INSECTICIDE RESISTANCE AND RESISTANCE MANAGEMENT

Efficacies of Spinosad and a Combination of Chlorpyrifos-Methyl and Deltamethrin Against Phosphine-Resistant *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) on Wheat

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J. Econ. Entomol. 106(5): 2208–2215 (2013); DOI: http://dx.doi.org/10.1603/EC13215

ABSTRACT  Highly phosphine-resistant populations of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) have recently been found in Oklahoma grain storage facilities. These findings necessitate development of a phosphine resistance management strategy to ensure continued effective use of phosphine. Therefore, we investigated the efficacies of two grain insecticides, namely, spinosad applied at label rate of 1 ppm and a mixture of chlorpyrifos-methyl and deltamethrin applied at label rates of 3 and 0.5 ppm, respectively, against highly phosphine-resistant *R. dominica* and *T. castaneum*. Adult mortality and progeny production suppression of spinosad- or chlorpyrifos-methyl/deltamethrin mixture-treated wheat that had been stored for 2, 84, 168, 252, and 336 d posttreatment were assessed. We found that both spinosad and chlorpyrifos-methyl/deltamethrin were effective against phosphine-resistant *R. dominica* and caused 83–100% mortality and also caused total progeny production suppression for all storage periods. Spinosad was not effective against phosphine-resistant *T. castaneum*; the highest mortality observed was only 3% for all the storage periods. Chlorpyrifos-methyl/deltamethrin was effective against phosphine-resistant *T. castaneum* only in treated wheat stored for 2 and 84 d, where it caused 93–99% mortality. However, chlorpyrifos-methyl/deltamethrin was effective and achieved total suppression of progeny production in *T. castaneum* for all the storage periods. Spinosad was not as effective as chlorpyrifos-methyl/deltamethrin mixture at suppressing progeny production of phosphine-resistant *T. castaneum*. These two insecticides can be used in a phosphine resistance management strategy for *R. dominica* and *T. castaneum* in the United States.

KEY WORDS  stored product, red flour beetle, lesser grain borer, insecticide resistance, phosphine resistance management

Oklahoma produced 4.2 million tonnes (155 million bushels) of winter wheat (*Triticum aestivum* L.) worth US$1.2 billion in 2012 (National Agricultural Statistics Service [NASS] 2013). In Oklahoma, phosphine gas (hydrogen phosphide or PH₃) is the method of choice for fumigating stored grain to manage stored-product insect pests. Stored wheat in commercial grain storage facilities in Oklahoma is fumigated by using phosphine, on average, three times each year (Cuperus et al. 1990). However, low levels of resistance to phosphine started to be documented in *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), the lesser grain borer, and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle, collected in Oklahoma in the 1980s (Zettler and Cuperus 1990). Resistance levels seem to have increased over the years because in 2009–2011, strong phosphine resistance was found in *R. dominica* and *T. castaneum* collected from commercial grain storage structures in Oklahoma (Opit et al. 2012a). A population of *T. castaneum* that was 119 times more resistant to phosphine compared with a susceptible population and three populations of *R. dominica* that were 254, 910, and 1,519 times more resistant than the susceptible population were found (Opit et al. 2012a). It is likely that resistant populations of these pest species occur in other parts of the United States as well.

The occurrence of phosphine resistance in pest populations presents challenges to the continued effective use of this fumigant. Phosphine fumigation is an important tool for the management of stored-grain pests. Governmental regulation of pesticides has significantly contributed to the common use of phosphine worldwide because it led to the loss of older fumigants (carbon tetrachloride, carbon disulfide, ethylene dichloride, and ethylene dibromide), the declining use of methyl bromide, reduced use of residual contact insecticides because of harmful residues they leave in food, and the lack of alternative fumi-
tants that are cost-effective, easy to apply, leave no residues, and can be used in a wide range of storage types and commodities like phosphine (Collins et al. 2001, Fields and White 2002, Nayak et al. 2003, Phillips and Throne 2010).

