Survival of Stored-Product Insect Natural Enemies in Spinosad-Treated Wheat

MICHAEL D. TOEWS¹ AND BHADRIRAJU SUBRAMANYAM

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

J. Econ. Entomol. 97(3): 1174-1180 (2004)

ABSTRACT The survival of stored product insect natural enemies in wheat treated with spinosad was investigated in laboratory and pilot scale experiments. The predator *Xylocoris flavipes* (Reuter), the warehouse pirate bug, and the parasitoids *Habrobracon hebetor* (Say), *Theocolax elegans* (Westwood), and *Anisopteromalus calandrae* (Howard) were exposed to wheat treated with aliquots of water or spinosad at 0.05–1 mg ([AI])/kg. *X. flavipes* was the only species that survived (92% survival) in spinosad-treated wheat at 1 mg/kg. *X. flavipes* suppressed populations of immature *Tribolium castaneum* (Herbst), the red flour beetle, by nearly 90% compared with a water-treated control, but 100% suppression of immatures was achieved in wheat receiving spinosad or spinosad + *X. flavipes* treatments. A 3-mo pilot scale experiment to evaluate *T. castaneum* suppression in drums holding 163.3 kg of wheat showed that the pest populations increased throughout the study in the control treatment, but peaked after 1 mo in the *X. flavipes*-treated drums. By comparison, better *T. castaneum* population suppression was achieved in spinosad or spinosad + *X. flavipes* can survive and reproduce in spinosad-treated wheat, under our test conditions spinosad alone provided adequate suppression of *T. castaneum* populations in stored wheat.

KEY WORDS Saccharopolyspora spinosa, stored-product insects, biological control, natural enemies

SPINOSAD, A COMMERCIAL PESTICIDE based on the fermentation products of the bacterium *Saccharopolyspora spinosa* Mertz & Yao, is efficacious against several insects associated with stored grain in laboratory (Fang et al. 2002a, Toews and Subramanyam 2003) and field evaluations (Fang et al. 2002b). Spinosad is currently labeled for use on vegetable crops, ornamentals, and forest trees (Thompson et al. 2000), but not on stored grain. In May 2002, an experimental use permit for use on grain at 1 mg([AI])/kg was approved by the U.S. Environmental Protection Agency (EPA Experimental Use Permit No. 62719-EUP-50) that facilitated full-scale field trials with this pesticide on farms in Kansas and other states.

The compatibility of low rates of spinosad with parasitoids of stored product insect pests is unknown. Bret et al. (1997) reported that spinosad was much less toxic to beneficial insects in field crops than synthetic pesticides. Schoonover and Larson (1995) reported that spinosad was practically nontoxic to the insidious flower bug, *Orius insidiosus* (Say) (Heteroptera: Anthocoridae); convergent lady beetle, *Hippodamia con*- vergens Guérin-Méneville (Coleoptera: Coccinellidae); phytoseid mite, *Phytoseiulus persimilis* Athias Henriot (Acari: Phytoseiidae); and common green lacewing, *Chrysoperla plorabunda* (Fitch) (Neuroptera: Chrysopidae). Boucher (1999) reported that spinosad applied to bell peppers effectively controlled the pepper maggot, *Zonosemata electa* (Say) (Diptera: Tephritidae), but it did not reduce populations of beneficial arthropods, including unspecified species of Coccinellidae, Chrysopidae, Cecidomyiidae, Syrphidae, Nabidae, and hymenopteran-parasitized Aphididae. Mason et al. (2002) found that spinosad was toxic to the parasitoids *Trichogramma inyoense* Pinto & Oatman (Hymenoptera: Trichogrammatidae) and *Microplitis mediator* Haliday (Hymenoptera: Braconidae).

In the present investigation, we evaluated the susceptibility of several parasitoids and a predator of stored product insect pests to spinosad-treated wheat in the presence and absence of hosts or prey. Additionally, we determined the effectiveness of spinosad in combination with a predator to suppress the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), in hard red winter wheat stored in 208-liter plastic drums. *T. castaneum* was used in experiments because the adults are relatively less susceptible than other stored-product insect species to spinosad at 1 mg/kg (Fang et al. 2002a,b; Toews and Subramanyam 2003).

