comparing the field efficacy of spinosad and aeration in managing insects in farm bins. Our results suggest that spinosad is very effective in suppressing *R. dominica*, *C. ferrugineus*, and *T. castaneum* populations in stored wheat.

KEY WORDS *Cryptolestes ferrugineus*, *Tribolium castaneum*, *Rhyzopertha dominica*, stored grain

SPINOSAD IS A COMMERCIAL BACTERIAL insecticide that is registered for use on >100 crops in 24 countries, including the United States (Thompson et al. 2000). Several laboratory studies and one field study have shown spinosad to be highly effective in controlling insects associated with stored wheat (Fang et al. 2002a,b; Toews and Subramanyam 2003; Toews et al. 2003). In field crops, spinosad loses activity after a week (Brunner and Doerr 1996) due to breakdown caused by UV radiation from sunlight (Saunders and Bret 1997). In farm bins, where most of the wheat is not exposed to sunlight, spinosad degraded very little during 12 mo of storage without appreciable loss of insecticidal activity against the lesser grain borer, *Rhyzopertha dominica* (F.), and red flour beetle, *Tribolium castaneum* (Herbst) (Fang et al. 2002b).

Spinosad is presently not registered for use on stored wheat, but an experimental use permit for treating grain at 1 mg ([AI])/kg was approved by the U.S. Environmental Protection Agency on 31 May 2002 (EPA Experimental Use Permit No. 62719-EUP-50). Evaluation of spinosad as a candidate stored grain protectant is timely, because the future of the organophosphate chlorpyrifos-methyl (Reldan), currently approved for use on stored wheat, remains uncertain under the 1996 Food Quality Protection Act (Anonymous 1997). Furthermore, *R. dominica*, a destructive pest of stored wheat in Kansas and neighboring states (Reed et al. 1991, Vela-Coiffier et al. 1997) is resistant to chlorpyrifos-methyl (Zettler and Cuperus 1990).

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In most of the United States, controlled aeration is effective in suppressing insect population growth in bins of stored wheat (Flinn et al. 1997, Reed and Harner 1998, Arthur and Flinn 2000). This method uses a simple thermostat controller that turns on an electrical fan. The fan pushes air through the grain mass when conditions are appropriate for cooling the grain. The predominant beetle species that infest stored wheat fail to develop and reproduce at temperatures below 15°C (Fields and Muir 1996). The sooner wheat can be cooled after storage, the slower insects will develop and reproduce in the grain. Unaerated grain also will cool in the fall, but the core of grain will remain warm during much of the winter. This allows insects to develop and reproduce continuously in such warm areas, leading to major losses in grain quality.

A field test was conducted between July 2002 and January 2003 in bins holding stored wheat to evaluate the effectiveness of controlled aeration and spinosad in suppressing insect populations. The 6-mo study was conducted because in the northern plains of the United States, most wheat is harvested around July and is stored on-farm for 3–9 mo (Martin et al. 1997). Therefore, spinosad or aeration should be effective in controlling insects during this period. Insect populations in unaerated bins generally reach high levels under Kansas weather conditions (Flinn et al. 1997, Arthur and Flinn 2000). Our objectives were to determine whether spinosad was a viable product for use on wheat stored in unaerated bins and to compare spinosad with controlled aeration.

Materials and Methods

Grain Treatment. Six cylindrical steel bins (4.72 m in diameter by 3.35 m in height at the eaves) were used in the study. The bins had concrete floors with "Y" tube aeration ducts. The bins were thoroughly cleaned of grain dust and debris by using a vacuum cleaner and brooms. All seams at the eaves were sealed with silicone caulk and bin roof vents and aeration fans were covered with a wire mesh screen (177-μm openings) to prevent insects from entering or leaving the bins. The three treatments (untreated wheat [control], spinosad, and aeration) were randomly assigned to the six bins. Four of the six bins (control and aeration bins) were filled with newly harvested (2002) hard red winter wheat on 9 and 10 July 2002. The grain temperature and moisture at the time of storage were 36°C and 10.8%, respectively. Wheat added to the remaining two bins (on 10 July 2002) was treated with spinosad (SpinTor 2 SC of 240 mg([AI])/ml purity, Dow AgroSciences, Indianapolis, IN) to obtain a deposit level of 1 mg ([AI])/kg on the wheat kernels. Wheat was treated with spinosad diluted in water as it was augered into the bin. The amount of water used was comparable with that used for chlorpyrifos-methyl, an organophosphate approved for use on stored wheat (Sloderbeck et al. 2002). Each bin contained ≈30.7 metric tons (1,100 bu) of grain (grain depth ≈2.5 m). After filling, the grain in each bin was leveled. In the two bins receiving the aeration treatment, aeration controllers were set to run the fans when ambient air temperature fell below 23.9, 18.3, and 7.2°C for the first, second, and third cooling cycles, respectively. The fans generated an airflow rate of 0.0026 $m^3/s/m^3$ through the grain mass. This is a normal rate used to cool wheat during fall storage. Each cooling cycle took ≈100–120 h of fan time to move the cooling front through the grain mass. When one cooling cycle was completed, the control was manually set to the next lower temperature threshold.

Grain temperatures were monitored using cables inserted into the grain mass at the center of the bin, and at a distance of 0.61 m from the side wall in each of the four cardinal directions by using five HOBO H8 four-channel data loggers, each connected to four TCMx-HA sensors (Onset Computer Corporation, Bourne, MA). The sensors in each bin were inserted into the grain mass at depths of 0.15, 0.81, 1.45, and 2.11 m from the grain surface.

