Susceptibility of Laboratory and Field Strains of Four Stored-Product Insect Species to Spinosad

FANGNENG HUANG,[1] BHADRIRAJU SUBRAMANYAM, AND MICHAEL D. TOEWS[2]

Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506

J. Econ. Entomol. 97(6): 2154–2159 (2004)

ABSTRACT Two field strains of the Indianmeal moth, *Plodia interpunctella* (Hübner); red flour beetle, *Tribolium castaneum* (Herbst); and lesser grain borer, *Rhyzopertha dominica* (F.), and one field strain of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), were collected from hard red winter wheat stored on farms in northeastern Kansas. Fifty eggs of *P. interpunctella* and 25 beetle adults of each species were exposed to 100 g of untreated wheat or wheat treated with various rates of spinosad, to determine susceptibility of the field and corresponding insecticide-susceptible laboratory strains. Mortality of beetle adults and *P. interpunctella* larvae was assessed after 7 and 21 d postinfestation, respectively. Field strains of *P. interpunctella*, *C. ferrugineus*, and *T. castaneum* were less susceptible to spinosad than the corresponding laboratory strains. The LD_{50} and LD_{95} values for *P. interpunctella* and *C. ferrugineus* field strains were 1.7–2.5 times greater than values for corresponding laboratory strains. Adults of both laboratory and field strains of *T. castaneum* were tolerant to spinosad, resulting in <88% mortality at 8 mg/kg. The LD_{50} and LD_{95} values for the field strains of *T. castaneum* were 2.0–7.5 times greater compared with similar values for the laboratory strain. The field and laboratory strains of *R. dominica* were highly susceptible to spinosad, and one of the field strains was relatively less susceptible to spinosad than the laboratory strain. Our results confirm a range of biological variability in field populations, which is consistent with findings for other compounds, and underscores the need to adopt resistance management programs with stored grain insect pests. The baseline data generated on the susceptibility of the four insect species to spinosad will be useful for monitoring resistance development and for setting field rates.

KEY WORDS stored-product insects, wheat, spinosad, baseline susceptibility

SPINOSAD IS A REDUCED RISK insecticide based on metabolites of a soil bacterium, *Saccharopolyspora spinosad* Mertz & Yao (Mertz and Yao 1990). This bacterial insecticide has a unique mode of action with a very low mammalian toxicity compared with other insecticides (Bert et al. 1997; Salgado 1997; Thompson et al. 1997, 2000). Laboratory and field data have shown spinosad at 1 mg([AI])/kg to be effective against several major stored-grain insects (Fang et al. 2002a, b; Toews and Subramanyam 2003; Toews et al. 2003; Huang and Subramanyam 2004). Dow AgroSciences (Indianapolis, IN), the manufacturer of spinosad, was granted an experimental use permit (EUP) by the United States Environmental Protection Agency (U.S. EPA) (experimental use permit no. 62719-EUP-50) on 31 May 2002, for conducting trials on farms to document the effectiveness of spinosad at 1 mg/kg on wheat, barley, oats, sorghum, and corn against stored grain insect pests. A registration application has been submitted to the U.S. EPA to use spinosad at 1 mg/kg as a grain protectant, and a decision is expected some time before December 2004.

Notable geographical variation in susceptibility to spinosad has been reported in several field crop insects, such as the cotton bollworm, *Helicoverpa armigera* (Hübner) (Ahmad et al. 2003); diamondback moth, *Plutella xylostella* (L.) (Shelton et al. 2000, Zhao et al. 2002); and beet armyworm, *Spodopera exigua* (Hübner) (Mascarenhas et al. 1998, Moulton et al. 2000). The variation in susceptibility of field strains of stored-grain insects to spinosad is not known. The objective of this study was to determine the susceptibility of field strains of four stored product insect species, collected from two locations in northeastern Kansas, to spinosad.

Materials and Methods

Insects. Adults of the Indianmeal moth, *Plodia interpunctella* (Hübner); rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); red flour beetle, *Tribolium castaneum* (Herbst); and lesser grain borer, *Rhyzoper-*

This paper reports research results only. Mention of a proprietary product name does not constitute an endorsement for its use by Kansas State University or the USDA.