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 United States Department of Agriculture.

¹Current address: USDA-ARS, Grain Marketing and Production Research Center, 1515 College Ave., Manhattan, KS 66502 (e-mail: mtoews@gmprc.ksu.edu).

Materials and Methods

Wheat. Hard red winter wheat, previously stored for 24 mo in a round metal bin at the Kansas State University Grain Storage Training Center, Manhattan, KS, was used in tests. Four weeks before use in experiments, the wheat was fumigated with aluminum phosphide (Pestcon Systems, Inc., Raleigh, NC) to kill any live insects. Wheat (≈ 2 kg) was removed for use in experiments from the bin by using a 1.2-m-long grain trier (Seedburo Equipment Co., Chicago, IL). The dockage content, shrunken/broken kernels, foreign material, damaged kernels, and total defects of wheat, determined following official methods (GIPSA 1997), was 0.1, 1.0, 0.3, 0.6, and 1.9% by weight, respectively. The test weight or bulk density of wheat was 79.1 kg/hl, and the moisture content was 12.1%. Before use in laboratory experiments, the wheat was frozen for 7 d at -13°C to kill any insects that survived the fumigation. Moisture content of the wheat used in all experiments ranged from 11.7 to 13.3%.

Insects. All natural enemies of stored-product insects were reared in the laboratory to produce sufficient numbers of known ages. Cultures of the Indianmeal moth, Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae), were reared on a turkeymash diet (Subramanyam and Cutkomp 1987). The rice weevil, Sitophilus oryzae (L.) (Coleoptera: Curculionidae), was reared on whole hard red winter wheat, and T. castaneum was reared on whole wheat flour plus 5% (by weight) brewer's yeast. The parasitoids Theocolax elegans (Westwood) (Hymenoptera: Pteromalidae) and two strains (Savannah and Bamberg) of Anisopteromalus calandrae (Howard) (Hymenoptera: Pteromalidae), were obtained from the USDA-ARS, Grain Marketing and Production Research Center, Manhattan, KS. The Bamberg strain is resistant to malathion (Baker 1994, Baker and Weaver 1993), whereas the Savannah strain is not. T. elegans and A. calandrae were reared by introducing 100 adult wasps (<3 d old) into 100 g of whole wheat containing approximately 100 fourth instars of S. oryzae. Habrobracon hebetor (Say) (Hymenoptera: Braconidae) and a predator, the warehouse pirate bug, Xylocoris flavipes (Reuter) (Heteroptera: Anthocoridae), were obtained from BioFac Crop Care (Mathis, TX). H. hebetor was reared using the wandering stage larvae (fifth instars) of *P. interpunctella*, whereas *X. flavipes* was reared on *T. castaneum* eggs ($\leq 3 d \text{ old}$) placed in rolled oats. We placed 40 X. flavipes adults in 100 g of rolled oats with ≈ 200 T. castaneum eggs, twice each week. All insects were reared in 0.95-liter glass jars placed in environmental growth chambers (model I-36VL, Percival Scientific, Boone, IA) maintained at 28°C and 65% RH with a photoperiod of 16:8 (L:D) h. All natural enemy and T. castaneum adults used in experiments, unless otherwise specified, were ≤ 3 and ≤ 7 d old, respectively.

Spinosad. SpinTor 2SC (Dow AgroSciences, Indianapolis, IN) formulation containing 240 mg([AI]) spinosad/ml was diluted in distilled water to the appropriate concentration for grain treatment.