Insects. To simulate natural insect immigration that occurs during the summer under Kansas weather conditions, we added 400 adults each of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); *R. dominica;* and *T. castaneum* monthly between 16 July and 8 October 2002 to each bin. These are the three most common insect pests found in wheat stored on farms in Kansas (Reed et al. 1991). Unsexed adults of mixed ages were used to infest the wheat in bins. Insects were obtained from cultures maintained at Kansas State University in the Department of Grain Science and Industry. The rearing procedures for these species have been described in detail by Fang et al. (2002a). All insect cultures were reared at 30°C, 65% RH, and a photoperiod of 14:10 h. Adults were placed in 0.45-liter glass jars and then were gently and evenly sprinkled onto the grain surface.

Grain Sampling. The grain in each of the bins was sampled for insects at monthly intervals starting 16 July 2002 by using a pneumatic grain sampler (Probe-A-Vac, Cargill, Minneapolis, MN). Seven 3-kg samples in each of the three 0.8-m layers of wheat were taken as follows: at three points 0.3 m from the bin center and at four points 0.6 m from the bin wall. In total, 21 samples (3 kg each) were taken from each bin. An additional grain sample for quality assessment and extracting spinosad residues was obtained in each bin by taking one continuous 3-kg sample as the probe was pushed down from the grain surface to the bottom of the bin. All of the samples, except the grain quality and residue samples, were processed once with an Insectomat, a motorized inclined sieve (Samplex LTD, Willow Park, United Kingdom), to separate insects from the grain. The number of live adult insects in each sample was counted immediately after extraction.

Grain Quality. A 1-kg sample was submitted to the Kansas Grain Inspection Service in Topeka, KS, for grain quality assessment. Grain quality parameters (dockage, test weight, moisture, total damage, foreign material, insect damaged kernels, broken, and de-

fects) were assessed following official inspection procedures (GIPSA 1997).

Spinosad Residue Analysis. Samples (1.5 kg) of wheat taken in July and October 2002 and January 2003 were sent to Dow AgroSciences for analysis of spinosad residues. Spinosad residues were extracted using analytical methods developed by Hastings and Clements (2000) that were described in detail in a recent article (Fang et al. 2002b).

Laboratory Bioassays. After insect extraction, the three samples taken near the bin center at each depth were pooled for each of the three layers. Similarly, the four samples taken near the bin periphery at each depth were pooled for each of the three layers. Therefore, on each sampling occasion we obtained six samples from each bin. Wheat samples from each of the six locations were divided into six 100-g lots, and each 100-g lot was placed in a 0.45-liter glass jar. Three of the jars were used for conducting bioassays with adults of *C. ferrugineus*, *R. dominica*, and *T. castaneum*, whereas three additional jars were used for conducting bioassays with eggs of *R. dominica*, *T. castaneum*, and the Indianmeal moth, *Plodia interpunctella* (Hübner). Eggs of *C. ferrugineus* were not included in the bioassays because it was difficult to collect the eggs necessary for conducting bioassays. Furthermore, a rate of 0.5 mg/kg is sufficient to kill all adults and completely suppress progeny production of *C. ferrugineus* (Bh.S., unpublished data). Adults for bioassays were obtained from cultures at the USDA–ARS Grain Marketing and Production Research Center. Eggs were obtained from cultures maintained at the Kansas State University's Department of Grain Science and Industry. Eggs for *R. dominica* and *T. castaneum* were collected by placing 100 adults of each species in separate 0.45-liter glass jars ($n = 20$ jars for each species) containing wheat flour that was initially passed through a wire mesh sieve with 177-μm openings. After 2 d, the same sieve was used to extract eggs from the flour. Eggs retained on top of the sieve were gently placed in 9-cm-diameter glass petri dishes.

Wheat in glass jars was infested with 20 adults or 40 eggs of a species. After infestation, jars were closed with lids fitted with filter paper and wire mesh screen and incubated at 27°C and 60% RH for adults and at 28°C and 65% RH for immatures. Wheat infested with adults was examined after 14 d to count the number of live and dead adults. Wheat infested with eggs of *T. castaneum* and *P. interpunctella* was checked 21 d later to count the number of live larvae. Wheat infested with *R. dominica* eggs was checked after 49 d to count the number of live adults that emerged from eggs because *R. dominica* larvae and pupae complete development inside the kernels (Potter 1935). Adult mortality was determined by the number of dead adults out of the 20 original adults. In tests with eggs, mortality was expressed as the number of dead larvae (40 eggs − number of live larvae) out of the 40 original eggs. *R. dominica* mortality was based on number of adults that failed to emerge from the 40 original eggs added to wheat in each glass jar.

Data Analysis. The general linear model (GLM) (SAS Institute 1988) was used to compare treatment effects on insect density by date, and treatment effects on grain quality by date. Means were compared using Fisher's protected least significant difference (LSD) test. The insect bioassay data (proportion dead, x) were transformed using an arcsine $(x)^{0.5}$ transformation to stabilize heteroscedastic variances. Insect mortality differences by date, location, and depth were determined for each species separately using the GLM procedure followed by Fisher's protected LSD for separating means (SAS Institute 1988). After pooling mortality data for the three depths and the two locations at each depth, comparisons were made by examining treatment differences by species for each sample date using the GLM procedure and Fisher's protected LSD test (SAS Institute 1988). Spinosad residue data from the two bins were subjected to one-way analysis of variance (ANOVA) (SAS Institute 1988) to determine effects of sampling date, location, and depth on residue levels. All treatment comparisons were considered significant at the $\alpha = 0.05$ level.