Phosphine kills insects by causing respiratory stress because of its disruption of the oxidative process occurring within living cells, a process by which the chemical energy of organic molecules is released in a series of metabolic steps involving the consumption of oxygen and the liberation of carbon dioxide and water (Chefurka et al. 1976, Chaudhry 1997, Schlipalus et al. 2008). Resistance limits the effectiveness of phosphine as a stored-product insect pest management tool, and this has become a problem in various parts of the world (Collins et al. 2001, Pimentel et al. 2010, Opit et al. 2012a). To ensure continued effective use of phosphine in the future, a phosphine resistance management strategy for the United States needs to be developed to maintain a high proportion of susceptible insects in pest populations.

An important component of phosphine resistance management involves the elimination of phosphine-resistant insects. Examples of ways that could be explored to eliminate phosphine-resistant insects include alternative fumigant gases (sulfuryl fluoride) and residual long-acting insecticides such as spinosad and a mixture of chlorpyrifos-methyl (21.6%) and deltamethrin (3.7%). Spinosad is a biologically derived insecticide from a soil actinomycete, Saccharopolyspora spinosa (Mertz and Yao (Bacteria: Actinobacteri- dae) (Mertz and Yao 1990), which is toxic to insects by contact as well as ingestion (Toews and Subramanyam 2003). It acts on the nicotinic acetylcholine and gamma amino butyric acid receptor sites of the insect nervous system initially causing involuntary muscle contractions and tremors by hyperexcitation of the central nervous system, and after continuous hyperexcitation, insects become paralyzed because of neuromuscular exhaustion (Salgado 1998). Spinosad was registered by the U.S. Environmental Protection Agency for use on stored grains in 2005, but it has not yet been made commercially available because of the delay in approval of all international trade agreements (Hertlein et al. 2011). A mixture of chlorpyrifos-methyl (21.6%) and deltamethrin (3.7%) is labeled for use on stored wheat and for structural treatment of grain storages. The active ingredient chlorpyrifos-methyl is an organophosphate that acts as an acetylcholinesterase inhibitor causing hyperexcitation leading to paralysis of insect neurons (O’Brien 1966), and deltamethrin is a pyrethroid that affects the insect neuromuscular system by acting as a sodium channel modulator causing hyperexcitation and tremors followed by paralysis (Narahashi 1971). The fact that spinosad, organophosphates, and pyrethroids kill insects in different ways than phosphine means they have a greater likelihood of eliminating phosphine-resistant insects.

The effectiveness of spinosad as a grain protectant against various species of stored-product insect pests on different grains is well established (Fang et al. 2002a,b; Nayak et al. 2005; Subramanyam 2006; Vayias et al. 2010), but these studies have not specifically investigated efficacy against phosphine-resistant stored-product insect pests. In addition, there are no published studies on efficacy of chlorpyrifos-methyl + deltamethrin mixture against phosphine-resistant stored-product insect pests. Therefore, this study was initiated to evaluate efficacies of a liquid formulation of spinosad and a mixture of chlorpyrifos-methyl and deltamethrin against phosphine-resistant and -susceptible adult R. dominica and T. castaneum collected from Oklahoma.

Materials and Methods

Insects. One phosphine-resistant population and one phosphine-susceptible strain of R. dominica and T. castaneum were used in this study. The phosphine-resistant and -susceptible R. dominica will subsequently be referred to as Rd-res and Rd-sus, respectively. In the case of T. castaneum, these will be referred to as Tc-res and Tc-sus, respectively. Cultures of Rd-sus and Tc-sus were started by using insects collected from concrete silos in Garfield Co., OK, in 2009. Cultures of Tc-sus and Rd-sus were started by using insects obtained from laboratory cultures maintained at the Center for Grain and Animal Health Research of the USDA Agricultural Research Service, Manhattan, KS. Cultures of these susceptible strains have been maintained since 1958 and 1972, respectively. T. castaneum were reared on a mixture of 95% all-purpose wheat flour and 5% Brewer’s yeast (wt/wt) at 28°C and 65% relative humidity (RH), and R. dominica were reared on a mixture of 95% whole-wheat kernels and 5% Brewer’s yeast at 28°C and 65% RH. Voucher specimens of Rd-res, Rd-sus, Tc-res, and Tc-sus that were used in this study were deposited in the K. C. Emerson Entomology Museum at Oklahoma State University under lot numbers 122, 126, 129, and 136, respectively.

