Variation in Susceptibility of Field Strains of Three Stored Grain Insect Species to Spinosad and Chlorpyrifos-Methyl Plus Deltamethrin on Hard Red Winter Wheat

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ABSTRACT Spinosad and chlorpyrifos-methyl plus deltamethrin efficacy at labeled rates on hard red winter wheat were evaluated against 11 field strains of the red flour beetle, *Tribolium castaneum* (Herbst); six strains of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); and two strains of the lesser grain borer, *Rhyzopertha dominica* (F.), collected mostly from farm-stored grain in Kansas. Adults were exposed to wheat treated with spinosad at 1 mg(active ingredient)/kg or chlorpyrifos-methyl plus deltamethrin at three plus 0.5 mg(active ingredient)/kg. Adult mortality was assessed after 7 and 14 d and progeny production after 42 d. Spinosad did not provide complete mortality or progeny suppression of *T. castaneum* and *O. surinamensis* field strains, but was effective against *R. dominica* strains. Chlorpyrifos-methyl plus deltamethrin produced complete mortality and progeny suppression of field strains all three species. The two least susceptible *T. castaneum* and *O. surinamensis* strains and the two *R. dominica* strains were chosen for dose–response tests only with spinosad. The LD_{99} values for *T. castaneum* and *R. dominica* field strains were similar to that of the corresponding laboratory strains. Corresponding values for the two *O. surinamensis* field strains were significantly greater (≈6 times) than the laboratory strain. The effective dose for progeny reduction (ED_{99}) of only one *R. dominica* field strain was significantly greater (≈2 times) than the laboratory strain. The baseline susceptibility data of field strains of three insect species to spinosad will be useful for monitoring resistance development when this product is commercially released as a grain protectant.

KEY WORDS stored-grain insects, spinosad, chlorpyrifos-methyl plus deltamethrin, field strains, mortality

The application of an insecticide to newly harvested grain as it is loaded into a bin is an important preventive integrated pest management (IPM) approach (Arthur and Subramanyam 2012). Insecticides applied directly to grain are called grain protectants. Spinosad was approved as a grain protectant by the United States Environmental Protection Agency (US–EPA) in 2005 for use on barley, corn, millets, oats, rice, sorghum, and wheat (Hertlein et al. 2011), and commercial release of formulations for grain treatment is expected soon. Chlorpyrifos-methyl plus deltamethrin was registered by the US–EPA on 27 October 2004 for use on barley, oats, rice, sorghum, and wheat as well as empty bins receiving these grains. This combination product replaced chlorpyrifos-methyl (Reldan 4E) used at 6 mg(active ingredient [AI])/kg after the product registrants (Dow AgroSciences, Indianapolis, IN, and Bayer CropScience, Research Traingle Park, NC) petitioned US–EPA to revoke existing tolerances and cancel product registrations, which became effective 5 December 2007 (Anonymous 2007).

Spinosad at the labeled rate of 1 mg(AI)/kg is effective against a broad range of stored-grain insects (Fang et al. 2002b, Subramanyam et al. 2007, Hertlein et al. 2011, Subramanyam et al. 2012), on barley, corn, sorghum, and wheat (Chintzoglou et al. 2008, Getchell and Subramanyam 2008, Athanassiou et al. 2010, Kavallieratos et al. 2010, Vayias et al. 2010). Previous studies have shown the lesser grain borer, *Rhyzopertha dominica* (F.), to be highly susceptible to spinosad (Fang et al. 2002a,b; Flinn et al. 2004; Huang et al. 2004; Subramanyam et al. 2007). The adults of sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), and red flour beetle, *Tribolium castaneum* (Herbst), are moderately susceptible to spinosad (Subramanyam et al. 1999, Fang et al. 2002a, Nayak et al. 2005), but first instars of these species are highly susceptible to spinsoad as evidenced by lack of adult progeny production

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on spinosad-treated grain (Toews and Subramanyam 2003, Flinn et al. 2004, Athanassiou et al. 2010).

Chlorpyrifos-methyl plus deltamethrin at the labeled rate of three plus 0.5 mg(AI)/kg is effective against several species of psocids on stored wheat (Athanassiou et al. 2009). It is also effective against *R. dominica*, *T. castaneum*, the rice weevil, *Sitophilus oryzae* (L.); and Indianmeal moth, *Plodia interpunctella* (Hübner), on wheat and *R. dominica* and *S. oryzae* on short-grain rice and long-grain rice (Subramanyam et al. 2012).

Huang et al. (2004) tested the efficacy of spinosad against two field strains each of *R. dominica*, *T. castaneum*, and *P. interpunctella*, and one strain of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), collected from bins of stored hard red winter wheat in Kansas. Athanassiou et al. (2008) tested six strains of the confused flour beetle, *Tribolium confusum* Jacquelin du Val, collected from different countries of Europe. In general, these two studies showed field strains to be less susceptible than corresponding laboratory-reared strains. To our knowledge, field strains of stored-grain insects have not been evaluated for their susceptibility to chlorpyrifos-methyl plus deltamethrin. Field strains of insects may differ widely in their susceptibility to insecticides applied to grains because of natural tolerance or resistance (Subramanyam et al. 1989, Subramanyam and Hagstrum 1996, Huang et al. 2004, Kljajić and Perić 2006, Vayias et al. 2009). Therefore, evaluation of insecticides against field strains is necessary to confirm whether or not an approved insecticide at the labeled rate will work in practical field situations. In the present investigation, we determined susceptibility of adults of *T. castaneum*, *O. surinamensis*, and *R. dominica* field strains collected mostly from farm-stored grain in Kansas to spinosad and chlorpyrifos-methyl plus deltamethrin applied to hard red winter wheat. Data on effectiveness of an insecticide against field strains are important to make recommendations to farmers and grain managers; in addition such data provide baseline information on insect susceptibility that is important in pest management and resistance management programs.

Materials and Methods

Collection of Field Strains and Insect Rearing. Adults of *T. castaneum*, *O. surinamensis*, and *R. dominica* were collected from farm bins at cooperating farm sites (Table 1) between July and November 2011 by inserting five perforated probe traps (Subramanyam et al. 1993) just below the stored-grain surface to capture live adults. The traps were removed after 1–2 wk. In addition, 1–2 kg sample of mostly wheat, and some corn and sorghum were collected in 37.5 by 30.5 cm plastic Ziploc bags (Assorted Bag Company, Dallas, TX). In the laboratory, live adults of each species (≈50–200) were separated from traps and grain samples. In addition, four strains of *T. castaneum* and one strain of *R. dominica* collected from flour mills in the United States before 2011, and one strain of *T. castaneum* collected in 2011 from a rice-processing facility were also included in this study. In total, 11 field strains of *T. castaneum*, six strains of *O. surinamensis*, and two strains of *R. dominica* were used in the study along with corresponding laboratory strains, that have been in rearing, without insecticide exposure, since 1999 in the Department of Grain Science and Industry, Kansas State University. These laboratory strains served as the standard reference strains and assumed to be insecticide-susceptible.