Spinosad Toxicity to Natural Enemies. The survival of natural enemies in spinosad-treated wheat was determined in the presence and absence of host insects in separate laboratory experiments. In the first experiment, each 0.95-liter glass jar, filled with 100 g of uninfested wheat, was treated with 100 μ l of distilled water (control) or 100 μ l of spinosad solution to provide rates of 0-1 mg/kg. Each natural enemy by rate treatment was replicated eight times, and each replicate was treated separately. After adding distilled water or spinosad solution to the grain, the jars were fitted with wire mesh and filter paper lids and tumbled for 20 min on a ball-mill roller (Morse Manufacturing Co., model 200VS, East Syracuse, NY), to ensure uniform insecticide coverage on kernels. Twenty-four hours after tumbling, 20 adults each of A. calandrae (Savannah strain), H. hebetor, T. elegans, or X. flavipes were introduced into untreated- or spinosad-treated wheat in a jar. All jars were held at 28°C, 65% RH, and a photoperiod of 16:8 (L:D) h. After 24-h exposure, natural enemies were separated from the wheat using a standard testing sieve with 1.68-mm mesh openings (No. 12, Seedburo Equipment Co.). Natural enemies that could walk or fly when prodded with a fine camel's-hair brush were considered to be alive.

In the second experiment, adults of A. calandrae and T. elegans were exposed to wheat infested with S. oryzae and treated with water or spinosad solution. Some members of the Pteromalidae require host feeding for normal oögenesis (Clausen 1940); thus, we investigated the potential that host feeding may increase parasitoid survival in spinosad-treated wheat. Wheat was first infested with S. *oryzae* and held in an environmental chamber until fourth instars were present, based on published developmental data (Sharifi and Mills 1971). The wheat was then treated with either water or 1 mg/kg spinosad solution. H. hebetor was not included in this study because S. oryzae is not a host for this parasitoid. In this experiment, the Bamberg strain of A. calandrae was included to determine whether malathion resistance would confer any survival benefits to parasitoids in spinosad-treated wheat. Survival was assessed as described above.

X. flavipes Suppression of T. castaneum in Spinosad-Treated Wheat. Because X. flavipes exhibited significant survival in the previous experiments, we investigated the ability of X. flavipes to suppress T. castaneum immatures in spinosad-treated wheat. Individual 0.95-liter jars containing 100 g of wheat received one of the following four treatments: distilled water, 1 mg/kg spinosad, 1 mg/kg spinosad + three female and two male X. flavipes adults, or distilled water + three female and two male X. *flavipes* adults. Wheat in jars was tumbled on a ball-mill roller for 20 min and held at room conditions for 24 h before introduction of insects. Each jar (n = 7 per treatment)was infested with 100 T. castaneum eggs ($\leq 3 \text{ d old}$), followed by the introduction of natural enemies. After 7 d in the growth chamber at 28°C and 65% RH with a photoperiod of 16:8 (L:D) h, all insects were separated from the wheat and the number of live *T. castaneum* larvae was recorded. In the second experiment, 50 first and second instars of *T. castaneum* were placed in separate 0.94-liter jars (n = 7 per treatment) containing 100 g of wheat that received the same treatments as described above. *X. flavipes* introduction occurred 4 h after introduction of *T. castaneum* larvae into the wheat. After 7 d, insects were separated from the grain as described above, and the number of live *T. castaneum* larvae was recorded.

X. flavipes nymphs were observed in the spinosadtreated replications described above in which only predator adults were introduced. Therefore, we investigated the ability of X. flavipes to reproduce in spinosad-treated wheat in the third test. Glass jars (0.95 liter), each containing 400 g of wheat, were treated with either distilled water (control treatment, n = 7) or 1 mg/kg spinosad solution (treatment, n =7) and then infested with 10 female and 10 male T. castaneum adults. Jars were placed in the growth chamber for 2 wk after which five female and two male X. flavipes were introduced into each jar. Jars were examined after 8 wk, and the number of live T. castaneum adults and X. flavipes adults and nymphs was counted.