Insecticides. Efficacies of spinosad (Sensat; 88.33 [AI] Conc.; Bayer CropScience) applied at a label rate of 1 ppm and chlorpyrifos-methyl + deltamethrin mixture (Storicide II; 253 [AI] Conc.; 21.6% chlorpyrifos-methyl and 3.7% deltamethrin; Bayer CropScience) applied at a label rate of 3 ppm of chlorpyrifos-methyl and 0.5 ppm of deltamethrin for control of phosphine-resistant and -susceptible adult R. dominica and T. castaneum were evaluated. Both Sensat and Storicide II were diluted by using distilled water and their solutions used to treat wheat.

Insecticide Application. Three 3.8-liter jars and three 2.5-kg batches of wheat were assigned to each of the aforementioned insecticides (spinosad or chlorpyrifos-methyl + deltamethrin). The application of spinosad or chlorpyrifos-methyl + deltamethrin will be referred to as “treatment,” although they are not true treatments as defined in statistics. Adequate insecticide treatment of each 2.5-kg batch of wheat added to each jar required 2.5 ml of insecticide solution (Bonjour and Opit 2012). Therefore, to treat 2.5 kg of wheat added to each of the jars assigned to the
spinosad treatment at a label rate of 1 ppm, 0.6 ml of pesticide was mixed with 50 ml of water and 2.5 ml of the solution was taken and applied to the sides of each jar. For the chlorpyrifos-methyl + deltamethrin mixture treatment, 0.7 ml of pesticide was mixed with 50 ml of water and 2.5 ml of the solution was taken and applied to the sides of each jar; to attain an application rate of 3 ppm of chlorpyrifos-methyl and 0.5 ppm of deltamethrin. Additional three 3.8-liter jars and three 2.5-kg batches of wheat were assigned to the control and 2.5 ml of distilled water was taken and applied to the sides of each jar (Bonjour and Opit 2012).

After the application of 2.5 ml of insecticide solution or water to the sides of each 3.8-liter jar, 2.5 kg of wheat was added to each jar and the jar was closed with its lid. Each jar was then turned end-for-end 10 times and then rotated a full revolution 10 times (Bonjour and Opit 2012). Jars were left to sit on the bench for 2 h and then they were turned and rotated as before (Bonjour and Opit 2012). Sealed jars of treated wheat were kept in an incubator maintained at 28 ± 1°C, 65% ± 5% RH, and 24 h of darkness for storage during the experiment.

The experiment had five posttreatment storage periods, namely, 2, 84, 168, 252, and 336 d (referred to as storage periods hereafter). These storage periods corresponded to spinoas- or chlorpyrifos-methyl + deltamethrin mixture-treated wheat that was stored for 2, 84, 168, 252, and 336 d posttreatment before use; wheat for the control treatment was also stored for the same storage periods. Before using treated wheat after each storage period, the 3.8-liter jars were rotated end-for-end 10 times before removing wheat for the experiment. For each storage period, three replications for each strain or population of R. dominica and T. castaneum (Rd-res, Rd-sus, Tc-res, and Tc-sus) were set up. One replication came from the grain in each of 3.8-liter jars for each treatment (e.g., three 3.8-liter jars were treated with spinoas and 100 g of grain were then taken from each of the three different 3.8-liter jars for a given treatment and separately placed in a 236.6-ml jar).

Bioassays. The experimental unit for R. dominica and T. castaneum strain or population used in the experiment was a 236.6-ml glass jar containing 100 g of treated wheat. For jars receiving R. dominica, 100 g of treated whole kernels was used, and the jar lids were fitted with a circular piece of U.S. Standard #40 mesh copper screen sandwiched between two pieces of filter paper. For jars receiving T. castaneum, 95 g of treated whole kernels and 5 g of ground treated kernels were used, and jar lids were fitted with two pieces of filter paper. Ground kernels were obtained by grinding kernels for 30 s by using an electric blender (Hamilton Beach 909 Clanshell Commercial Blender, Hamilton Beach/Proctor–Silex, Inc., Southern Pines, NC).