Table 1. Sites and years of collection of field strains of adult *T. castaneum*, *O. surinamensis*, and *R. dominica*

Species	County, state	Commodity	Strain ID	Collection year
T. castaneum	Dickinson, KS	Wheat	AB1	2011
	Maricopa, AZ	Flour mill	AZ[a]	2009
	Washington, KS	Wheat	CF	2011
	McPherson, KS	Wheat	CN	2011
	Stafford, KS	Flour mill	HN[a]	2001
	Cook, IL	Rice facility	IL[b]	2011
	Jackson, MO	Flour mill	KC[a]	2005
	Ottawa, KS	Wheat	MN1	2011
	Russell, KS	Wheat	PD1	2011
	Dickinson, KS	Flour mill	SR[a]	2001
	Mitchell, KS	Wheat	TP	2011
O. surinamensis	Dickinson, KS	Wheat	AB1	2011
	Dickinson, KS	Wheat	AB2	2011
	Washington, KS	Wheat	CF	2011
	Ottawa, KS	Wheat	MN1	2011
	Russell, KS	Wheat	PD1	2011
	Mitchell, KS	Wheat	TP	2011
R. dominica	Washington, KS	Wheat	CF	2011
	Riley, KS	Flour mill	RL[a]	2007

[a] These strains were collected before 2011 and were provided by James Campbell, USDA–ARS, Center for Grain and Animal Health Research, Manhattan, KS.

[b] This strain was collected by one of the authors (B.S.) during a visit to a rice-processing facility in 2011.

Laboratory and field strains were reared on standard diets in a growth chamber set at 28°C and 65% relative humidity (RH). Organic white wheat flour (Heartland Mills, Marienthal, KS) plus 5% (by wt) brewer's yeast diet was used for rearing *T. castaneum*. Organic, hard red winter wheat (Heartland Mills) and rolled oats (Heartland Mills) plus 5% brewer's yeast diet were used for rearing *R. dominica* and *O. surinamensis*, respectively.

Insecticides. The liquid formulations of spinosad (Contain II, a precommercial release formulation) and chlorpyrifos-methyl plus deltamethrin (Storcide II) were supplied by Bayer CropScience (Research Triangle Park, Raleigh, NC). Liquid spinosad formulation had a purity of 232 mg(AI)/ml and the purity of chlorpyrifos-methyl plus deltamethrin was 216 mg(AI)/ml of chlorpyrifos-methyl and 37 mg(AI)/ml of deltamethrin. Stock solutions and dilutions for grain treatment were made in distilled water.

Wheat Treatment. Organic, hard red winter wheat was cleaned manually by sieving it over a 2-mm round hole sieve (Seedburo Equipment Co., Chicago, IL) to remove dockage and broken kernels. Cleaned wheat was frozen for 1 wk at −13°C to kill any live insects present. The moisture content of grain samples was equilibrated to 12 ± 1% in environmental growth

chambers maintained at 28°C and 65% RH and quantified with a Moisture Analyzer model 930 (Shore Sales Co., Rantoul, IL).

Tests at Labeled Rates. Spinosad and chlorpyrifos-methyl plus deltamethrin were evaluated at their respective labeled rates of 1 mg(AI)/kg and three plus 0.5 mg(AI)/kg against laboratory and field strains of *T. castaneum*, *O. surinamensis*, and *R. dominica*.

Spinosad or chlorpyrifos-methyl plus deltamethrin solution (1 ml) was applied to 1 kg lots of wheat to provide the respective labeled rates. Each lot of wheat was treated with an insecticide in a 5 kg capacity stainless steel drum that was rotated mechanically for 10 min to ensure uniform coverage of the insecticide on kernels. Wheat treated with aliquots of distilled water served as the control treatment (0 mg [AI]/kg). Treated wheat (50 g) was weighed in separate 473 ml glass jars and 25 unsexed 1–3 wk old adults of an insect species were introduced into each jar. The jars after adult introduction were closed with metal lids fitted with wire-mesh screens and filter papers and kept in an environmental chamber at 28°C and 65% RH.

The mortality of introduced adults was examined at 7 and 14 d and adult progeny production after 42 d. Separate sets of jars were used for each observation time. Adults unable to move when prodded with a fine brush were considered dead. Each combination of insecticide, observation time, and insect species was replicated five times and each replicate was treated separately as explained above. The original number of introduced adults (25) was subtracted from the number of adult progeny produced before subjecting data to statistical analysis.

Dose-Response Bioassays. Initially bioassays were carried out on laboratory strains for standardization of doses to be used in dose–response tests only with spinosad, because laboratory and field strains of the three species exposed to chlorpyrifos-methyl plus deltamethrin at the labeled rate showed complete mortality and progeny suppression. Based on the tests at labeled rates, two least susceptible field strains of each species (CF and HN strains of *T. castaneum*, AB1 and AB2 strains of *O. surinamensis*, and CF and RL strains of *R. dominica*) as well as the corresponding laboratory strains were chosen for dose–response tests. The spinosad rates used were 0, 1, 2, 5, 10, 15, 20, 25, 30, 35, and 40 mg(AI)/kg for *T. castaneum* laboratory and field strains; 0, 0.2, 0.5, 0.7, 1, and 2 mg(AI)/kg for the *O. surinamensis* laboratory strain; 1, 2, 5, 7, and 10 mg(AI)/kg for *O. surinamensis* field strains, and 0, 0.005, 0.007, 0.01, 0.02, 0.03, 0.04, 0.06 mg(AI)/kg for *R. dominica* laboratory and field strains.

Wheat (50 g) was taken in separate 473 ml glass jars and 50 μl of each insecticide solution was applied to obtain the desired insecticide rate. After adding the insecticide solution, wheat in the glass jars was shaken by hand for 1 min to facilitate insecticide coverage on the kernels. Wheat treated with aliquots of distilled water served as the control treatment (0 mg[AI]/kg). Twenty-five unsexed, 1–3 wk old adults of an insect species were introduced into each jar. The jars were closed with metal lids fitted with wire-mesh screens and filter papers and kept at 28°C and 65% RH. Each combination of insecticide and rate for an insect species was replicated three times and each replication was treated separately. After 7 d, the grain samples were sifted over a 2-mm diameter sieve (Seedburo Equipment Co., Chicago, IL) to count the number of live and dead adults. The contents (wheat and insects) were transferred back into the jars and were returned to the growth chamber to determine progeny production at 42 d postinfestation. The original number of introduced adults (25) was subtracted from the number of adult progeny produced before subjecting data to statistical analysis.