Results

Insect Suppression in Bins. Insects were not found in any of the wheat samples taken immediately after the bins were filled, which indicated that the wheat was not infested at the beginning of the study. In bins with spinosad-treated wheat, insect populations were very low compared with the control or aerated bins (Fig. 1). Insect density in wheat treated with spinosad was significantly less ($P < 0.05$) than insect density in the control bins for all sample dates after August. No live *T. castaneum* or *C. ferrugineus* were found in any of the bins with spinosad-treated wheat during the entire 6-mo sampling period. Throughout the study, only very low densities of *R. dominica* (<0.008 adults per kilogram) were found in the bins with spinosad-treated wheat.

Insect densities in bins of wheat subjected to controlled aeration were also low, especially for *C. ferrugineus* and *T. castaneum*, which did not exceed two adults per kilogram of wheat during the 6 mo of storage (Fig. 1). By January, the density of *C. ferrugineus* and *T. castaneum* dropped to <0.1 adult per kilogram of wheat in the aerated bins. *R. dominica* density was not as low as the other two species in the aerated bins. This species reached a density of 12 adults per kilogram of wheat on 5 November; however, by January the density was only three adults per kilogram of wheat.

In the control bins, *R. dominica* density increased faster than that of *C. ferrugineus* or *T. castaneum* (Fig. 1). Interestingly, *R. dominica* reached its peak density of 58 adults per kilogram of wheat in October, but decreased in subsequent months. *T. castaneum* reached a density of 10 adults per kilogram in October and then continued to increase to a density of 78 adults per kilogram on the final sampling date (8 January). The density of *C. ferrugineus* was only six adults per kilogram of wheat by January.

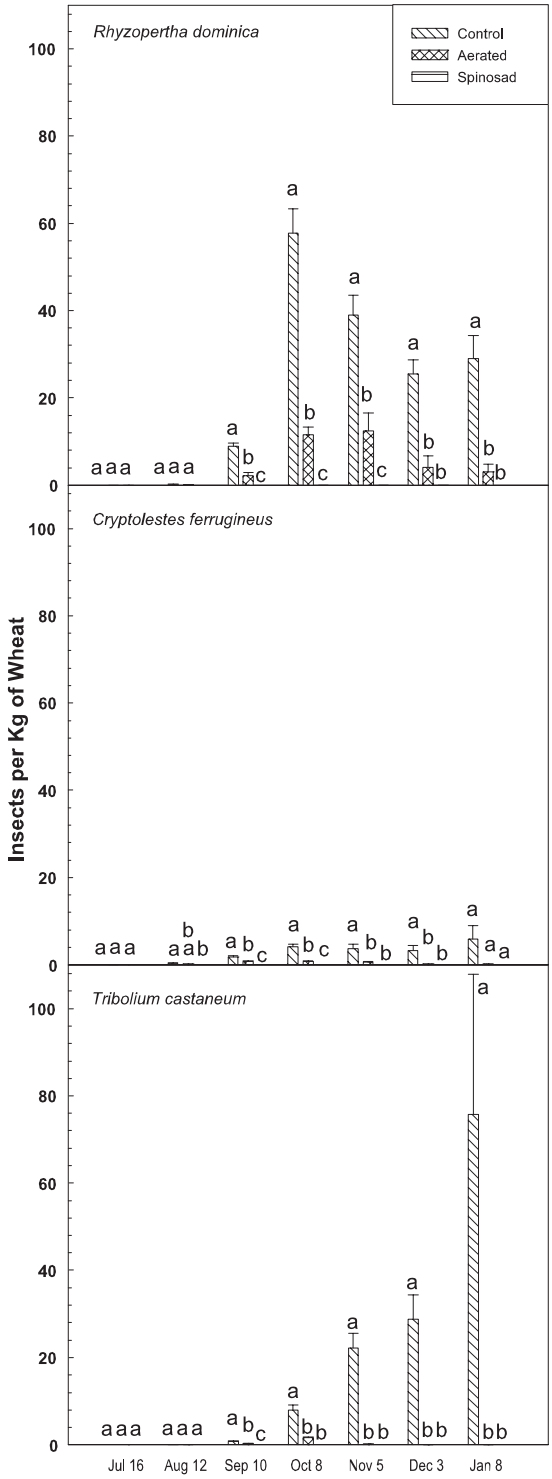

Fig. 1. Seasonal changes in density of *R. dominica*, *C. ferrugineus*, and *T. castaneum* adults in 30.7-metric ton (1,100-bu) bins of stored wheat treated with either spinosad, controlled aeration, or no treatment (control). Vertical bars indicate standard errors.

Temperature Changes in Bins. Grain temperatures in the bins varied over time, depending on the treatment (Fig. 2). We did not have grain temperature measurements for two of the control bins and one of the spinosad-treated bins during the first 4 wk of the study, because of unexpected delays in obtaining the HOBO data loggers. However, the temperatures in these bins should have been very similar to the temperatures in the spinosad-treated bin, because insect density in the control bins would not have been high enough to cause heating during the first month. The mean grain temperature at the start of the study in all of the bins was ≈36°C. Temperatures in the two aerated bins decreased rapidly during the first week of storage to ≈28°C. The first cooling cycle was completed by 1 August, and the controllers on the two aerated bins were switched to a threshold of 18.3°C. On 20 September, the second cooling cycle was completed and the controllers were switched to the final threshold of 7.2°C for the third cooling cycle, which was completed by 23 October. Grain temperature in the control and spinosad-treated bins remained at ≈36°C until mid-August. Thereafter, mean grain temperature in bins with spinosad-treated wheat decreased steadily by ≈7°C/mo. However, in the control bins, heat produced by very high insect densities caused mean grain temperature to increase from 32°C on 5 September to a maximum of 37°C on 7 October. In comparison, mean grain temperature in the spinosad bins decreased to 26°C on 7 October.