For each storage period, 50 adult insects were added to treated grain in each 236.6-ml jar and held for 1 wk. Beetles were 2–3 mo old and were obtained from our laboratory colonies. Jars were randomly placed in a plastic box containing a saturated solution of sodium nitrite below perforated false floors to maintain 65 ± 5% RH (Greenspan 1977). The box was placed in an incubator maintained at 28 ± 1°C. After 1 wk, adult mortality was determined. Adult insects were removed from the jars and counted as live, moribund, or dead. Moribund and dead adults were then placed in a 9-cm petri dish containing a piece of filter paper moistened with 0.5 ml of water. Those insects were reevaluated after 24 h for recovery. Jars were then held for an additional 6 wk at the incubator conditions previously mentioned, after which the number of progeny was counted. Environmental conditions in the incubator were monitored by using a temperature and relative humidity sensor (HOBO U12, Onset Computer Corporation, Bourne, MA) and a digital thermometer (Mini-alarm thermometer with probe, Fisher 15-007-32).

Data Analyses. Control mortality did not exceed 3% in all cases, and treatment mortalities were corrected by using Abbott’s formula (Abbott 1925). All statistical procedures were accomplished by using Statistical Analysis System software (SAS Institute 2010). The mortality data were analyzed separately for each species by using General Linear Models Procedure for a three-way analysis of variance for treatment, storage period, and resistance status as main effects. Percent-age mortality data were transformed by using the arcsine square root transformation to stabilize variances. Untransformed means and standard errors are reported to simplify interpretation. Least significant difference test was used to determine differences among mean adult mortalities. Despite storage period being a quantitative independent variable, we did not use regression for data analyses. This is because regression equations were not particularly meaningful, as responses from our experiments usually were either minimal or not in patterns that were easily described by regression equations. In progeny production data (number of progeny) analyses, the control treatment was included. For R. dominica progeny production data, only the control treatment data were analyzed, because spinoas and chlorpyrifos + deltamethrin resulted in total suppression of progeny production. Spinoas and chlorpyrifos + deltamethrin mixture were considered effective if the insecticides attained adult mortality and progeny production suppression of at least 80%.

Results

R. dominica. For mortality counts, all main effects and all interactions were significant at P < 0.05, with the exception of resistance status × treatment and resistance status × storage period (Fig. 1; Table 1). Spinoas (1 ppm) was effective against phosphine-resistant R. dominica for all storage periods, and adult mortality ranged from 96 to 98% for all storage periods (Fig. 1A); similar results were obtained for phosphine-susceptible R. dominica, where mortality was 99% for all storage periods (Fig. 1A). Chlorpyrifos-methyl (3 ppm) + deltamethrin (0.5 ppm) was effective against both phosphine-resistant and -susceptible R. dominica.
for all storage periods (Fig. 1B). However, effectiveness of chlorpyrifos-methyl /H11001 deltamethrin declined from 100 to 83% from the first to last storage periods in the phosphine-resistant R. dominica and from 100 to 84% in phosphine-susceptible insects of this species (Fig. 1B). Both spinosad and chlorpyrifos-methyl /H11001 deltamethrin resulted in total suppression of progeny production in the phosphine-resistant and -susceptible R. dominica for all storage periods. In relation to R. dominica progeny production in the control treatment, there were significantly more susceptible R. dominica than their resistant counterparts at all storage periods except 336 d (Fig. 2; Table 2). Despite the lack of a significant difference in the 336-d storage period, the number of progeny in the susceptible R. dominica (135 /H11006 46) was numerically higher than in the resistant R. dominica (91 /H11006 10) (Fig. 2). For all storage periods, the number of progeny in the susceptible R. dominica ranged from 135 to 587 and in the resistant R. dominica from 70 to 228 (Fig. 2).