Data Analysis. In tests at labeled rates, the number of dead insects out of the total exposed to untreated and insecticide-treated wheat after 7 and 14 d were calculated as a percentage. Mortality data on insecticide-treated wheat were corrected for mortality on untreated (control) wheat (Abbott 1925). Corrected mortality data were transformed to angular values (Zar 1984) and adult progeny production data were transformed to $\log_{10}(x+1)$ scale for statistical analysis. Mortality data, by species and insecticide, were subjected to two-way analysis of variance (ANOVA) to determine differences in mortality between 7 and 14 d and among the insect strains. To determine differences among strains, corrected mortality data at 7 or 14 d were subjected to one-way ANOVA and means were separated using Bonferroni *t*-tests at $\alpha = 0.05$ (SAS Institute 2008). The adult progeny produced on the untreated (control) and spinosad-treated wheat for each insect species and strain were compared using two-sample *t*-tests (SAS Institute 2008), because of wide variation in progeny production among strains.

The mean mortality data from dose–response tests for each insect species on spinosad-treated wheat were corrected for mortality on untreated wheat (Abbott 1925). Corrected dose-mortality data were subjected to probit analysis (SAS Institute 2008) for determining the dose producing 50% (LD_{50}) and 99% (LD_{99}) mortality of insects and associated statistics. The LD_{99} values for any two strains of a species were compared using a ratio test (Robertson and Preisler 1992). The percent reduction in progeny production in the spinosad-treated wheat relative to the control treatment was calculated. These data were subjected to probit analysis, where possible, to determine effective dose for 50% (ED_{50}) and 99% (ED_{99}) reduction in progeny production (SAS Institute 2008). The ED_{99} values for any two strains of a species were compared using a ratio test. The LD_{99} or ED_{99} values between any two strains were considered significantly different ($P < 0.05$) if the 95% CI for the ratio did not include one (Robertson and Preisler 1992).

Results

Tests at Labeled Rates. The 7 and 14 d mortality of all *T. castaneum* strains on untreated wheat ranged from 0 to 4% and from 0 to 9%, respectively. The 7 and 14 d mortality of all the strains on spinosad-treated wheat ranged from 2 to 18% and 4 to 58%, respectively

Table 2. Corrected mortality of adults of *T. castaneum* strains exposed for 7 and 14 d to wheat treated with spinosad at 1 mg(AI)/kg and chlorpyrifos-methyl (C-methyl) plus deltamethrin at 3 plus 0.5 mg(AI)/kg

Strain	Mean ± SE mortality (%)[a]			
	Spinosad		C-methyl plus deltamethrin	
	7 d[b]	14 d[b]	7 d	14 d
Lab.	4.8 ± 0.8ab	35.5 ± 2.6ab	100.0 ± 0.0	100.0 ± 0.0
AB1	12.3 ± 2.5ab	57.7 ± 8.3a	100.0 ± 0.0	100.0 ± 0.0
CF	1.8 ± 1.3b	4.3 ± 3.5c	100.0 ± 0.0	100.0 ± 0.0
CN	14.0 ± 4.2ab	25.3 ± 3.0abc	100.0 ± 0.0	100.0 ± 0.0
HN	7.2 ± 4.3ab	23.2 ± 6.0abc	100.0 ± 0.0	100.0 ± 0.0
MN1	2.1 ± 1.0ab	43.4 ± 3.5ab	100.0 ± 0.0	100.0 ± 0.0
PD1	5.6 ± 2.4ab	25.6 ± 10.5bc	100.0 ± 0.0	100.0 ± 0.0
SR	11.2 ± 3.2ab	28.8 ± 5.3ab	100.0 ± 0.0	100.0 ± 0.0
TP	17.8 ± 4.3a	24.4 ± 7.9abc	100.0 ± 0.0	100.0 ± 0.0
KC	8.0 ± 4.4ab	21.6 ± 7.0bc	100.0 ± 0.0	100.0 ± 0.0
IL	11.2 ± 1.5ab	13.7 ± 3.6bc	100.0 ± 0.0	100.0 ± 0.0
AZ	5.6 ± 2.0ab	11.2 ± 2.3bc	100.0 ± 0.0	100.0 ± 0.0

Each mean is based on $n = 5$.

[a] Mean ± SE 7 and 14 d mortality on untreated wheat (control) for all strains ranged from 0 to 4.4 ± 1.8% and 0 to 8.8 ± 4.6%, respectively.

[b] Means among strains at 7 or 14 d followed by different letters are significantly different ($P < 0.05$; by Bonferroni *t*-tests).

(Table 2), and the laboratory strain was as susceptible as several field strains. Two-way ANOVA showed that the 14 d mortality of *T. castaneum* strains when exposed to spinosad-treated wheat was greater than the 7 d mortality ($F = 79.73$; df = 1, 96; $P < 0.0001$). Mortality differences among strains were also significant ($F = 6.3$; df = 11, 96; $P < 0.0001$) as was the interaction of strain and day ($F = 3.51$; df = 11, 96; $P = 0.0004$). One-way ANOVA showed significant differences among strains for mortality data collected on day 7 ($F = 3.10$; df = 11, 48; $P = 0.0032$) and day 14 ($F = 6.0$; df = 11, 48; $P < 0.0001$). The 7 d mortality of CF strain was significantly different ($P < 0.05$) from that of TP strain, whereas no differences were observed among all other strains. A similar trend was observed for the 14 d mortality among strains. The CF strain mortality was significantly different ($P < 0.05$) from that of the AB1 strain, and despite wide variation in mortality among strains, significant differences were not detected. Chlorpyrifos-methyl plus deltamethrin was extremely effective against all *T. castaneum* strains with 100% mortality at 7 and 14 d.

Adult progeny production among *T. castaneum* strains in the control treatment ranged from 10 to 86 adults/jar (Table 3). There was no progeny production in four out of the 11 field strains exposed to spinosad-treated wheat. Except for the CF strain, reduction in progeny production relative to production on untreated (control) wheat ranged from 90 to 100%. In six strains, where minimal progeny production was observed, the numbers produced were significantly lower ($P < 0.05$) than in the corresponding control treatment. The progeny production of the CF strain exposed to spinosad was similar ($P > 0.05$) to production on untreated wheat. None of the strains exposed to chlorpyrifos-methyl plus deltamethrin produced progeny.