Grain Quality. In the bins treated with spinosad or aeration, grain quality remained high during the entire 6 mo of the study (Table 1). Of the eight quality factors, insect-damaged kernels (IDK) was affected the most by high insect density. Of the three species used in this study, only *R. dominica* causes IDK. Significant differences were detected as early as August between the control and spinosad-treated wheat. The highest numbers of IDK were found in the December and January grain samples in the control bins. In the December samples there were 30 IDK per 100 g of wheat; in January samples, IDK increased to 70.5 IDK per 100 g. Federal Grain Inspection Standards classify wheat as "sample grade" with 32 or greater IDK per 100 g of wheat. Sample grade wheat cannot be used for human consumption and is often disposed of or sold for use in animal feed.

Test weight is another important factor that millers use to judge grain quality. In December, test weight was significantly lower in the control samples than in the aerated or spinosad-treated wheat. Over the 6-mo storage period, test weight in the aerated and spinosad-treated bins decreased by only 0.5 kg/hl, whereas in the control bins, the test weight decreased by ≈1.5 kg/hl.

Spinosad Residues. Spinosad residues decreased slightly over time ($F = 8.58$; df = 2, 18; $P = 0.024$) (Table 2). Spinosad residue immediately after treatment was 0.70 mg ([AI])/kg of wheat, and decreased to 0.51 mg/kg after 6 mo. Residues also degraded slightly faster in the periphery of the grain compared with the center ($F = 6.18$; df = 1, 18; $P = 0.023$).

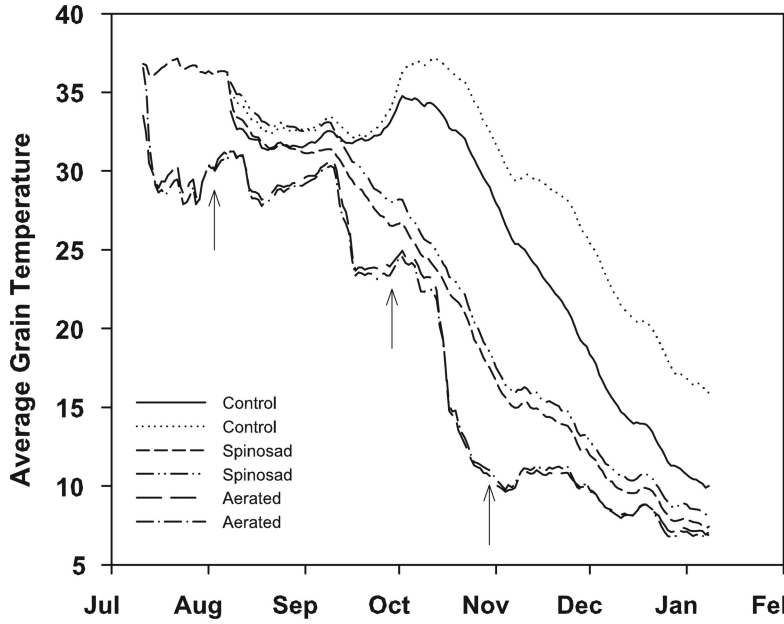

Fig. 2. Seasonal changes in mean grain temperature in six, 30.7-metric ton (1,100-bu) bins of stored wheat treated with either spinosad, controlled aeration, or no treatment (control). Arrows indicate end of the first, second, and third aeration cooling cycles.

Residues in wheat samples taken from the bin periphery and center immediately after grain treatment were 0.80 and 0.61 mg/kg, respectively. After 6 mo of storage, residues in samples taken from the bin periphery and center were 0.52 and 0.49 mg/kg, respectively. Therefore, there was a larger decrease in residues in the peripheral samples than in the center samples. There were no significant differences between residues in samples taken in the top, center or bottom positions of the bin ($F = 0.82$; df = 2, 18; $P = 0.455$).

Bioassays. In bioassays with immature insects, the mortality of *P. interpunctella*, *R. dominica*, and *T. cas-*

Table 1. Grain quality parameters (mean ± SE) of wheat samples in control, aeration, and spinosad treatments from July 2002 to January 2003