[1] E-mail: bhs@wheat.ksu.edu.

[2] Current address: USDA–ARS, Grain Marketing and Production Research Center, Manhattan, KS 66502.

0022-0493/04/2154–2159$04.00/0 © 2004 Entomological Society of America

Table 1. Sources of stored-product insect strains used in bioassays with spinosad

Insect species	Strain	Date	Source	No. adults collected
P. interpunctella	Laboratory	>10 yr	Department of Grain Science and Industry, Kansas State University	
	Abilene	16 Sept. 2002	Farm bin with 2002 crop year hard red winter wheat treated on 8 July 2002 with spinosad at 1 mg/kg	65
	Morganville	16 Sept. 2002	Farm bin with untreated hard red winter wheat	43
C. ferrugineus	Laboratory	>10 yr	Department of Grain Science and Industry, Kansas State University	
	Abilene	13 Feb. 2002	Farm bin with 2002 crop year hard red winter wheat treated on 8 July 2002 with chlorpyrifos-methyl at 3 mg/kg	>200
T. castaneum	Laboratory	>10 yr	Department of Grain Science and Industry, Kansas State University	
	Abilene	16 Sept. 2002	Farm bin with untreated 2002 crop year hard red winter wheat	>200
	Morganville	16 Sept. 2002	Farm bin with untreated 2002 crop year hard red winter wheat	>200
R. dominica	Laboratory		Department of Grain Science and Industry, Kansas State University	
	Abilene	16 Sept. 2002	Farm bin with 2002 crop year untreated hard red winter wheat	>200
	Abilene	13 Feb. 2002	Farm bin with 2002 crop year hard red winter wheat treated on 8 July 2002 with chlorpyrifos-methyl at of 3 mg/kg	>200

tha dominica (F.), were collected from round metal bins holding wheat at two locations, Abilene and Morganville, in northeastern Kansas (Table 1). Field-collected insects were reared in 0.95-liter glass jars at 28°C, 65% RH, and a photoperiod of 14:10 (L:D) h in the laboratory. Field strains of *P. interpunctella* were reared on a poultry-mash diet (Subramanyam and Cutkomp 1987). Eggs of *P. interpunctella*, produced by the first generation of moths from the field-collected strains, were used in bioassays. Cultures of *C. ferrugineus* strains were reared on a mixture of 95% rolled oats and 5% (by weight) brewer's yeast; *T. castaneum* was reared on a mixture of 95% wheat flour and 5% (by weight) brewer's yeast; and *R. dominica* was reared on whole, hard red winter wheat. Bioassays with field strains of *C. ferrugineus* and *R. dominica* were conducted using the third generation adults, whereas the second generation adults were used for *T. castaneum.* Laboratory strains of the four insect species, maintained in cultures for over a decade without insecticide exposure, were assayed simultaneously and served as standard insecticide-susceptible strains. All laboratory strains were reared on diets mentioned above for each species.

Insecticide. Spinosad (SpinTor 2 SC) containing 240 mg ([AI])/ml was provided by Dow AgroSciences. Insecticide dilutions were made in distilled water.

Grain. Hard red winter wheat (variety Jagger) was obtained from the Milling Laboratory in the Department of Grain Science and Industry, Kansas State University (Fang et al. 2002a). Dockage and broken kernels were removed by manual sieving over a 0.21-mm round-holed aluminum sieve (Seedburo Equipment Company, Chicago, IL). Cleaned wheat was frozen for 1 wk at −13°C to kill any residual insect infestation. Wheat was tempered and equilibrated to 13% moisture in environmental growth chambers maintained at 28°C and 65% RH.

Hatchability of *P. interpunctella* Eggs. Three replicates of 200 eggs each of laboratory and field strains of *P. interpunctella* were placed in glass petri dishes (25 mm diameter × 10 mm high). These dishes were placed on the surface of 100 g of untreated wheat (treated with 0.1 ml of distilled water only) held in 0.45-liter glass jars, so that larvae hatching from eggs could infest the wheat. These jars were placed in a growth chamber maintained at the same conditions as described above. Eggs hatching in each dish were examined after 7 d. Percentage of egg hatchability was calculated from the number of eggs that hatched out of the total (200).