Table 3. Adult progeny production of *T. castaneum* strains exposed continuously for 42 d to spinosad at 1 mg(AI)/kg on wheat

Strain	Mean ± SE adult progeny[a] (% reduction)[b]		*t* (df)	*P* value
	Control	Spinosad		
Lab.	39.2 ± 5.5	0.0 ± 0.0 (100)	—[c]	—
AB1	10.4 ± 2.3	0.0 ± 0.0 (100)	—	—
CF	69.2 ± 19.8	40.4 ± 4.2 (41.6)	−1.38 (8)[d]	0.205
CN	30.2 ± 12.4	0.2 ± 0.2 (99.3)	−2.84 (4.22)[e]	0.044*
HN	46.2 ± 2.7	0.0 ± 0.0 (100)	—	—
MN1	10.2 ± 2.7	0.0 ± 0.0 (100)	—	—
PD1	15.4 ± 6.9	0.6 ± 0.4 (96.1)	−2.74 (8)[d]	0.026*
SR	41.2 ± 1.7	0.0 ± 0.0 (100)	—	—
TP	86.2 ± 5.5	8.8 ± 2.0 (89.8)	−9.98 (4.57)[e]	0.0003*
KC	72.2 ± 3.9	0.4 ± 0.2 (99.4)	−22.51 (4.8)[e]	<0.0001*
IL	78.4 ± 4.3	2.4 ± 0.7 (96.9)	−14.86 (4.56)[e]	<0.0001*
AZ	75.0 ± 10.4	7.2 ± 1.7 (90.4)	−8.00 (8)[d]	<0.0001*

[a] None of the strains produced adult progeny in chlorpyrifos-methyl plus deltamethrin treatment.

[b] Percent reduction in adult progeny production of field strains relative to production on untreated (control) wheat.

[c] Data were not subjected to two sample *t*-tests because progeny were not produced in spinosad treatments.

[d] Variances were equal (*F*, range = 1.69–7.82; df = 4,4; *P*, range = 0.071–0.623).

[e] Variances were unequal (*F*, range = 9.93–37.07; df = 4,4; *P*, range = 0.004–0.047).

* Significant ($P < 0.05$).

The mean 7 and 14 d mortality of *O. surinamensis* strains on untreated wheat was <2 and 9%, respectively. All six field strains of *O. surinamensis* after 7–14 d exposures were less susceptible to spinosad (4–20% mortality) when compared with the laboratory strain (77–80% mortality) (Table 4). There were significant differences in mortality among the strains ($F = 62.22$; df = 6, 56; $P < 0.0001$), but mortality was similar between 7 and 14 d ($F = 3.08$; df = 1, 56; $P = 0.085$). The strain and exposure time interaction was not significant ($F = 0.92$; df = 6, 56; $P = 0.488$). One-way ANOVA showed differences among strains at both 7 d

Table 4. Corrected mortality of adults of *O. surinamensis* strains exposed for 7 and 14 d to wheat treated with spinosad at 1 mg(AI)/kg and chlorpyrifos-methyl (C-methyl) plus deltamethrin at 3 plus 0.5 mg(AI)/kg

Strain	Mean ± SE mortality (%)[a]			
	Spinosad		C-methyl plus deltamethrin	
	7 d[b]	14 d[b]	7 d[b]	14 d[c]
Lab.	80.2 ± 2.0a	77.1 ± 4.5a	100.0 ± 0.0a	100.0 ± 0.0
AB1	7.2 ± 2.0b	10.0 ± 2.5bc	83.7 ± 5.9b	93.6 ± 2.0
AB2	4.0 ± 0.0b	3.5 ± 1.2c	96.0 ± 4.0a	100.0 ± 0.0
CF	9.6 ± 2.0b	19.5 ± 5.0b	100.0 ± 0.0a	100.0 ± 0.0
MN1	9.7 ± 1.6b	16.9 ± 3.4bc	100.0 ± 0.0a	100.0 ± 0.0
PD1	4.8 ± 1.5b	13.0 ± 3.8bc	100.0 ± 0.0a	100.0 ± 0.0
TP	20.0 ± 6.6b	16.9 ± 2.5bc	100.0 ± 0.0a	100.0 ± 0.0

Each mean is based on $n = 5$.

[a] Mean ± SE 7 and 14 d mortality on untreated (control) wheat ranged from 0 to 1.7 ± 1.7 and 0 to 8.6 ± 1.4%, respectively.

[b] For each insecticide means among strains at 7 or 14 d followed by different letters are significantly different ($P < 0.05$; by Bonferroni *t*-tests).

[c] One-way ANOVA was significant (*F*-value = 13.67; df = 6, 28; $P < 0.0001$).

Table 5. Adult progeny production of *O. surinamensis* strains exposed continuously for 42 d to spinosad at 1 mg(AI)/kg on wheat

Strain	Mean ± SE adult progeny[a] (% reduction)[b]		*t* (df)	*P* value*
	Control	Spinosad		
Lab.	96.0 ± 5.47	10.2 ± 3.7 (89.4)	−8.97 (4.39)[d]	0.0005
AB1	70.6 ± 10.2	21.2 ± 2.5 (70.0)	−6.37 (8)[c]	0.0002
AB2	98.4 ± 2.3	31.2 ± 4.9 (68.3)	−8.50 (4.24)[d]	0.0008
CF	79.0 ± 6.7	11.0 ± 3.6 (86.1)	−7.28 (4.81)[d]	0.0009
MN1	66.2 ± 5.1	7.2 ± 0.9 (89.1)	−16.42 (8)[c]	<0.0001
PD1	112.4 ± 9.2	31.8 ± 4.0 (71.7)	−8.42 (8)[c]	<0.0001
TP	38.0 ± 2.2	7.0 ± 1.1 (81.6)	−10.32 (8)[c]	<0.0001

[a] There were no adult progeny produced by all strains in chlorpyrifos-methyl plus deltamethrin treatment except AB1 strain where 0.2 ± 0.2 adults/jar were produced.

[b] Percent reduction in adult progeny production of field strains relative to production on untreated (control) wheat.

[c] Variances were equal (*F*, range = 1.67–6.29; df = 4, 4; *P*, range = 0.103–0.633).

[d] Variances were unequal (*F*, range = 9.77–33.19; df = 4, 4; *P*, range = 0.005–0.049).

* All *P* values were significant ($P < 0.05$).

($F = 36.11$; df = 6, 28; $P < 0.0001$) and 14 d ($F = 27.37$; df = 6, 28; $P < 0.0001$). The significant effect observed is because of mortality of the laboratory strain being higher ($P < 0.05$) than that of the field strains. The 7 d mortality of all field strains was similar ($P > 0.05$), whereas the 14 d mortality of strain AB2 was different ($P < 0.05$) from that of CF strain but was not different ($P > 0.05$) among all other field strains.

Exposure to chlorpyrifos-methyl plus deltamethrin for 14 d resulted in complete mortality of five of the six *O. surinamensis* field strains and the laboratory strain (Table 4). The 7 d mortality of five strains was 100% and that of other two field strains was 84–96%. Mortality differences were observed among strains ($F = 19.48$; df = 6, 56; $P < 0.0001$), but not between 7 and 14 d ($F = 3.5$; df = 1, 56; $P = 0.066$). The strain and exposure time interaction also was not significant ($F = 1.62$; df = 6, 56; $P = 0.159$). One-way ANOVA showed mortality differences among strains at 7 d ($F = 9.79$; df = 6, 28; $P < 0.001$) and 14 d ($F = 13.67$; df = 6, 28; $P < 0.0001$). This difference was primarily because of AB1 strain showing significantly lower ($P < 0.05$) mortality than the other strains.