T. castaneum. For mortality counts, only treatment, storage period, and treatment × storage period were significant at P < 0.05, that is, resistance status had no effect on mortality (Fig. 1; Table 1). Spinosad (1 ppm) was not effective against phosphine-resistant and -susceptible T. castaneum; mortality ranged from 0.2 to 3% for all the storage periods (Fig. 1C). Chlorpyrifos-methyl (3 ppm) /H11001 deltamethrin (0.5 ppm) was effective against phosphine-resistant and -susceptible T. castaneum only in the 2- and 84-d storage periods, where mortality ranged from 93 to 100%; thereafter, mortality significantly declined and ranged between 26 and 45% (Fig. 1D). In relation to progeny production, all main effects and interactions were significant at P < 0.05, with the exception of storage period and treatment × storage period (Table 2). Chlorpyrifos-methyl /H11001 deltamethrin resulted in total suppression of progeny production in both phosphine-resistant and -susceptible T. castaneum populations at all storage periods. Spinosad caused total progeny suppression of phosphine-susceptible T. castaneum in the 168-, 252-, and 336-d storage periods (Fig. 3A). In the 2- and 84-d storage periods, phosphine-susceptible T. castaneum progeny production declined with storage time from

Fig. 1. Mortality (%) (mean ± SE) of R. dominica adults on wheat treated with spinosad (A) and chlorpyrifos-methyl + deltamethrin (B). Mortality (%) (mean ± SE) of T. castaneum adults on wheat treated with spinosad (C) and chlorpyrifos-methyl + deltamethrin (D) (for each insecticide for each species, means with the same letter are not significantly different). Storage periods correspond to the duration wheat was stored posttreatment before use.

<table>
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<th>Source</th>
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<th>R. dominica</th>
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<td>P</td>
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<tr>
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15 to 2 (Fig. 3A). In the spinosad treatment, phosphine-resistant *T. castaneum* progeny were produced at all storage periods, but progeny production generally declined as storage period increased (Fig. 3A). In the control treatment, phosphine-resistant *T. castaneum* produced significantly more progeny than their susceptible counterparts in all storage periods except 252 d (Fig. 3B; Table 2). Despite the lack of a significant difference in the 252-d storage period, the number of progeny in the resistant *T. castaneum* (147 ± 8) was numerically higher than in the susceptible *T. castaneum* (118 ± 5) (Fig. 3B). The number of progeny in the former, for all storage periods, ranged from 147 to 207 and in the latter from 59 to 118 (Fig. 3B).

**Discussion**

Phosphine-resistant and -susceptible *R. dominica* and *T. castaneum* can be effectively controlled by using a mixture of chlorpyrifos-methyl (3 ppm) and deltamethrin (0.5 ppm). However, only phosphine-resistant and -susceptible *R. dominica* can be effectively controlled by using spinosad (1 ppm). Our results are in agreement with earlier observations on the effectiveness of spinosad against *R. dominica* (Fang et al. 2002a, Nayak et al. 2005, Subramanyam et al. 2012). Based on our data, spinosad was not effective against phosphine-resistant and -susceptible *T. castaneum*. Low efficacy of spinosad against *T. castaneum* has previously been reported (Nayak et al. 2005, Subramanyam et al. 2012). Although spinosad (1 ppm) was not effective against adult *T. castaneum*, it resulted in significant suppression of progeny production in phosphine-resistant *T. castaneum*. Progeny production of phosphine-resistant *T. castaneum* in the 168-, 252-, and 336-d storage periods decreased by 98, 99, and 98%, respectively, relative to progeny production on untreated wheat. In phosphine-susceptible *T. casta-

![Fig. 2. Progeny production (number of individuals per jar ± SE) of *R. dominica* in the control treatment (means with the same letter are not significantly different). Storage periods correspond to the duration wheat was stored posttreatment before use.](image1)

![Fig. 3. Progeny production (number of individuals per jar ± SE) of *T. castaneum* on wheat treated with spinosad (A) and in the control treatment (B) (means with the same letter are not significantly different). Storage periods correspond to the duration wheat was stored posttreatment before use.](image2)

<table>
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<td><strong>P</strong></td>
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<td>Resistance status × treatment × storage period</td>
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Table 2. ANOVA results for main effects and interactions for progeny production of phosphine-resistant and -susceptible populations of *R. dominica* and *T. castaneum*. 