Month	Treatment	Dockage (%)	Test wt (kg/hl)	Moisture (%)	Total damage (%)	Foreign Material (%)	Broken and shrunken kernels (%)	Total defects (%)	No. insect damaged kernels/100 g
July	Control	0.2 ± 0.0[a]	55.5 ± 0.1[a]	11.1 ± 0.0a	0.1 ± 0.1[a]	0.0 ± 0.0[a]	1.6 ± 0.0[a]	1.7 ± 0.1[a]	0.0 ± 0.0[a]
	Aeration	0.2 ± 0.0	55.6 ± 0.2	10.9 ± 0.1b	0.2 ± 0.0	0.1 ± 0.1	1.7 ± 0.1	2.0 ± 0.1	0.0 ± 0.0
	Spinosad	0.2 ± 0.1	55.4 ± 0.0	10.9 ± 0.0b	0.2 ± 0.2	0.0 ± 0.0	1.9 ± 0.1	2.1 ± 0.3	0.0 ± 0.0
August	Control	0.1 ± 0.0[a]	55.1 ± 0.1[a]	10.9 ± 0.1[a]	0.5 ± 0.1[a]	0.1 ± 0.1[a]	1.6 ± 0.0[a]	2.1 ± 0.0[a]	0.0 ± 0.0a
	Aeration	0.1 ± 0.0	55.2 ± 0.0	10.6 ± 0.2	0.4 ± 0.1	0.1 ± 0.0	1.4 ± 0.2	1.9 ± 0.3	0.0 ± 0.0a
	Spinosad	0.1 ± 0.0	55.2 ± 0.1	10.7 ± 0.1	0.4 ± 0.1	0.0 ± 0.0	1.6 ± 0.2	2.0 ± 0.2	1.5 ± 0.5b
September	Control	0.2 ± 0.0[a]	55.6 ± 0.0[a]	11.1 ± 0.0a	0.4 ± 0.2[a]	0.2 ± 0.1[a]	1.0 ± 0.8[a]	1.5 ± 0.6[a]	5.0 ± 1.0a
	Aeration	0.2 ± 0.0	55.4 ± 0.3	10.7 ± 0.0c	0.3 ± 0.1	0.1 ± 0.0	1.8 ± 0.1	2.2 ± 0.1	0.5 ± 0.5b
	Spinosad	0.2 ± 0.1	55.4 ± 0.4	11.0 ± 0.1b	0.3 ± 0.1	0.1 ± 0.1	2.1 ± 0.3	2.4 ± 0.3	1.0 ± 0.0b
October	Control	0.3 ± 0.1[a]	55.2 ± 0.0[a]	11.2 ± 0.0a	0.6 ± 0.1[a]	0.1 ± 0.0[a]	1.9 ± 0.1[a]	2.6 ± 0.1[a]	12.0 ± 2.0a
	Aeration	0.2 ± 0.0	55.4 ± 0.1	10.6 ± 0.1c	0.5 ± 0.1	0.1 ± 0.1	1.8 ± 0.0	2.3 ± 0.1	0.5 ± 0.5b
	Spinosad	0.2 ± 0.0	56.0 ± 0.4	10.9 ± 0.1b	0.5 ± 0.1	0.1 ± 0.1	1.8 ± 0.2	2.4 ± 0.4	2.5 ± 0.5b
November	Control	0.3 ± 0.1[a]	55.2 ± 0.1[a]	11.4 ± 0.2a	0.5 ± 0.0[a]	0.1 ± 0.0[a]	1.7 ± 0.1[a]	2.3 ± 0.1[a]	16.5 ± 5.5a
	Aeration	0.2 ± 0.0	55.6 ± 0.0	10.2 ± 0.1b	0.4 ± 0.1	0.1 ± 0.0	1.8 ± 0.1	2.2 ± 0.1	0.5 ± 0.5b
	Spinosad	0.2 ± 0.0	55.4 ± 0.1	10.9 ± 0.0a	0.4 ± 0.1	0.1 ± 0.1	1.9 ± 0.1	2.3 ± 0.2	2.0 ± 1.0ab
December	Control	0.3 ± 0.1[a]	54.8 ± 0.1b	11.5 ± 0.3a	0.7 ± 0.0[a]	0.1 ± 0.0a	1.8 ± 0.1[a]	2.6 ± 0.1[a]	30.0 ± 5.0a
	Aeration	0.2 ± 0.0	55.4 ± 0.0a	10.2 ± 0.2b	0.5 ± 0.1	0.1 ± 0.0a	1.8 ± 0.1	2.3 ± 0.0	1.5 ± 1.5b
	Spinosad	0.2 ± 0.0	55.2 ± 0.0a	10.8 ± 0.1b	0.4 ± 0.2	0.0 ± 0.0b	1.9 ± 0.0	2.3 ± 0.2	0.5 ± 0.5b
January	Control	0.3 ± 0.0[a]	54.0 ± 0.5[a]	11.8 ± 0.5a	1.2 ± 0.6[a]	0.2 ± 0.1[a]	1.8 ± 0.1[a]	5.8 ± 2.1[a]	70.5 ± 4.5a
	Aeration	0.3 ± 0.0	54.9 ± 0.1	10.3 ± 0.0b	0.6 ± 0.0	0.1 ± 0.1	1.9 ± 0.0	2.6 ± 0.1	6.0 ± 1.0b
	Spinosad	0.3 ± 0.1	55.1 ± 0.1	10.8 ± 0.0ab	0.5 ± 0.1	0.1 ± 0.0	1.9 ± 0.3	2.4 ± 0.2	1.5 ± 0.5b

Each mean is based on two replications. Means for each quality factor, within the same month, followed by different letters are significantly different ($P \leq 0.05$; Fisher's protected LSD).

[a] Differences among the three treatments in each quality parameter at the indicated months were not significant (F, range among months and parameters, 0.06–9.0; df = 2, 5; P, range 0.054–0.94; one-way ANOVA).

Table 2. Variation in spinosad residues (mean ± SE) within the grain mass immediately after treatment and at 3 and 6 mo after treatment

Month	Bin location	Bin depth	Residue (mg/kg)
0	Center	Top	0.40 ± 0.06
		Middle	0.76 ± 0.11
		Bottom	0.67 ± 0.04
	Periphery	Top	0.88 ± 0.19
		Middle	0.77 ± 0.04
		Bottom	0.76 ± 0.09
3	Center	Top	0.53 ± 0.05
		Middle	0.56 ± 0.004
		Bottom	0.71 ± 0.03
	Periphery	Top	0.68 ± 0.20
		Middle	0.71 ± 0.04
		Bottom	0.59 ± 0.002
6	Center	Top	0.53 ± 0.02
		Middle	0.35 ± 0.06
		Bottom	0.60 ± 0.04
	Periphery	Top	0.52 ± 0.01
		Middle	0.48 ± 0.05
		Bottom	0.58 ± 0.07

Each mean is based on two replications.