Adult progeny production of each *O. surinamensis* strain was significantly lower ($P < 0.05$) on spinosad-treated wheat when compared with production on untreated wheat (Table 5). Percent reduction in progeny production on spinosad-treated grain relative to that on untreated wheat ranged from 68 to 89%. No progeny were produced in strains exposed to chlorpyrifos-methyl plus deltamethrin.

The mean 7 and 14 d mortality of *R. dominica* strains in control treatment was <10%. Spinosad was extremely effective against all *R. dominica* strains with 99–100% mortality after 7 and 14 d of exposure. Therefore, difference among strains ($F = 1.0$; df = 2, 24; $P = 0.383$) and between exposure times ($F = 1.0$; df = 1, 24; $P = 0.327$) were not significant. The strain and exposure time interaction also was not significant ($F = 1.0$; df = 2, 24; $P = 0.383$).

Like spinosad, chlorpyrifos-methyl plus deltamethrin was effective against *R. dominica.* There were no significant differences among strains ($F = 2.67$; df = 2, 24; $P = 0.090$) and between the exposure times ($F = 2.67$; df = 1, 24; $P = 0.116$). The strain and exposure time interaction was not significant ($F = 2.67$; df = 2, 24; $P = 0.09$). Adult progeny production of *R. dominica* on the untreated wheat for the laboratory strain was 357 adults/jar, whereas for the CF and RL strains, it was 35 and 36 adults/jar, respectively. No progeny production was observed in strains exposed to either spinosad or chlorpyrifos-methyl plus deltamethrin.

Dose–Response Tests. The LD_{50} and LD_{99} values and associated statistics for the *T. castaneum*, *O. surinamensis*, and *R. dominica* strains exposed to spinosad are shown in Table 6. Based on LD_{50} and LD_{99} values, the adults of *R. dominica*, in general, were highly susceptible to spinosad followed by *O. surinamensis*, and *T. castaneum.* Ratio tests using the LD_{99} values showed that there were no differences ($P \geq 0.05$) between the laboratory and field strains of *T. castaneum* or *R. dominica* (Table 7). The two field strains of *O. surinamensis* were not significantly different from one another ($P > 0.05$), but each field strain had significantly higher ($P < 0.05$) LD_{99} than the laboratory strain.

Table 6. Probit regression estimates for laboratory and field strains of three insect species exposed to spinosad-treated wheat

Species	Strain	*N*[a]	Mean ± SE		LD (95% CL) (mg[AI]/kg)		χ^2 (df)	*P* value*
			Intercept	Slope	LD_{50}	LD_{99}		
T. castaneum[b]	Lab	975	−0.97 ± 0.21	1.93 ± 0.21	3.17 (2.26–4.14)	51.00 (33.51–94.57)	5.15 (9)	0.821
	CF	900	−2.42 ± 0.40	2.88 ± 0.35	6.92 (5.24–8.49)	44.56 (33.27–69.32)	7.94 (8)	0.440
	HN	825	−1.89 ± 0.27	2.60 ± 0.26	5.31 (4.09–6.66)	41.70 (29.69–66.95)	2.79 (7)	0.904
O.surinamensis[b]	Lab	525	0.99 ± 0.21	5.04 ± 0.92	0.64 (0.54–0.73)	1.84 (1.39–3.26)	1.20 (3)	0.753
	AB1	600	−1.08 ± 0.25	3.14 ± 0.43	2.21 (1.71–2.73)	12.16 (8.49–21.79)	4.11 (4)	0.392
	AB2	600	−1.93 ± 0.33	4.24 ± 0.56	2.86 (2.35–3.42)	10.12 (7.62–15.79)	1.42 (4)	0.842
R. dominica[c]	Lab	975	8.97 ± 0.97	4.54 ± 0.49	0.011 (0.009–0.012)	0.03 (0.03–0.05)	10.89 (9)	0.284
	CF	600	6.32 ± 0.92	3.24 ± 0.47	0.011 (0.009–0.014)	0.06 (0.04–0.12)	1.76 (4)	0.780
	RL	600	6.60 ± 0.98	3.35 ± 0.49	0.011 (0.009–0.013)	0.05 (0.04–0.11)	1.97 (4)	0.742

[a] Total no. of insects used in bioassays.

[b] Mortality of *T. castaneum* and *O. surinamensis* strains on untreated (control) wheat was 0%.

[c] Mean ± SE mortality of three *R. dominica* strains on untreated (control) wheat ranged from 2.7 ± 2.7 to 6.7 ± 6.7%.

* Goodness-of-fit of the probit model to data was not significant ($P > 0.05$), indicating good fit of the model to data.

Table 7. Comparison of LD_{99} values between field and corresponding laboratory strains of three insect species exposed to spinosad-treated wheat

Species	Strains compared[a]	LD_{99} ratio (95% CL)
T. castaneum	Lab. vs. CF	1.14 (0.62–2.13)
	Lab. vs. HN	1.22 (0.64–2.34)
O. surinamensis	AB1 vs. Lab.	6.60 (3.66–11.93)*
	AB2 vs. Lab.	5.49 (3.27–9.24)*
R. dominica	CF vs. Lab.	1.71 (0.96–3.07)
	RL vs. Lab.	1.54 (0.86–2.76)

[a] The strain mentioned first has the larger LD_{99} value in the pair being compared.

* The LD_{99} values within a pair being compared are significantly different ($P < 0.05$) from one another because the ratio does not include 1.

Adult progeny production of *T. castaneum* on the untreated wheat was 55 adults/jar, and that of the CF and HN strains was 52 and 61 adults/jar, respectively. Progeny production was observed only with the two field strains exposed to 1 mg(AI)/kg of spinosad; the CF strain produced eight adults/jar while the HN strain produced two adults/jar. Reduction in progeny production at 1 mg(AI)/kg of these strains compared with production of the same strains on untreated wheat was 87–96%.