Refer to the original document for detailed discussion and analysis.
neum, spinosad significantly reduced progeny production in the 2- and 84-d storage periods and caused total progeny suppression in the 168-, 252-, and 336-d storage periods. These results indicate that spinosad is toxic to *T. castaneum* immatures, and are in agreement with data from a study by Toews and Subramanyam (2003). Furthermore, Bonjour et al. (2006) demonstrated that the effectiveness of spinosad against adult *T. castaneum* decreased over time but caused total or nearly total progeny production suppression for storage periods ≤252 d. Finally, Subramanyam et al. (2007, 2012) have reported low progeny production by *T. castaneum* on wheat treated with spinosad; progeny production was assessed after 56 and 42 d, respectively.

The mixture of 3 ppm of chlorpyrifos-methyl and 0.5 ppm of deltamethrin was highly effective against phosphine-resistant and -susceptible *R. dominica* for all storage periods. Subramanyam et al. (2012) also reported that chlorpyrifos-methyl (3 ppm) plus deltamethrin (0.5 ppm) was effective against *R. dominica* on wheat with 100% adult mortality after both 7- and 14-d exposure, and that study did not find any adult progeny on the treated wheat when progeny production was assessed after 42 d. Subramanyam et al. (2007) found that application of chlorpyrifos-methyl alone at 3 ppm was ineffective against *R. dominica*. Therefore, the effectiveness of chlorpyrifos-methyl + deltamethrin against *R. dominica* can probably be attributed to deltamethrin or the possibility of a synergistic effect of deltamethrin and chlorpyrifos-methyl.

In the current study, we found that the effectiveness of chlorpyrifos-methyl + deltamethrin against adults of both phosphine-resistant and -susceptible *R. dominica* declined significantly as posttreatment period increased. A study conducted by Arthur (2012) where *R. dominica* adults were exposed to wheat treated at 0, 25, 50, 75, and 100% of the label rate of a mixture of chlorpyrifos-methyl (3 ppm) and deltamethrin (0.5 ppm) for 2, 4, 8, 16, or 32 h showed that parental adult mortality increased as the concentration and exposure interval increased. Similarly, for progeny production that was assessed after 7 wk, it decreased with increasing concentration of chlorpyrifos-methyl + deltamethrin mixture and increasing exposure time. Given that all adult *R. dominica* in the current study were exposed to wheat treated with 100% of the label rate of chlorpyrifos-methyl (3 ppm) + deltamethrin (0.5 ppm) mixture for much longer than 32 h (7 d) before mortality was assessed, the decline in effectiveness as posttreatment storage period increased is most likely because of insecticide degradation over time.

Although chlorpyrifos-methyl + deltamethrin was highly effective against phosphine-resistant and -susceptible *T. castaneum* in treated wheat stored for 2 and 84 d, its effectiveness significantly declined over the 168-, 252-, and 336-d storage periods. Subramanyam et al. (2007) reported that the organophosphate component of chlorpyrifos-methyl + deltamethrin, chlorpyrifos-methyl, applied at 3 ppm was effective against *T. castaneum* in stored wheat. Arthur (1994) suggested that chlorpyrifos-methyl is more effective against *T. castaneum* compared with deltamethrin. In that study, it was shown that for up to 8 mo of storage, no *T. castaneum* adults and progeny survived on corn treated with twice (6 ppm) the rate of chlorpyrifos-methyl used in the current study. In corn treated with three different rates (0.5, 0.75, or 1 ppm) of deltamethrin, survival of *T. castaneum* adults was observed at all the storage periods; however, there were no progeny. The significant decline observed in our study in the efficacy of chlorpyrifos-methyl + deltamethrin as the posttreatment period increased may be because of degradation of chlorpyrifos-methyl, which breaks down rapidly at high grain temperatures and moisture contents; residues of deltamethrin are more persistent on grains (Noble et al. 1982, Arthur et al. 1992, Afridi et al. 2001).

Subramanyam et al. (2012) reported 100% *T. castaneum* mortality and significant reduction of progeny production on wheat treated by using 3 ppm of chlorpyrifos-methyl and 0.5 ppm of deltamethrin. Our data show that chlorpyrifos-methyl + deltamethrin resulted in total suppression of progeny production in both phosphine-resistant and -susceptible *T. castaneum* populations for all storage periods, thereby suggesting that it is highly effective against the immature stages.