Bioassays. Wheat kernels were treated with spinosad to provide nominal rates of 0, 0.0078, 0.016, 0.031, 0.063, 0.125, 0.25, 0.5, 1, and 2 mg/kg in bioassays with *P. interpunctella*, *C. ferrugineus*, and *R. dominica.* Two additional rates (4 and 8 mg/kg) were used in bioassays with *T. castaneum*, because adults of this species are known to be less susceptible to spinosad than the other species (Fang et al. 2002a, b; Toews and Subramanyam 2003).

Wheat (100 g), placed in separate 0.45-liter glass jars, was treated with 0.1 ml of the insecticide solution. Wheat treated with aliquots of distilled water served as the control treatment (0 mg/kg). Jars containing grain treated with the insecticide or distilled water were placed in a plastic drum (38-liter capacity) that was tumbled on a ball-mill roller for 10 min to ensure uniform coverage of insecticide on the kernels. After tumbling, 50 eggs (≤24 h old) of *P. interpunctella* or 25 unsexed adults of each beetle species were introduced into each jar containing 100 g of untreated or spinosad-treated wheat. Infested jars were closed with wire mesh and filter paper lids. Jars were incubated at 28°C, 65% RH, and a photoperiod of 14:10 (L:D) h. Wheat infested with insects was sieved and examined 21 d after egg introduction to count the number of live *P. interpunctella* larvae or after 7 d to count the number

Table 2. Probit regression estimates (mean ± SE) for laboratory and field strains of four stored-product insect species exposed to spinosad-treated wheat

Strain	Total no. insects	Intercept	Slope	LD_{50} (95% CL) (mg/kg)	LD_{95} (95% CL) (mg/kg)	χ^2 (df)
P. interpunctella						
Laboratory	1,698	3.69 ± 0.38	2.72 ± 0.27	0.04 (0.03–0.06)	0.18 (0.12–0.30)	29.6 (5)*
Abilene	1,650	2.33 ± 0.20	1.97 ± 0.15	0.07 (0.05–0.08)	0.45 (0.32–0.73)	24.8 (7)*
Morganville	1,728	2.80 ± 0.25	2.37 ± 0.20	0.07 (0.05–0.08)	0.33 (0.24–0.51)	28.2 (7)*
C. ferrugineus						
Laboratory	875	3.12 ± 0.19	2.76 ± 0.16	0.07 (0.07–0.08)	0.29 (0.25–0.36)	4.5 (5)
Abilene	875	2.42 ± 0.15	2.69 ± 0.15	0.12 (0.11–0.14)	0.52 (0.44–0.64)	8.0 (5)
T. castaneum						
Laboratory	1,125	−0.06 ± 0.04	1.14 ± 0.06	1.10 (0.93–1.32)	30.54 (20.36–50.24)	8.5 (7)
Abilene	625	−1.12 ± 0.15	1.55 ± 0.27	5.31 (3.22–15.89)	61.18 (18.80–2981)	9.7 (3)*
Morganville	1,000	−1.00 ± 0.08	1.15 ± 0.12	8.33 (4.73–14.16)	196.6 (69.6–1129)	11.1 (6)
R. dominica						
Laboratory	857	4.57 ± 0.89	2.25 ± 0.50	0.009 (0.003–0.015)	0.050 (0.029–0.233)	33.7 (5)*
Abilene[a]	500	7.85 ± 1.15	3.41 ± 0.57	0.005 (0.004–0.006)	0.016 (0.013–0.020)	1.2 (2)
Abilene[b]	1,000	4.57 ± 1.96	2.22 ± 1.09	0.009[c]	0.048[c]	191.0 (6)*

* Goodness-of-fit of the probit model to dose/response data was significant ($P < 0.05$), indicating poor fit of the model to data.
[a] Strain collected from a bin holding untreated wheat.
[b] Strain collected from a bin holding wheat treated with 3 mg/kg of chlorpyrifos-methyl.
[c] The 95% CL for the lethal values could not be computed.

of live and dead beetle adults. Each insect strain by spinosad rate combination was replicated five times, and each replicate was treated separately.