The progeny production of *O. surinamensis* on untreated wheat was essentially similar between the laboratory and the two field strains, and ranged from 29 to 33 adults/jar. The laboratory strain of *O. surinamensis* failed to produce adult progeny at spinosad rates of 2 mg(AI)/kg and above. Progeny production was observed at 0.2, 0.5, 0.7, and 1 mg(AI)/kg of spinosad (7–24 adults/jar). Even at the labeled rate of 1 mg(AI)/kg, the reduction in progeny production of the laboratory strain when compared with production on untreated wheat was 76%. The field strains did not produce progeny at spinosad rates of 7–20 mg(AI)/kg, but those exposed to 1, 2, and 5 mg(AI)/kg produced 1–13 adults/jar. In the two field strains, reduction in progeny production on spinosad-treated wheat was 57–99% relative to production of the same strains on untreated wheat. The ED_{99} values for progeny reduction were six to eight times higher than the ED_{50} values (Table 8). In general, for the field strains the spinosad rate for 50 and 99% progeny reduction was slightly lower than the rate required to produce 50 and 99% adult mortality. The ED_{99} values for progeny reduction of the AB1 and AB2 strains were not significantly different from one another ($P > 0.05$) based on the ratio test (ratio [95% CL] was 1.39 [0.81–2.38]). Each of the field strains, AB1 (1.89 [0.88–4.07]) and AB2 (1.36 [0.64–2.89]), were also not different from the laboratory strain.

Adult progeny production of the laboratory and two field strains of *R. dominica* on untreated wheat ranged from 176–254 adults/jar. Progeny production was inversely related to spinosad rate for all strains, and ranged from 0 to 170 adults/jar. At the highest spinosad rate (0.06 mg[AI]/kg), the laboratory strain failed to produce any progeny, but 8 to 10 adults/jar were observed in the two field strains. In the two field strains, reduction in progeny production on spinosad-treated wheat was 31–97% relative to production of the same strains on untreated wheat. The ED_{99} values for progeny reduction of *R. dominica* strains were 7 to 15 times higher than the corresponding ED_{50} values (Table 9). The ED_{99} values for progeny reduction of the three strains was ≤0.1 mg(AI) of spinosad per kilogram of grain. Comparison of the ED_{99} values for the CF and RL field strains using the ratio test showed that they were not significantly different ($P > 0.05$) from one another (1.40 [0.78–2.50]). The ED_{99} value of the CF strain was significantly different ($P < 0.05$) from that of the laboratory strain (2.22 [1.38–3.57]), but the ED_{99} value of RL and laboratory strains were not different ($P > 0.05$) from one another (1.59 [0.93–2.71]).

Discussion

Spinosad is a promising grain protectant (Hertlein et al. 2011) and it has not been commercially released yet. In the current study, a 32-fold variation in adult mortality among *T. castaneum strains* and six-fold variation among *O. surinamensis* strains was observed when exposed to spinosad-treated wheat. Variation in susceptibility among different insect species, and different strains of the same species, to an insecticide is common (Subramanyam et al. 1989, Subramanyam and Hagstrum 1996, Kljajić and Perić 2006). Huang et al. (2004) reported the field strains of *T. castaneum*, *P. interpunctella*, and *C. ferrugineus* were two to six times less susceptible to spinosad than the corresponding laboratory strains based on LD_{95} values. Strains of *T. confusum* from different locations in Europe differed markedly in their susceptibility to spinosad with adult mortalities ranging from 1 to 81% after a 7 d exposure on wheat treated with a dry spinosad formulation applied at a rate of 0.19 mg(AI)/kg (Athanassiou et al.

Table 8. Probit regression estimates (mean ± SE) showing effective dose (ED) for progeny reduction for the laboratory and field strains of *O. surinamensis* exposed to spinosad-treated wheat

Strain	N[a]	Mean ± SE		ED (95% CL) (mg[AI]/kg)		χ^2 (df)	P value
		Intercept	Slope	ED_{50}	ED_{99}		
Lab.	525	0.75 ± 0.19	2.95 ± 0.53	0.56 (0.36–0.79)	3.43 (1.83–20.23)	21.45 (4)	0.0003*
AB1	600	0.19 ± 0.12	2.63 ± 0.34	0.85 (0.64–1.03)	6.49 (4.75–10.72)	1.61 (2)	0.448
AB2	600	0.25 ± 0.12	3.11 ± 0.45	0.83 (0.63–0.99)	4.66 (3.50–7.61)	0.27 (2)	0.872

[a] Total no. of insects used in bioassays.

* Goodness-of-fit of the probit model to dose-response data was significant ($P < 0.05$), indicating poor fit of the model to data.

Table 9. Probit regression estimates (mean ± SE) showing effective dose (ED) for progeny reduction for the laboratory and field strains of *R. dominica* exposed to spinosad-treated wheat

Strain	N^a	Mean ± SE		ED (95% CL) (mgAI/kg)		χ^2 (df)	*P* value
		Intercept	Slope	ED_{50}	ED_{99}		
Lab.	975	5.54 ± 0.42	2.55 ± 0.21	0.007 (0.006–0.008)	0.05 (0.04–0.08)	6.96 (5)	0.224
CF	600	4.16 ± 0.30	2.00 ± 0.16	0.008 (0.007–0.010)	0.12 (0.09–0.19)	1.94 (5)	0.858
RL	600	4.85 ± 0.49	2.38 ± 0.26	0.009 (0.007–0.011)	0.09 (0.06–0.19)	11.12 (5)	0.049*

[a] Total no. of insects used in bioassays.
* Goodness-of-fit of the probit model to dose-response data was significant ($P < 0.05$), indicating poor fit of the model to data.

2008). The large variation in susceptibility among strains may be because of differences in mobility, insecticide absorption, penetration, and metabolism.

In our study, both the laboratory and field strains of *T. castaneum* were less susceptible to spinosad even at rates much higher than the labeled rate. Previous studies have shown T. castaneum adults to be less susceptible to spinosad on wheat (Fang et al. 2002a,b; Flinn et al. 2004; Subramanyam et al. 2007). However, susceptibility of *T. castaneum* adults to spinosad at the labeled rate varies with the wheat class. For example, the 14 d mortality of *T. castaneum* adults ranged from 2 to 55% on hard red spring, soft red winter, hard red winter, and durum wheats (Fang et al. 2002a). Our results showed that even though there was wide variation in adult mortality among strains, there was 90–100% reduction in progeny production relative to that on untreated wheat, except in the CF strain. The low adult progeny production on spinosad-treated wheat in 10 out of the 11 field strains indicated that the neonates are highly susceptible to spinosad. Toews and Subramanyam (2003) reported 100% suppression of egg-to-adult emergence of *T. castaneum* exposed as eggs to wheat treated with spinosad at the labeled rate after 14 d. Athanassiou et al. (2008) found the third to fourth instars of six *T. confusum* strains from different locations of Europe to be generally more susceptible to spinosad than adults. The high progeny production in the CF strain may be an anomaly and not an indicator of resistance because the LD_{99} value of this strain was similar to that of the laboratory strain in dose–response tests.