Table 3. Mortality (%mean ± SE) of immatures of three insect species exposed to wheat samples obtained from untreated control bins, aerated wheat bins, or spinosad-treated wheat bins from July 2002 to January 2003

Species	Month	Control	Aerated	Spinosad
P. interpunctella	July	55.6 ± 0.2b	54.2 ± 1.7b	100.0 ± 0.0a
	Aug.	54.8 ± 0.6b	52.5 ± 3.3b	100.0 ± 0.0a
	Sept.	31.0 ± 0.2b	28.3 ± 2.5b	100.0 ± 0.0a
	Oct.	36.7 ± 4.2b	40.9 ± 3.5b	100.0 ± 0.0a
	Nov.	36.7 ± 4.2b	30.6 ± 3.1b	100.0 ± 0.0a
	Dec.	29.2 ± 0.4b	22.9 ± 5.0b	100.0 ± 0.0a
	Jan.	24.4 ± 4.8b	23.8 ± 1.7b	100.0 ± 0.0a
R. dominica	July	27.8 ± 8.1b	14.2 ± 4.0b	99.6 ± 0.0a
	Aug.	31.7 ± 16.7b	30.8 ± 6.7b	100.0 ± 0.0a
	Sept.	31.6 ± 3.4b	41.7 ± 5.8b	100.0 ± 0.0a
	Oct.	40.8 ± 1.7b	44.2 ± 0.4b	100.0 ± 0.0a
	Nov.	53.5 ± 3.5b	54.6 ± 1.3b	100.0 ± 0.0a
	Dec.	59.4 ± 1.9b	57.9 ± 2.9b	100.0 ± 0.0a
	Jan.	31.7 ± 6.3b	48.3 ± 6.7b	99.8 ± 0.2a
T. castaneum	July	16.0 ± 0.2b	15.4 ± 2.1b	100.0 ± 0.0a
	Aug.	22.7 ± 3.1b	18.3 ± 3.8b	100.0 ± 0.0a
	Sept.	25.2 ± 0.2b	20.8 ± 7.5b	99.8 ± 0.2a
	Oct.	24.6 ± 2.1b	25.2 ± 1.0b	100.0 ± 0.0a
	Nov.	29.9 ± 0.9b	32.9 ± 3.8b	97.5 ± 0.0a
	Dec.	27.5 ± 8.3b	22.2 ± 0.7b	97.5 ± 0.4a
	Jan.	18.8 ± 1.7b	20.0 ± 0.4b	100.0 ± 0.0a

Each mean is based on two replications. Means for each species, within the same month, followed by different letters are significantly different ($P \leq 0.05$; Fisher's protected LSD).

taneum was significantly different among the sampling months (F, range among species = 2.50–12.04; df = 6, 240; $P = 0.023–0.0001$) and the three treatments ($F = 601.89–2349.67$; df = 2, 240; $P = 0.0001$). Immature mortality was not significantly different when insects were exposed to samples taken from the bin periphery and bin center (F, range among species = 0.42–3.39; df = 1, 240; $P = 0.067–0.518$) or samples taken from the top, middle, and bottom positions of the bin ($F = 0.10–1.96$; df = 2, 240; $P = 0.140–0.904$). Therefore, mortality data for each species were pooled across the two bin locations and three depths to make comparisons among the three treatments.

The mortality of each species exposed to spinosad-treated wheat ranged from 98 to 100% throughout the study (Table 3). Although there was some variation from month to month in mortality of the three species exposed to untreated wheat from the control and aerated bins, it was significantly lower ($P < 0.05$) than mortality of insects exposed to spinosad-treated wheat.

In bioassays with adults, the mortality of *C. ferrugineus*, *R. dominica*, and *T. castaneum* varied over the 6-mo study period (F range among species = 4.52–24.29; df = 6, 240; $P < 0.0002$) and among the three treatments ($F = 94.80–691.18$; df = 2, 240; $P = 0.0001$). Mortality of *C. ferrugineus* or *R. dominica* was not significantly different when exposed to wheat samples taken from the bin periphery and bin center (F, range among species = 0.97–1.22; df = 1, 240; $P = 0.270–0.325$) or when exposed to samples taken at the three bin depths ($F = 0.02–0.23$; df = 2, 240; $P = 0.797–0.982$). In the case of *T. castaneum*, mortality was significantly different between peripheral and center samples ($F = 5.47$; df = 1, 240; $P = 0.020$), but not among the three bin depths ($F = 0.75$; df = 2, 240; $P = 0.475$). Mortality data were pooled across peripheral and center samples and the three depths to make comparisons among the three treatments, to be consistent with the procedures used for analyzing immature bioassay data.

Mortality of *C. ferrugineus* and *R. dominica* was 100% on spinosad-treated wheat throughout the study, and mortality of these two species on spinosad-treated wheat was much higher compared with that on control or aerated wheat samples (Table 4). The high mortality of *R. dominica* observed in the control and aeration bioassays conducted with samples taken in November and January may have been caused by the high susceptibility of *R. dominica* to very small amounts of spinosad (Fang et al. 2002a,b; Toews and Subramanyam 2003). We suspect that the higher mortality during these months in the control and aeration grain samples may have been caused by spinosad cross-contamination in sieves and jars used for conducting the bioassays.