In the fourth experiment, the ability of X. flavipes to suppress T. castaneum populations over a 3-mo period was evaluated in 208-liter plastic drums, each holding 163.3 kg of wheat. Wheat for this study could not be frozen due to practical constraints on freezer space. Two unbaited probe traps (Storgard WB-II, Trécé Inc., Salinas, CA), inserted into the wheat bulk for 48 h before grain treatment, did not capture any live insects. This indicated that the wheat was relatively insect-free before use in our experiments. The wheat in each drum (n = 3 per treatment) was treated with distilled water, water + X. flavipes, spinosad at 1 mg/ kg, or spinosad at 1 mg/kg + X. flavipes. Wheat was preweighed and then moved into an overhead bin with a pneumatic conveyor where a gate regulated the flow out of the overhead bin. Distilled water or aqueous spinosad suspension was applied to the falling grain stream (0.7 ml/kg of grain) with a sprayer (model 1126, Cummins Industrial Tools, Spring Hill, KS) powered by compressed air at 103.4-kPa pressure. From the overhead bin, wheat fell 1 m into a grain hopper that fed the wheat into a 4.9-m-long electric grain auger (10.2 cm diameter), positioned 30° from horizontal that dropped the grain into the plastic drums.

Forty unsexed adults of *T. castaneum* (≤ 2 wk old) were introduced into each drum 5 d after grain treatment. Three weeks after *T. castaneum* introduction, five female and five male *X. flavipes* adults (≤ 1 wk old) were released into each of the appropriate treatment drums. Plastic drums were then individually sealed and stored in the research flour mill located in the third floor of the Department of Grain Science and Industry, Kansas State University.

Four separate grain samples (\approx 350 g per sample) were taken monthly from each drum with a 1.2-m-long grain trier (Seedburo Equipment Co.) to determine

the number of live *T. castaneum* adults and *X. flavipes* adults and nymphs. Each of the four samples spanned from the top to the bottom of the grain mass. The four samples from each drum were combined and the composite sample was sieved to separate live insects from the grain. At the termination of the test (3 mo), the wheat in each drum was sieved over an inclined sieve (White 1983) to determine the absolute numbers of live *T. castaneum* adults and *X. flavipes* adults and nymphs.

Temperature and relative humidity of grain in a drum were recorded 30 cm below the grain surface near the drum center using a HOBO data logger (Onset Computer Corp., Bourne, MA). A second HOBO unit was placed on top of a drum lid to record ambient temperature and relative humidity in the room. All of the drums were stored adjacent to each other so we assumed conditions to be similar among drums. Mean \pm SE grain temperature 30 cm below the grain surface inside the drums was 19.6 \pm 0.4°C. The temperature and relative humidity of the ambient air in the room was 20.3 \pm 1.0°C and 66.4 \pm 5.0%, respectively.

Data Analyses. A completely random design was used for all tests. Survival of natural enemies was adjusted for mortality in the control treatments by using the method of Abbott (1925). The response variables were the proportion of the sample population that survived in the laboratory (jar) experiments and actual number of live insects recovered in the drum study. Before statistical procedures, proportions were transformed using arcsine square root, whereas the number of live insects was transformed to logarithmic scale (Zar 1984). Statistical inferences were made after subjecting data to PROC MIXED (SAS Institute 1999), with degree of freedom adjustments for the variance components following the methods of Satterthwaite (1946). The analysis of variance (ANOVA) for repeated measures was used for analyzing drum data as the same experimental units (drums) were sampled monthly. Treatment means were separated using pairwise comparisons of least squares means (LSMEANS) at the $\alpha = 0.05$ level. Untransformed means and standard errors are presented in tables and figures.

Results

Spinosad Toxicity to Natural Enemies. On untreated wheat (control), the survival (mean \pm SE) of natural enemies was 72.2 \pm 2.8% for *T. elegans*, 87.6 \pm 2.7% for *X. flavipes*, 90.7 \pm 2.4% for *A. calandrae*, and 71.0 \pm 4.8% for *H. hebetor*. Survival of these natural enemies in spinosad-treated wheat, adjusted for mortality in the control treatments, is shown in Fig. 1. *X. flavipes* was the only species that had >90% survival in wheat treated with 1 mg/kg spinosad. All hymenopteran species experienced <30% survival in wheat treated with 0.1 mg/kg spinosad, and none survived in wheat treated with 1 mg/kg spinosad.