The field strains of *O. surinamensis* showed reduced susceptibility to spinosad when compared with the laboratory strain. This was confirmed in dose–response tests as the LD_{99} values of the two field strains were about six times higher than that of the laboratory strain. However, progeny production was not adversely affected as shown by similar ED_{99} values of the laboratory and field strains. Therefore, there may be natural tolerance or resistance in *O. surinamensis* field strains with no reproductive disadvantage. Complete progeny suppression in *O. surinamensis* could not be achieved which may be because of lower susceptibility of neonates to spinosad. Toews and Subramanyam (2003) reported that the 14 d mortality of *O. surinamensis* larvae exposed as eggs to labeled rate of spinosad on whole hard red winter wheat was ≈80% indicating reduced susceptibility of first instars. Effectiveness of spinosad against *O. surinamensis* varies depending on the type of commodity. Mortality of *O. surinamensis* adults ranged from 3 to 76% among different classes of wheat treated with spinosad at the labeled rate after 7 and 14 d of exposure (Fang et al. 2002a). Subramanyam et al. (1999) also found *O. surinamensis* to be moderately susceptible to spinosad on hard red spring wheat based on adult mortality, and significant reduction in adult progeny production was observed only at rates ≥3 mg(AI)/kg. Spinosad was highly effective against *O. surinamensis* on corn based on >98% mortality after 12 d of exposure and complete progeny suppression (Huang and Subramanyam 2007). Daglish et al. (2003) found the adult mortality of *O. surinamensis* was <10% after 14 d of exposure to spinosad-treated wheat resulting in only 7% reduction in progeny production.

Spinosad was effective against *R. dominica* adults of all strains as evidenced by complete mortality within 7 d and complete progeny suppression. These findings are consistent with previous studies, which showed *R. dominica* to be highly susceptible to spinosad even at low rates of 0.1–0.5 mg(AI)/kg (Fang et al. 2002a,b; Flinn et al. 2004, Huang et al. 2004, Daglish and Nayak 2006, Getchell and Subramanyam 2008, Subramanyam et al. 2012). The high susceptibility of *R. dominica* adults to spinosad coupled with delayed mortality effects on adults after brief exposures to spinosad-treated wheat (Getchell and Subramanyam 2008, Boina et al. 2012) contribute to spinosad's overall effectiveness against this species.

Chlorpyrifos-methyl plus deltamethrin was a much better protectant on wheat against laboratory and field strains of *T. castaneum, O. surinamensis,* and *R. dominica* based on 100% or close to 100% mortality of adults after 7 d of exposure and total progeny suppression. Lack of progeny production could be because of rapid mortality of introduced adults before they have had a chance to lay eggs, or high susceptibility of first instars hatching from eggs laid by the adults. Chlorpyrifos-methyl plus deltamethrin was effective against the psocid species *Lepinotus reticulatus* Enderlein, *Liposcelis entomophila* (Enderlein), *Liposcelis bostrychophila* Badonnel, and *Liposcelis paeta* Pearman on rice and wheat (Athanassiou et al. 2009). It was also effective against *R. dominica, S. oryzae,* and *T. castaneum* on wheat (Subramanyam et al. 2012). The combined treatment of chlorpyrifos-methyl at 6 mg(AI)/kg and deltamethrin at 0.5 or 1 mg(AI)/kg resulted in 100% mortality of *R. dominica* and *S. oryzae* on wheat and *T. castaneum* on corn (Arthur 1994).

Our study provides baseline data on susceptibility of laboratory and field strains of three stored-grain insect species to two of the insecticides approved as grain protectants. This information would be helpful in screening changes in susceptibility of these species to insecticides once these insecticides are used in the field on a continuous basis. Baseline data generated here can be used to detect development of incipient resistance. Additional work is needed to determine the behavioral or physiological factors responsible for the differential performance of spinosad among strains.

In summary, our results show that chlorpyrifos-methyl plus deltamethrin is an ideal insecticide to use on stored wheat for controlling adults of *R. dominica*, *T. castaneum*, and *O. surinamensis* strains and for suppression of progeny production. There are several reports of resistance in stored-grain insects to deltamethrin (Lorini and Galley 1999, Perez–Mendoza 1999, Ribeiro et al. 2003) and to chlorpyrifos-methyl (Beeman and Wright 1990, Zettler and Cuperus 1990, Collins et al. 1993, Guedes et al. 1996, Ribeiro et al. 2003), but not to the combination product. Therefore, it is advisable to use the combination product in rotation with spinosad or other approved grain protectants (Arthur and Subramanyam 2012) as part of an integrated insect pest management and resistance management programs.

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References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Anonymous. 2007. Chlorpyrifos-methyl; product cancellation order. Environmental Protection Agency [EPA-HQ-OPP-2004-0043; FRL-8155-5]. Fed. Reg. 72: 68580–68581.

Arthur, F. H. 1994. Efficacy of unsynergised deltamethrin and deltamethrin plus chlorpyrifos-methyl combinations as protectants of stored wheat and stored corn (maize). J. Stored Prod. Res. 30: 87–94.

Arthur, F. H., and Bh. Subramanyam. 2012. Chemical control in stored products, pp. 95–100. *In* D. W. Hagstrum, T. W. Phillips, and G. Cuperus (eds.), Stored Product Protection. Kansas State University, Manhattan, KS.

Athanassiou, C. G., N. G. Kavallieratos, and G. J. Chintzoglou. 2008. Effectiveness of spinosad dust against different European populations of the confused flour beetle, *Tribolium confusum* Jacquelin du Val. J. Stored Prod. Res. 44: 47–51.

Athanassiou, C. G., F. H. Arthur, and J. E. Throne. 2009. Efficacy of grain protectants against four psocid species on maize, rice and wheat. Pest Manag. Sci. 65: 1140–1146.

Athanassiou, C. G., F. H. Arthur, and J. E. Throne. 2010. Effects of short exposures to spinosad-treated wheat or maize on four stored-grain insects. J. Econ. Entomol. 103: 197–202.

Beeman, R. W., and V. F. Wright. 1990. Monitoring for resistance to chlorpyrifos-methyl, pirimiphos-methyl and malathion in Kansas populations of stored-product insects. J. Kans. Entomol. Soc. 63: 385–392.

Boina, D. R., Bh. Subramanyam, and K. Mutambuki. 2012. Delayed mortality responses of *Rhyzopertha dominica* (F.) adults subjected to short exposures on spinosad-treated wheat. J. Stored Prod. Res. 48: 149–152.

Chintzoglou, G. J., C. G. Athanassiou, A. N. Markoglou, and N. G. Kavallieratos. 2008. Influence of commodity on the effect of spinosad dust against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Intl. J. Pest Manag. 54: 277–285.

Collins, P. J., T. M. Lambkin, B. W. Bridgeman, and C. Pulvirenti. 1993. Resistance to grain-protectant insecticides in coleopterous pests of stored cereals in Queensland, Australia. J. Econ. Entomol. 86: 239–245.