Mortality of adult *T. castaneum* on spinosad-treated wheat samples was much lower than for *R. dominica* or *C. ferrugineus* and ranged from 4.2 to 45.4% (Table 4). Mortality of *T. castaneum* was 0–4% when exposed to wheat from control and aerated bins. *T. castaneum* mortality among the three treatments was not significantly different in bioassays conducted with September, October, November, and December samples (F, range among months = 2.05–9.09; df = 2, 3; $P = 0.057–0.274$).

Discussion

Extremely low densities of live adult *C. ferrugineus*, *R. dominica*, and *T. castaneum* were found in bins with spinosad-treated wheat, even though 400 insects of each species were added to each bin every month for the first 4 mo. Spinosad is less effective against

Table 4. Mortality (%mean ± SE) of adults of three insect species exposed to wheat samples obtained from untreated control bins, aerated wheat bins, or spinosad-treated wheat bins from July 2002 to January 2003

Species	Month	Control	Aerated	Spinosad
C. ferrugineus	July	25.4 ± 8.8b	15.8 ± 3.3b	100.0 ± 0.0a
	Aug.	41.7 ± 10.8b	41.3 ± 14.6b	100.0 ± 0.0a
	Sept.	8.3 ± 0.0b	12.9 ± 3.8b	100.0 ± 0.0a
	Oct.	7.9 ± 1.3b	34.6 ± 21.3b	100.0 ± 0.0a
	Nov.	10.4 ± 2.1c	30.8 ± 1.7b	100.0 ± 0.0a
	Dec.[a]	30.8 ± 27.5	21.3 ± 10.4	100.0 ± 0.0
	Jan.	5.4 ± 0.4b	30.0 ± 1.7b	100.0 ± 0.0a
R. dominica	July[b]	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0
	Aug.	50.8 ± 22.5b	24.2 ± 6.7b	100.0 ± 0.0a
	Sept.	5.8 ± 2.5b	31.3 ± 29.6b	100.0 ± 0.0a
	Oct.[c]	40.0 ± 15.0	42.1 ± 36.3	100.0 ± 0.0
	Nov.	84.2 ± 1.7b	63.8 ± 5.4c	100.0 ± 0.0a
	Dec.	35.0 ± 23.3b	29.2 ± 3.3b	100.0 ± 0.0a
	Jan.	82.1 ± 2.1c	91.3 ± 0.4b	100.0 ± 0.0a
T. castaneum	July	0.0 ± 0.0b	0.4 ± 0.4b	22.9 ± 2.1a
	Aug.	0.8 ± 0.8b	0.0 ± 0.0b	9.6 ± 2.1a
	Sept.[d]	0.8 ± 0.0	4.2 ± 2.5	45.4 ± 18.8
	Oct.[d]	1.7 ± 0.8	2.9 ± 0.4	6.7 ± 0.8
	Nov.[d]	2.9 ± 2.9	2.1 ± 2.1	16.3 ± 8.8
	Dec.[d]	1.3 ± 1.3	1.7 ± 1.7	10.8 ± 0.8
	Jan.	0.0 ± 0.0b	0.0 ± 0.0b	4.2 ± 0.8a

Each mean is based on two replications. Means for insect species, within the same month, followed by different letters are significantly different ($P \leq 0.05$; Fisher's protected LSD).

[a] Differences among treatments were not significant ($F = 8.59$; df = 2, 3; $P = 0.07$; one-way ANOVA).

[b] Data were not analyzed because of 0 or 100% mortality in treatments.

[c] Differences among treatments were not significant ($F = 4.00$; df = 2, 3; $P = 0.14$).

[d] Differences among treatments, for each month, were not significant (F, range among months, 2.05–9.09; df = 2, 3; P, range 0.057–0.27).

T. castaneum adults compared with other species (Fang et al. 2002a, Toews and Subramanyam 2003). A similar finding was confirmed during this study with adult bioassays. However, laboratory tests have shown spinosad to be extremely effective in suppressing *T. castaneum* progeny production (Fang et al. 2002a). The presence of low densities of *T. castaneum* adults in spinosad-treated grain bins could be due to high immature mortality so that none of the eggs laid survived to become adults. Both *R. dominica* and *C. ferrugineus* adults are susceptible to spinosad at 1 mg/kg (Fang et al. 2002a, Bh. Subramanyam, unpublished data), and *R. dominica* is highly susceptible to spinosad at rates as low as 0.1 mg/kg (Fang et al. 2002b, Toews and Subramanyam 2003). Bioassay results with adult *C. ferrugineus* and *R. dominica* in the current study were similar to those reported by Fang et al. (2002a,b). Furthermore, spinosad at 1 mg/kg completely suppresses progeny production in these two species (Fang et al. 2002a, Bh. Subramanyam, unpublished data). Although, bioassays were not conducted with eggs of *C. ferrugineus* in the current study, the results obtained in bioassays with *R. dominica* eggs suggest that *C. ferrugineus* immature mortality also would have been high. The absence of *C. ferrugineus* adults and the presence of extremely low densities of *R. dominica* adults in spinosad-treated wheat was probably due to both high adult and immature mortality.

R. dominica population density in bins with controlled aeration were suppressed by 80, 68, 84, and 90% relative to populations in the control bins during October, November, December, and January, respectively. *T. castaneum* populations in bins with controlled aeration were suppressed by 80, 99, 100, and 100% compared with the control bins during October, November, December, and January, respectively. *C. ferrugineus* populations in bins with controlled aeration were suppressed by 83, 84, 95, and 98% compared with the control bins during October, November, December, and January, respectively.