The presence of hosts did not affect the survival of *A. calandrae* and *T. elegans* in the control and spinosad-



Fig. 1. Mean \pm SE proportion of natural enemies surviving 24-h exposure to spinosad-treated wheat (no hosts present). Data were adjusted for mortality in the control replicates.

treated wheat. Survival in the control treatments with hosts present was 79.2 ± 11.6 and $87.6 \pm 2.2\%$ for the Bamberg and Savannah strains of *A. calandrae*, respectively, and $75.6 \pm 6.0\%$ for *T. elegans*. The survival of these species in wheat treated with 1 mg/kg spinosad was <2%.

X. flavipes Suppression of T. castaneum in Spinosad-Treated Wheat. In the test with T. castaneum eggs, many more T. castaneum larvae were recovered in the control treatment than in the other treatments (Fig. 2). Spinosad treatment alone decreased T. castaneum larval survival by 90%, whereas no larvae were recovered in the X. flavipes and spinosad + X. flavipes treatments. Similar results were observed in experiments starting with T. castaneum first and second instars (Fig. 3). There was an 88% decrease in T. castaneum larval numbers in spinosad and X. flavipes only treatments relative to the control treatment. No T. castaneum larvae were recovered from the combination treatment.



Fig. 2. Mean \pm SE proportion of live *T. castaneum* larvae recovered after 7 d in jars of wheat initially infested with 100 eggs. Means followed by different letters are significantly different (P < 0.05; LSMEANS test).



Fig. 3. Mean \pm SE proportion of live *T. castaneum* larvae recovered after 7 d in jars of wheat initially infested with 50 first and second instars. Means followed by different letters are significantly different (P < 0.05; LSMEANS test).

In the 8-wk study, we observed significant survival of *T. castaneum* adults and survival and reproduction of *X. flavipes* (Fig. 4). The mean number of *T. castaneum* adults recovered at the end of the study was similar between the control and spinosad treatments (F = 0.41; df = 11, 1; P = 0.534). Likewise, the number of *X. flavipes* adults (F = 1.88; df = 1, 11; P = 0.197) and nymphs (F = 4.07; df = 1, 11; P = 0.069) recovered also was similar between these two treatments. The presence of *X. flavipes* nymphs clearly indicates adult reproduction and nymphal survival on spinosad-treated wheat, but it is not clear whether the adult insects recovered from the experiment represent survivors of the initial cohort (introduced insects) or progeny that matured to adulthood.

Overall repeated measures ANOVA showed that there were significant differences among treatments in the number of live *T. castaneum* adults recovered from the grain trier samples (F = 44.6; df = 3, 8; P =0.001). The control treatment had 7.6 \pm 2.1 *T. castaneum* adults per kilogram of grain, which was statis-



Fig. 4. Mean \pm SE number of *T. castaneum* adults, *X. flavipes* adults, and *X. flavipes* nymphs recovered after 8 wk in jars of *T. castaneum*-infested wheat treated with aliquots of distilled water (control treatment) or spinosad at 1 mg/kg. Treatment means within species and life stage are not significantly different from one another (see text for details).