Data Analysis. Proportion of *P. interpunctella* eggs (x) of laboratory and field strains that hatched on untreated wheat was transformed using arcsine ($x^{0.5}$) to normalize treatment variances (Zar 1984), followed by one-way analysis of variance (ANOVA) using the GLM procedure (SAS Institute 1999), to detect differences among insect strains. Treatment means were separated using Fisher's protected least significant difference (LSD) test at the $\alpha = 0.05$ level.

The mean mortality for each insect species on spinosad-treated wheat was corrected for mortality on untreated wheat (Abbott 1925). Corrected dose/mortality data were subjected to probit analysis (SAS Institute 1999) for determining spinosad dose producing 50% (LD_{50}) and 95% (LD_{95}) mortality of insects and the associated 95% confidence limits (CL). Significant differences ($\alpha = 0.05$) among strains of each insect species to spinosad were verified by comparing individual probit regression models to a pooled model (Draper and Smith 1981).

Results

Hatchability of *P. interpunctella* Eggs. Egg hatchability varied among the three *P. interpunctella* strains ($F = 40.72$; df = 2, 3; $P = 0.0003$). The egg hatchability (mean ± SE) of the laboratory strain was 97.0 ± 1.0%, which was significantly greater than that of the Abilene (73.3 ± 1.4%) and Morganville (76.8 ± 2.9%) strains. Egg hatchability of the two field strains was similar ($P > 0.05$).

Responses of *P. interpunctella*. Mortality (mean ± SE) of larvae of the laboratory, Abilene, and Morganville strains on untreated wheat was 32.0 ± 3.4, 29.6 ± 3.3, and 22.4 ± 1.5%, respectively. The LD_{50} and LD_{95} values among the field and laboratory strains ranged from 0.04 to 0.45 mg/kg (Table 2). The LD values for the two field strains were 2–2.5 times greater than that of the laboratory strain. The probit regression models of the two field strains were not significantly different from one another ($F = 0.37$; df = 2, 14; $P = 0.7$). However, the probit regression model of the laboratory strain was significantly different from that of the Abilene ($F = 11.3$; df = 2, 12; $P < 0.002$) and Morganville strains ($F = 10.81$; df = 2, 12; $P = 0.002$).

Responses of *C. ferrugineus*. The mean ± SE adult mortality of the laboratory and field strains of *C. ferrugineus* on untreated wheat was 1.6 ± 1.0 and 0.8 ± 0.8%, respectively. Although adults of both *C. ferrugineus* strains were highly susceptible to spinosad, the LD_{50} or LD_{95} value of the field strain was approximately two-fold higher than that of the laboratory strain (Table 2). The probit regression model of the field strain was significantly different from that of the laboratory strain ($F = 4.93$; df = 2, 10; $P = 0.032$).

Responses of *T. castaneum*. *T. castaneum* adults were relatively less susceptible to spinosad compared with the other species tested. Adult mortality of the three strains on untreated wheat was <4%. The LD_{50} and LD_{95} values among strains ranged from a low of 1.1 to a high of 196.6 mg/kg (Table 2). Adults of the two field strains were less susceptible to spinosad than the laboratory strain. For example, the LD_{50} values of the two field strains were five- to eight-fold higher than that of the laboratory strain. Similarly, the LD_{95} values of the field strains were two- to six-fold greater than that of the laboratory strain. The probit regression model of the laboratory strain was significantly different from that of the Abilene ($F = 38.78$; df = 2, 10; $P < 0.0002$) and Morganville strains ($F = 47.22$; df = 2, 13; $P < 0.00001$). However, the probit regression models of the two field strains were not different from one another ($F = 3.30$; df = 2, 9; $P = 0.084$).