If *T. castaneum* is the key target pest of insecticide applications, we suggest that another control measure be applied 3 mo after the chlorpyrifos-methyl + deltamethrin treatment. In the case of *R. dominica*, this will not be required because chlorpyrifos-methyl + deltamethrin maintains mortality of at least 80% for up to 336 d. This is based on the fact that chlorpyrifos-methyl + deltamethrin was effective against phosphine-resistant and -susceptible *T. castaneum* for only up to 84 d posttreatment, whereas it was effective against *R. dominica* for up to 336 d posttreatment. Our study shows that spinosad is more effective against phosphine-resistant *R. dominica*, and chlorpyrifos-methyl + deltamethrin is effective against both phosphine-resistant *R. dominica* and *T. castaneum*. As previously mentioned, spinosad, chlorpyrifos-methyl, and deltamethrin have different modes of action than phosphine, and this most likely explains their effectiveness against phosphine-resistant *R. dominica* and *T. castaneum*. According to Opit et al. (2012b), successful elimination of phosphine-resistant insects by using alternative fumigants or grain protectant insecticides will have greater success if the alternative insecticides have a different mode of action and there is no cross-resistance.

In both *R. dominica* and *T. castaneum*, we found a difference in progeny production between the phosphine-resistant and -susceptible insects in the untreated wheat (control treatment). For *R. dominica*, the number of progeny produced by the phosphine-resistant insects was significantly higher than that by the phosphine-resistant insects for all storage periods except 336 d. The converse was true for *T. castaneum*, where significantly more progeny were produced by resistant insects for all storage periods except 252 d. These findings may indicate that in the
absence of phosphine treatments, there is a fitness cost to having phosphine resistance genes in *R. dominica*, whereas there may be a fitness benefit to having phosphine resistance genes in *T. castaneum*. This preliminary observation indicating that there may be a fitness cost associated with phosphine resistance in *R. dominica* and a fitness benefit in *T. castaneum* needs further investigation to provide confirmation that this is the case. Knowledge of the fitness effects of having phosphine resistance genes is important for the development of phosphine resistance management strategies (Opit et al. 2012b).

The goal of a phosphine resistance management strategy is to maintain the susceptibility of insects to phosphine so that a high level of mortality is attained each time phosphine is used for fumigation. Our findings show that the grain protectant insecticides spinosad and chlorpyrifos-methyl + deltamethrin mixture can be effective tools for the elimination of phosphine-resistant *R. dominica* and *T. castaneum*. A phosphine resistance management strategy seeks to delay the development of resistance to phosphine where it has not occurred and to mitigate resistance in populations where it occurs by infrequent use of phosphine and withholding use for long enough to mitigate resistance, respectively. Infrequent or suspended use of phosphine can be accomplished by integrating the use of alternative chemical and nonchemical control measures such as grain protectants, heat, aeration, sanitation, and other integrated pest management tools (Opit et al. 2012b).

Based on our study, we conclude that chlorpyrifos-methyl + deltamethrin and spinosad can be used to eliminate phosphine-resistant *R. dominica*, whereas only chlorpyrifos-methyl + deltamethrin can be used to eliminate phosphine-resistant *T. castaneum*. This means wheat infested by phosphine-resistant *R. dominica* can be treated by using chlorpyrifos-methyl + deltamethrin or spinosad to eliminate resistant insects. Wheat infested by phosphine-resistant *T. castaneum* and empty storage structures infested by resistant insects of both species can be treated by using chlorpyrifos-methyl + deltamethrin to eliminate these pests. Therefore, spinosad and chlorpyrifos-methyl + deltamethrin are effective insecticides for the management of phosphine-resistant *R. dominica* and *T. castaneum* and can be used in a phosphine resistance management strategy developed for stored-product insect pests in the United States.

Acknowledgments

We thank Kandara Shakya, Nirajan Bhattarai, and Edmond Bonjour for their technical support; we also thank Scott Armstrong, Georges Backouliou, and Hassan Melouk for reviewing an earlier draft of this manuscript. This work was funded by the Oklahoma Agricultural Experiment Station (Project No. OKL02695). Trade names or commercial products mentioned in this publication are solely for the purpose of providing specific information and do not imply recommendation or endorsement by Oklahoma State University.

References Cited


Received 5 May 2013; accepted 5 July 2013.