| Insect and life stage | Month | Treatment | | | |
|-----------------------|-------|-----------------|--------------------------|--------------------------|--------------------------|
| | | Control | X. flavipes | Spinosad | Spinosad + X. flavipes |
| T. castaneum adults | 1 | $3.3 \pm 0.6a$ | 2.8 ± 0.1 a | $0.0 \pm 0.0 \mathrm{b}$ | $0.0 \pm 0.0 \mathrm{b}$ |
| | 2 | $8.3 \pm 2.5a$ | $2.4 \pm 0.9 \mathrm{b}$ | $0.3\pm0.3\mathrm{c}$ | $0.0\pm0.0\mathrm{c}$ |
| | 3 | $11.3 \pm 5.2a$ | $2.4 \pm 1.0b$ | $0.0\pm0.0\mathrm{c}$ | $0.0\pm0.0\mathrm{c}$ |
| X. flavipes adults | 1 | a | $1.4 \pm 1.1a$ | _ | $0.0 \pm 0.0a$ |
| | 2 | _ | $0.3 \pm 0.3a$ | _ | $0.0 \pm 0.0a$ |
| | 3 | _ | $0.5 \pm 0.3a$ | _ | 0.0 ± 0.0 a |
| X. flavipes nymphs | 1 | _ | $0.2 \pm 0.2a$ | _ | 0.0 ± 0.0 a |
| 5 7 7 1 | 2 | _ | $1.0 \pm 0.7a$ | _ | 0.0 ± 0.0 a |
| | 3 | — | $0.5\pm0.3a$ | _ | $0.0\pm0.0a$ |

Table 1. Number (mean \pm SE) of live *T. castaneum* adults, *X. flavipes* adults, and *X. flavipes* nymphs per kilogram of wheat obtained using a grain trier during the 3-mo drum test

Means within a row (month) followed by different letters are significantly different (P < 0.05; LSMEANS test). ^{*a*} Not applicable.

tically different (P < 0.05) from the X. flavipes treatment (2.6 ± 0.4) (Table 1). The spinosad (0.1 ± 0.1) and the spinosad + X. flavipes treatments (0.0 ± 0.0) were statistically similar (P > 0.05).

Absolute densities derived from sieving all of the grain at the end of the study (Table 2) showed differences among treatments in the number of T. castaneum adults (F = 29.26; df = 3, 8; P = 0.001) or *X. flavipes* adults (F = 12.73; df = 1, 4; P = 0.023), but not *X. flavipes* nymphs (F = 2.92; df = 1, 4; P = 0.163). Contamination by 105 live sawtoothed grain beetles, Oryzaephilus surinamensis (L.) (Coleoptera: Silvanidae), was observed while sifting one drum of the X. flavipes treatment. Although exclusion of this replicate from the data set decreased the mean T. castaneum population in the X. flavipes treatment from 67.0 ± 52.7 to 14.5 ± 7.5 adults per drum, the number of T. castaneum adults present was still greater than that observed in the spinosad or spinosad + X. flavipes treatments (Table 2).

Discussion

Our results indicate that spinosad treatment of hard red winter wheat at 1 mg/kg greatly decreased the survival of all the hymenopteran parasitoids, but not that of the predator *X. flavipes*. A similar lack of survival of the parasitoid species in tests with and without hosts leads us to believe that spinosad poisoning adversely affected the parasitoids. The Savannah and Bamberg strains of *A. calandrae* were equally susceptible to spinosad, suggesting that malathion resistance in the Bamberg strain did not confer cross-resistance to spinosad. This result is not surprising, because spinosad has a completely different mode of action than the organophosphates (Salgado 1998, Sparks et al. 2001). Survivorship of hymenopteran species in the control (distilled water) treatments was not 100%, probably because of the sieving process, in which delicate parasitoid species may have suffered additional mortality. *X. flavipes* is a more stout-bodied insect, and was perhaps, less affected by the sieving. A general lack of survival among hymenopterans exposed to spinosad was also reported in the literature (Tillman and Mulrooney 2000, Mason et al. 2002, Michaud 2003).

There is limited evidence to suggest that *X. flavipes* is more pesticide-tolerant than parasitoids and pest insects. Baker and Arbogast (1995) reported X. flavipes to be 4- and 10-fold more tolerant to malathion than A. calandrae and H. hebetor, respectively. Press et al. (1978) reported that X. flavipes generally exhibited greater tolerance to the insecticides permethrin, fenitrothion, pirimiphos-methyl, pyrethrins + piperonyl butoxide, and malathion than three prey species, including T. castaneum; the cigarette beetle, Lasioderma serricorne (F.); and *P. interpunctella*. Tillman and Mulrooney (2000) found that counts of a hemipteran predator Geocoris punctipes (Say) (Hemiptera: Lygaidae), were not affected by spinosad in cotton fields. The exact mechanism by which *X. flavipes* is able to metabolize or tolerate moderately high doses of pesticides, including spinosad, is unknown and warrants further study.