Responses of *R. dominica*. Mortality of adults of the three strains on untreated wheat was <3%. Adults of all three strains of *R. dominica* were highly susceptible to spinosad, with the LD_{50} and LD_{95} values ranging

between 0.005 and 0.05 mg/kg (Table 2). The LD_{50} and LD_{95} values of the laboratory strain and the field strain collected from wheat treated with chlorpyrifos-methyl were essentially similar and 2–3 times greater than that of the field strain collected from the untreated bin. The probit regression model of the laboratory strain was different from that of the strain collected from the untreated bin ($F = 19.41$; df = 2, 7; $P = 0.0014$), but not from the strain collected from the chlorpyrifos-methyl treated bin ($F = 0.49$; df = 2, 11; $P = 0.625$). The probit regression models of the field strains were significantly different from one another ($F = 11.27$; df = 2, 8; $P < 0.005$).

Discussion

Field strains of *P. interpunctella*, *C. ferrugineus*, and *T. castaneum* were generally less susceptible to spinosad than the corresponding laboratory strains. Our findings are consistent with those reported in literature for field crop (Hasty et al. 1997, Shelton et al. 2000) and stored product insect pests (Subramanyam et al. 1989, Beeman and Wright 1990) tested with different insecticides. For example, Subramanyam et al. (1989) reported that four field strains of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), collected from farm-stored barley, were less susceptible to the grain protectant chlorpyrifos-methyl, compared with a laboratory strain. Stored barley from which the four field strains of *O. surinamensis* were collected had not been treated with chlorpyrifos-methyl, because at the time of insect collection this insecticide was not yet approved for grain treatment.

Differences in susceptibility observed among *R. dominica* strains is not of practical significance, because our current study and that of others (Fang et al. 2002a, b; Toews and Subramanyam 2003) has shown this species to be highly susceptible to spinosad at low rates. Although *T. castaneum* adults were less susceptible to spinosad, the 1 mg/kg rate is effective in preventing progeny production as shown in laboratory and field trials (Fang et al. 2002 a, Flinn et al. 2004), because larvae hatching from the eggs are highly susceptible to spinosad. Therefore, the 1 mg/kg rate should be effective in preventing population growth of *T. castaneum* despite survival of adults at this rate.

Resistance to various insecticides has been documented in *P. interpunctella* larvae and *C. ferrugineus* and *T. castaneum* adults (Subramanyam and Hagstrum 1995). The decreased susceptibility of field strains of these three species to spinosad is not likely caused by cross-resistance, because several field insects that are resistant to conventional insecticides do not show any cross-resistance to spinosad due to its novel mode of action (Scott 1998, Liu and Yue 2000, Scott et al. 2000, Hardee et al. 2001, Wei et al. 2001, Levot et al. 2002). Spinosad is toxic to insects by its action on the insect nervous system at the nicotinic acetylcholine receptor and GABA receptor sites (Salgado 1997, 1998).

Geographic variation in susceptibility to insecticides is common, and such variability also has been observed in several insect pests exposed to spinosad (Sparks et al. 1996, Mascarenhas et al. 1998, Moulton et al. 2000, Shelton et al. 2000, Zhao et al. 2002, Ahmad et al. 2003). For example, larvae of a strain of *S. exigua*, collected from a cotton field in Lyford, TX, was 3.6-fold more tolerant to spinosad than a laboratory strain (Sparks et al. 1996). Third instars of two of seven field strains of this moth pest collected from Alabama, California, Louisiana, Texas, and Rio Bravo, Mexico, were 2.3- to 3.3-fold more tolerant to spinosad than a laboratory strain that had been reared without exposure to insecticides for 5 yr (Mascarenhas et al. 1998). Moulton et al. (2000) also reported that second instars of seven field strains of *S. exigua* collected from United States and Thailand were 2.5- to 72-fold more tolerant to spinosad than the laboratory strain. The LC_{50} values for second instars of *P. xylostella* in seven of nine field-collected strains from California, where spinosad was not used before the collections were made, was 10.6- to 14.7-fold higher than that of a laboratory strain (Geneva 88). The Geneva-88 laboratory strain was reared for 199–206 generations before use in bioassays (Shelton et al. 2000).