Daglish, G. J., and M. K. Nayak. 2006. Long-term persistence and efficacy of spinosad against *Rhyzopertha dominica* (Coleoptera : Bostrychidae) in wheat. Pest Manag. Sci. 62: 148–152.

Daglish, G. J., M. K. Nayak, and V. S. Byrne. 2003. Is there a role for spinosad in protecting Australian grain from insects? *In* E. J. Wright, M. C. Webb, and E. Highley (eds.), Stored Grain in Australia 2003. Proceedings, Australian Postharvest Technical Conference, Canberra, 25–27 June 2003.

Fang, L., Bh. Subramanyam, and F. H. Arthur. 2002a. Effectiveness of spinosad on four classes of wheat against five stored-product insects. J. Econ. Entomol. 95: 640–650.

Fang, L., Bh. Subramanyam, and S. Dolder. 2002b. Persistence and efficacy of spinosad residues in farm stored wheat. J. Econ. Entomol. 95: 1102–1109.

Flinn, P. W., Bh. Subramanyam, and F. H. Arthur. 2004. Comparison of aeration and spinosad for suppressing insects in stored wheat. J. Econ. Entomol. 97: 1465–1473.

Getchell, A. I., and Bh. Subramanyam. 2008. Immediate and delayed mortality of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae) adults exposed to spinosad-treated commodities. J. Econ. Entomol. 101: 1022–1027.

Guedes, R.N.C., B. A. Dover, and S. Kambhampati. 1996. Resistance to chlorpyrifos-methyl, pirimiphos-methyl, and malathion in Brazilian and US populations of *Rhyzopertha dominica* (Coleoptera: Bostrichidae). J. Econ. Entomol. 89: 27–32.

Hertlein, M. B., G. D. Thompson, Bh. Subramanyam, and C. G. Athanassiou. 2011. Spinosad: a new natural product for stored grain protection. J. Stored Prod. Res. 47: 131–146.

Huang, F., and Bh. Subramanyam. 2007. Effectiveness of spinosad against seven major stored-grain insects on corn. Insect Sci. 14: 225–230.

Huang, F., Bh. Subramanyam, and M. D. Toews. 2004. Susceptibility of laboratory and field strains of four stored-product insect species to spinosad. J. Econ. Entomol. 97: 2154–2159.

Kavallieratos, N. G., C. G. Athanassiou, B. J. Vayias, S. Kotzamanidis, and S. D. Synodis. 2010. Efficacy and adherence ratio of diatomaceous earth and spinosad in three wheat varieties against three stored-product insect pests. J. Stored Prod. Res. 46: 73–80.

Kljajić, P., and I. Perić. 2006. Susceptibility to contact insecticides of granary weevil *Sitophilus granarius* (L.) (Coleoptera : Curculionidae) originating from different locations in the former Yugoslavia. J. Stored Prod. Res. 42: 149–161.

Lorini, I., and D. J. Galley. 1999. Deltamethrin resistance in *Rhyzopertha dominica* (F.) (Coleoptera : Bostrichidae), a pest of stored grain in Brazil. J. Stored Prod. Res. 35: 37–45.

Nayak, M. K., G. J. Daglish, and V. S. Byrne. 2005. Effectiveness of spinosad as a grain protectant against resistant beetle and psocid pests of stored grain in Australia. J. Stored Prod. Res. 41: 455–467.

Perez–Mendoza, J. 1999. Survey of insecticide resistance in Mexican populations of maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera : Curculionidae). J. Stored Prod. Res. 35: 107–115.

Ribeiro, B. M., R.N.C. Guedes, E. E. Oliveira, and J. P. Santos. 2003. Insecticide resistance and synergism in Brazilian populations of *Sitophilus zeamais* (Coleoptera : Curculionidae). J. Stored Prod. Res. 39: 21–31.

Robertson, J. L., and H. K. Preisler. 1992. Pesticide bioassays with arthropods. CRC, Boca Raton, FL.

SAS Institute. 2008. SAS/STAT 9.2 user's guide. SAS Institute, Cary, NC.

Subramanyam, Bh., P. K. Harein, and L. K. Cutkomp. 1989. Organophosphate resistance in adults of red flour beetle (Coleoptera, Tenebrionidae) and sawtoothed grain beetle (Coleoptera, Cucujidae) infesting barley stored on farms in Minnesota. J. Econ. Entomol. 82: 989–995.

Subramanyam, Bh., J. J. Nelson, R. A. Meronuck, and E. A. Flora. 1999. Evaluation of spinosad on stored-product insects, pp. 940–949. *In* Z. Jin, Q. Liang, Y. Liang, X. Tan, and L. Guan (eds.), Proceedings, 7th International Working Conference on Stored-Product Protection, 14–19 October 1998, Beijing, China. Sichuan Publishing House of Science and Technology, Chengdu, China.

Subramanyam, Bh., and D. W. Hagstrum. 1996. Resistance measurement and management, pp. 331–397. *In* Bh. Subramanyam and D. W. Hagstrum (eds.), Integrated management of insects in stored products. Marcel Dekker, Boston, MA.

Subramanyam, Bh., M. Hartzer, and D. R. Boina. 2012. Performance of pre-commercial release formulations of spinosad against five stored-product insect species on four stored commodities. J. Pest Sci. 85: 331–339.

Subramanyam, Bh., M. D. Toews, K. E. Ileleji, D. E. Maier, G. D. Thompson, and T. J. Pitts. 2007. Evaluation of spinosad as a grain protectant on three Kansas farms. Crop Prot. 26: 1021–1030.

Subramanyam, Bh., D. W. Hagstrum, and T. C. Schenk. 1993. Sampling adult beetles (Coleoptera) associated with stored grain: comparing detection and mean trap catch efficiency of two types of probe traps. Environ. Entomol. 22: 33–42.

Toews, M. D., and Bh. Subramanyam. 2003. Contribution of contact toxicity and wheat condition to mortality of stored-product insects exposed to spinosad. Pest Manag. Sci. 59: 538–544.

Vayias, B. J., C. G. Athanassiou, and C. T. Buchelos. 2009. Effectiveness of spinosad combined with diatomaceous earth against different European strains of *Tribolium confusum* du Val (Coleoptera: Tenebrionidae): influence of commodity and temperature. J. Stored Prod. Res. 45: 165–176.

Vayias, B. J., C. G. Athanassiou, D. N. Milonas, and C. Mavrotas. 2010. Persistence and efficacy of spinosad on wheat, maize and barley grains against four major stored product pests. Crop Prot. 29: 496–505.

Zar, J. H. 1984. Biostatistical analysis, 2nd ed. Prentice Hall, Englewood Cliffs, NJ.

Zettler, J. L., and G. W. Cuperus. 1990. Pesticide resistance in *Tribolium castaneum* (Coleoptera, Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera, Bostrichidae) in wheat. J. Econ. Entomol. 83: 1677–1681.

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