Suppression of *T. castaneum* in spinosad-treated wheat was variable. In experiments with *T. castaneum* eggs and small larvae, very few larvae were recovered after 7 d, which indicated high susceptibility of *T. castaneum* larvae to spinosad. In the 8-wk experiments, we recovered nearly one-half of the introduced

Table 2. Number (mean \pm SE) of live *T. castaneum* adults, *X. flavipes* adults, and *X. flavipes* nymphs recovered after sieving all of the grain in drums after 3 mo

| Insect and life stage | Treatment | | | | |
|--|-----------------|---|-----------------------|--|--|
| | Control | X. flavipes | Spinosad | Spinosad + X. flavipes | |
| <i>T. castaneum</i> adults <i>X. flavipes</i> adults <i>X. flavipes</i> nymphs | $396.0\pm50.0a$ | $67.0 \pm 52.7b$ 14.0 \pm 9.5a 21.0 \pm 18.1a | $1.0\pm0.6\mathrm{c}$ | $\begin{array}{c} 0.3 \pm 0.3 c \\ 0.0 \pm 0.0 b \\ 0.0 \pm 0.0 a \end{array}$ | |

Means within a row followed by different letters are significantly different (P < 0.05; LSMEANS test).

June 2004

Contamination by *O. surinamensis* in one replication of *X. flavipes* treated grain in the drum study likely decreased the observed suppression of *T. castaneum. O. surinamensis* attacks nearly all cereal grains and has a reproductive potential of 6–10 eggs per female per day (Howe 1956). Because *O. surinamensis* is a known prey of *X. flavipes* (Arbogast 1976), the reduced suppression of *T. castaneum* was likely due to *X. flavipes* feeding on the sizable *O. surinamensis* population in addition to the *T. castaneum* population. The exact time and source of the contamination remains unknown, but it could have resulted from immigration during the 4-wk window between fumigation of the grain and drum-filling.

Our results indicate that X. *flavipes* can survive, reproduce, and suppress T. *castaneum* populations in wheat treated with $\leq 1 \text{ mg/kg}$ spinosad, whereas the parasitoids were highly susceptible to spinosad poisoning. T. *castaneum* suppression with spinosad at 1 mg/kg alone was generally equal to or greater than the suppression achieved with X. *flavipes*. This finding leads us to believe there may be limited benefits in combining spinosad with X. *flavipes* for T. *castaneum* suppression in the storage situations tested.

Acknowledgments

We thank Jackie Rowan, Zeb Larson, and Glen Swartz for technical assistance. Paul Flinn, James Baker, David Weaver, and BioFac Crop Care graciously supplied starter material for the natural enemy cultures. Dow AgroSciences donated the spinosad sample. We thank R. T. Arbogast and J. P. Michaud for helpful comments on an earlier version of this manuscript. Research reported here was funded by Region VII EPA, Kansas City, MO, and partially, by CSREES–USDA (RAMP) under Agreement No. 00-51101–9674. This paper is Contribution No. 04-182-J of the Kansas Agricultural Experiment Station.

References Cited

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.
- Arbogast, R. T. 1976. Suppression of Oryzaephilus surinamensis (L.) (Coleoptera: Cucujidae) on shelled corn by the predator Xylocoris flavipes (Reuter) (Hemiptera: Anthocoridae). J. Ga. Entomol. Soc. 11: 67–71.
- Baker, J. E. 1994. Resistance mechanism in the Bamberg strain of Anisopteromalus calandrae (Hymenoptera: Pteromalidae). J. Entomol. Sci. 29: 580–584.
- Baker, J. E., and R. T. Arbogast. 1995. Malathion resistance in field strains of the warehouse pirate bug (Heteroptera: Anthocoridae) and a prey species *Tribolium castaneum* (Coleoptera: Tenebrionidae). J. Econ. Entomol. 88: 241– 245.