R. dominica reached higher insect densities earlier in the study than the other two species. This may have been why aeration was less effective in suppressing *R. dominica.* In the aerated bins, *T. castaneum* and *C. ferrugineus* never reached densities higher than 1.5 adults per kilogram and both were below 0.01 adults per kilogram by January. One of the factors that probably limited *C. ferrugineus* population growth was low grain moisture (10.8%). Previous studies have shown that low grain moisture causes high larval mortality in this species (Rilett 1949). It is likely that *T. castaneum* took longer to reach higher densities, because even though there was initially 2% shrunken and broken kernels, fine material in the grain may have been a limiting resource for this species (White 1982). As *R. dominica* populations increased, they produced more fine material (Potter 1935) so that it was no longer a limiting resource for *T. castaneum.*

Aeration was more effective against *T. castaneum* compared with *R. dominica.* This was because *R. dominica* increased rapidly during the warm summer in the aerated bins because unlike *T. castaneum,* it does not require fine material. *T. castaneum* probably did not increase as fast as *R. dominica* during the summer because the lack of fine material in the grain may have limited their growth. By October, sufficient fine material was available for *T. castaneum* to increase rapidly; however, by this time the grain temperature in the aerated bins was cool enough (Howe 1965) to suppress *T. castaneum* population growth.

It is interesting that in the control bins, *T. castaneum* replaced *R. dominica* as the dominant species during the study. It is known that *T. castaneum* is cannibalistic and feeds on eggs of other species (Sokaloff 1974). As the population of *T. castaneum* grew to high densities from November to January, adults and larvae may have fed on *R. dominica* eggs and reduced their population growth.

High population densities (>60 adults per kilogram) produced heating in the control bins from October onward. Although stored-product insects do not develop and reproduce at temperatures below 15°C (Fields and Muir 1996), this heating permitted population growth even during the winter. Controlled aeration lowers grain temperatures below 15°C and thus prevents insect-induced "hotspots."

Grain quality in the wheat treated with spinosad or aeration remained very high during the 6-mo study

period. The number of IDK was the grain quality factor most influenced by the treatments. *R. dominica* was the only species in our study that causes IDK, because the larvae develop inside the grain kernels and emerge as adults, making a hollowed-out kernel that is characteristic of IDK. *R. dominica* density was much higher in the control bins than in the aerated or spinosad-treated bins; thus, we expected IDK to be much higher in the control bins. In January, IDK was slightly higher in the aerated wheat than in the spinosad-treated wheat; however, this difference was not significant ($P > 0.05$). This was probably caused by the slightly higher *R. dominica* populations during the study in the aerated wheat, compared with the spinosad-treated wheat.

Spinosad residues decreased by ≈19% during the 6-mo study but remained effective against all three species (Fig. 1; Tables 3 and 4). Fang et al. (2002b) reported similar residual activity against adult *R. dominica* and *T. castaneum* over a 12-mo period. Spinosad residues degraded more rapidly on grain near the periphery of the bin than near the center, but the difference was slight. Although UV radiation is the primary cause of spinosad degradation (Saunders and Bret 1997), it is unlikely that UV light caused the differential degradation, because binned wheat is shielded from sunlight. It is more likely that higher grain temperatures near the periphery, produced by solar heating of the bin wall, was responsible for the slight degradation that occurred.

Some of the advantages of spinosad compared with traditional protectants such as chlorpyrifos-methyl are that it provides very good control of *R. dominica*, degrades very slowly in the grain, and has a very low mammalian toxicity (Thompson et al. 2000). If the product becomes available for stored wheat, it would be invaluable especially in situations where a high degree of insect control is needed, and/or in climates where aeration is not as effective in suppressing insects, such as in the more southern states (Arthur and Flinn 2000). The advantage of aeration is that it provides good insect suppression, with no insecticidal residues, at a relatively low cost (electrical costs are ≈1 cent/27 kg of wheat). Field studies in Kansas showed that cooling grain starting at harvest in July resulted in well-controlled insect populations; significantly more insects were found in bins that were not cooled until October (Reed and Harner 1998).

EPA registration and organic certification of spinosad is pending for stored grain. If an organic label of spinosad is approved for use on stored wheat, then it will provide a new tool for organic producers who have very limited options for stored-grain insect management. Aeration is also suitable for use by organic grain producers. An insecticide like spinosad may provide effective insect control for producers storing organic or nonorganic wheat.

Acknowledgments

We thank Paul Fields (Agriculture Canada, Winnipeg, Manitoba) and Carl Reed (Department of Grain Science and Industry, Kansas State University) for reviewing an earlier version of the manuscript. Terry Pitts (Gustafson, Plano, TX) and Jerry Keaton (Dow AgroSciences, Indianapolis, IN) provided guidance and assistance in treating wheat with spinosad. We appreciate the excellent technical assistance of Ken Friesen, Liang Fang, Andy Allen, Zeb Larson, Dhana Raj Boina, Jade Vardeman, Jackie Rowan, and Carmelita Goossen. This paper is Contribution No. 04-160-J of the Kansas Agricultural Experiment Station and was supported by funds from the Kansas Wheat Commission, and in part, by funds from Dow AgroSciences, and USDA–CSREES (RAMP) under Agreement No. 00-51101–9674.

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Received 10 December 2003; accepted 10 April 2004.