The laboratory insect strains used in the current study had been reared on insecticide-free diets for over a decade. Low genetic diversity due to inbreeding of laboratory strains could explain their increased susceptibility to spinosad when compared with the field strains. The decreased susceptibility to spinosad in field strains implies that the minimum effective rate of spinosad determined using laboratory strains may provide less than satisfactory control of the field strains. For example, spinosad at the currently proposed label rate of 1 mg/kg was effective against the laboratory strains of *P. interpunctella* (Fang et al. 2002a, 2002b), whereas our current study showed spinosad at this rate did not provide 100% egg-to-larval mortality of the two field strains. Spinosad rates that result in survival of a few individuals may, over time, lead to development of resistance to this insecticide.

Nearly 25% or more of the insecticide is lost during application to the grain (Thomas et al. 1987, le Patourel 1992, Collins and Cook 1998). Thus, wheat treated with the spinosad solution to obtain the proposed label rate of 1 mg/kg will likely result in less than the target dose immediately after application. Furthermore, uneven application and coating of insecticide residue on individual kernels, because of differences in kernel shape and size, could further reduce insecticide residues in some parts of the grain mass. In a recent field study, the actual spinosad deposit levels on wheat immediately after treatment at the 1 mg/kg application rate ranged from 0.4 to 0.8 mg/kg (Flinn et al. 2004). Therefore, field application rates should be carefully calibrated to minimize insecticide loss that occurs during application so that the target rate (e.g., 1 mg/kg) can be deposited on the grain. Our findings highlight the importance of determining the most appropriate rate suitable for controlling field strains of insects. The baseline data presented here for laboratory and field strains can be used to monitor changes in susceptibility of the four insect species in

Kansas, once spinosad is registered and used for managing insects in stored wheat.

Acknowledgments

We thank Gerald Wilde and Gary Thompson for reviewing an earlier draft of the manuscript. Muktinutalapati Laxminarayan assisted in conducting laboratory bioassays. This research was supported by Dow AgroSciences (Indianapolis, IN) and USDA–CSREES (RAMP) under Agreement No. 00-51101–9674. This article is Contribution No. 04-203-J of the Kansas Agricultural Experiment Station.

References Cited

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

Ahmad, M., M. I. Arif, and Z. Ahmad. 2003. Susceptibility of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to new chemistries in Pakistan. Crop Protection 22: 539–544.

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.

Bert, B. L., L. L. Larson, J. R. Schoonover, T. C. Sparks, and G. D. Thompson. 1997. Biological properties of spinosad. Down to Earth 52: 6–13.

Collins, D. A., and D. A. Cook. 1998. Periods of protection provided by different formulations of pirimiphos-methyl and etrimos, when admixed with wheat against four susceptible storage beetle pests. Crop Protection 17: 521–528.

Draper, N. R., and H. Smith. 1981. Applied regression analysis, 2nd ed. Wiley, New York.

Fang, L., Bh. Subramanyam, and F. 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.

Hardee, D. D., L. C. Adams, and G. W. Elzen. 2001. Monitoring for changes in tolerance and resistance to insecticide in bollworm/tobacco budworm in Mississippi, 1996–1999. Southwest. Entomol. 26: 364–372.

Hasty, M., E. Durham, and G. Payne. 1997. Evaluation of the susceptibility of tobacco budworm (*Heliothis virescens*) and cotton bollworm (*Helicoverpa zea*) populations in Georgia to various insecticides. 1997 Proceedings, Beltwide Cotton Conference, New Orleans, LA. 2: 1292–1293.

Huang, F., and Bh. Subramanyam. 2004. Responses of *Corcyra cephalonica* (Stainton) to pirimiphos-methyl, spinosad, and combinations of pirimiphos-methyl and synergized pyrethrins. Pest Manag. Sci. 60: 191–198.

le Patourel, G. 1992. Residues and efficacy of etrimfos and pirimiphos-methyl in wheat and malting barley stored in ventilated bins. Crop Protection 11: 470–476.

Levot, G. W., J. T. Rothwell, and N. Sales. 2002. Baseline laboratory bioassay data for spinosad against populations of Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae). Aust. J. Entomol. 41: 78–81.

Liu, N., and X. Yue. 2000. Insecticide resistance and cross-resistance in the house fly (Diptera: Muscidae). J. Econ. Entomol. 93: 1269–1275.