- Baker, J. E., and D. K. Weaver. 1993. Resistance in field strains of the parasitoid Anisopteromalus calandrae (Hymenoptera: Pteromalidae) and its host, Sitophilus oryzae (Coleoptera: Curculionidae), to malathion, chlorpyrifosmethyl, and pirimiphos-methyl. Biol. Control 3: 233–242.
- Boucher, J. 1999. Effect of spinosad on bell peppers pests and beneficial arthropods, 1998. Arthropod Manage. Tests 24: 144.
- Bret, 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.
- Clausen, C. P. 1940. Entomophagous insects. Hafner Publishing Co., Inc., New York.
- 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. Fate and efficacy of spinosad residues in farm-stored wheat. J. Econ. Entomol. 95: 1102–1109.
- GIPSA. 1997. Grain inspection handbook, book II, wheat. http://ww.usda.gov/gipsa/reference-library/handbooks/ grain-insp/grbook2/gihbk2.htm.
- Howe, R. W. 1956. The biology of the two common storage species of *Oryzaephilus* (Coleoptera, Cucujidae). Ann. Appl. Biol. 44: 341–355.
- Mason, P. G., M. A. Erlandson, R. H. Elliott, and B. J. Harris. 2002. Potential impact of spinosad on parasitoids of *Mamestra configurata* (Lepidoptera: Noctuidae). Can. Entomol. 134: 59–68.
- Michaud, J. P. 2003. Toxicity of fruit fly baits to beneficial insects in citrus. J. Insect Sci. 3:8, available online: insectscience.org/3.8.
- Press, J. W., B. R. Flaherty, and L. L. McDonald. 1978. Toxicity of five insecticides to the predaceous bug *Xylocoris flavipes* (Hemiptera: Anthocoridae). J. Ga. Entomol. Soc. 13: 177–181.
- 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.
- Satterthwaite, F. E. 1946. An approximate distribution of estimates of variance components. Biometrics Bull. 2: 110–114.
- Schoonover, J. R., and L. L. Larson. 1995. Laboratory activity of spinosad on non-target beneficial arthropods. Arthropod Manage. Tests 20: 357.
- Sharifi, S., and R. B. Mills. 1971. Developmental activities and behavior of the rice weevil inside wheat kernels. J. Econ. Entomol. 64: 1114–1118.
- Sparks, T. C., G. D. Crouse, and G. Durst. 2001. Natural products as insecticides: the biology, biochemistry and quantitative structure-activity relationships of spinosyns and spinosoids. Pest Manage. Sci. 57: 96–905.
- 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.
- Thompson, G. D., R. Dutton, and T. C. Sparks. 2000. Spinosad-a case study: an example from a natural products discovery programme. Pest Manage. Sci. 56: 696–702.
- Tillman, P. G., and J. E. Mulrooney. 2000. Effect of selected insecticides on the natural enemies *Coleomegilla maculata* and *Hippodamia convergens* (Coleoptera: Coccinellidae), *Geocoris punctipes* (Hemiptera: Lygaeidae), and *Bracon mellitor*, *Cardiochiles nigriceps*, and *Cotesia*

marginiventris (Hymenoptera: Braconidae) in cotton. J. Econ. Entomol. 93: 1638–1643.

- Toews, M. D., and Bh. Subramanyam. 2003. Contribution of contact toxicity and wheat condition to mortality of stored-product insects exposed to spinosad. Pest Manage. Sci. 59: 538–544.
- White, G. G. 1983. A modified inclined sieve for separation of insects from wheat. J. Stored Prod. Res. 19: 89–91.
- Zar, J. H. 1984. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, NJ.

Received 19 December 2003; accepted 6 March 2004.