Mascarenhas, V. J., J. B. Graves, B. R. Leonards, and E. Burris. 1998. Susceptibility of field population of beet armyworm (Lepidoptera: Noctuidae) to commercial and experimental insecticides. J. Econ. Entomol. 91: 827–833.

Mertz, E. P., and R. C. Yao. 1990. *Saccharopolyspora spinosa* sp. nov. isolated from soil collected in a sugar rum still. Int. J. Sust. Bacteriol. 40: 34–39.

Moulton, J. K., D. A. Pepper, and T. J. Dennehy. 2000. Beet armyworm (*Spodoptera exigua*) resistance to spinosad. Pest Manag. Sci. 56: 842–848.

Salgado, V. L. 1997. The modes of action of spinosad and other insect control products. Down to Earth 52: 35–43.

Salgado, V. L. 1998. Studies on the mode of action of spinosad: insect symptoms and physiological correlates. Pestic. Biochem. Physiol. 60: 91–102.

SAS Institute. 1999. SAS/STAT user's guide, version 8. SAS Institute, Cary, NC.

Scott, J. G. 1998. Toxicity of spinosad to susceptible and resistant strains of house flies, *Musca domestica.* Pestic. Sci. 54: 131–133.

Scott, J. G., T. G. Alefantis, P. E. Kaufman, and D. A. Rutz. 2000. Insecticide resistance in house flies from caged-layer poultry facilities. Pest Manag. Sci. 56: 147–153.

Shelton, A. M., F. V. Sances, J. Hawley, J. D. Tang, M. Boune, D. Jungers, H. L. Collins, and J. Farias. 2000. Assessment of insecticide resistance after the outbreak of diamondback moth (Lepidoptera: Plutellidae) in California in 1997. J. Econ. Entomol. 93: 931–936.

Sparks, A. N., J. W. Norman, and D. A. Wolfenbarger. 1996. Efficacy of selected insecticides against the beet armyworm, *Spodoptera exigua*-field and laboratory evaluations. 1996 Proceedings of the Beltwide Cotton Conference, Nashville, TN. 2: 844–864.

Subramanyam, Bh., and L. K. Cutkomp. 1987. Total lipid and free fatty acid composition in male and female larvae of Indian-meal moth and almond moth (Lepidoptera: Pyralidae). Great Lakes Entomol. 20: 99–102.

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., and D. W. Hagstrum. 1995. Resistance measurement and management, pp. 331–397. *In* Bh. Subramanyam and D. W. Hagstrum [eds.], Integrated management of insects in stored products. Marcel Dekker, New York.

Thomas, K. P., D. B. Pinniger, and R. W. Wilkin. 1987. An assessment of chlorpyrifos-methyl, etrimfos, fenitrothion, and pirimiphos-methyl. Pestic. Sci. 21: 57–72.

Thompson, G. D., K. H. Michel, R. C. Yao, J. S. Mynderse, C. T. Mosburg, T. V. Worden, E. H. Chio, T. C. Sparks, and S. H. Hutchins. 1997. The discovery of *Saccharopolyspora spinosa* and a new class of insect control products. Down to Earth 52: 1–5.

Thompson, G. D., R. Dutton, and T. C. Sparks. 2000. Spinosad-a case study: an example from a natural products discovery programme. Pest Manag. Sci. 56: 696–702.

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.

Toews, M. D., Bh. Subramanyam, and J. Rowan. 2003. Knockdown and mortality of eight stored-product beetles exposed to four surfaces treated with spinosad. J. Econ. Entomol. 96: 1967–1973.

Wei, Y., A. G. Appel, W. J. Moar, and N. Liu. 2001. PBO and pyrethrins resistance and cross resistance in the German

cockroach, *Blattella germanica* (L). Pest Manag. Sci. 57: 1055–1059.

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

Zhao, J. Z., Y. X. Li, H. L. Collins, L. Gusukuma-Minuto, R.F.L. Mau, G. D. Thompson, and A. M. Shelton. 2002. Monitoring and characterization of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad. J. Econ. Entomol. 95: 430–436.

Received 29 January 2004; accepted 25 July 2004.