PHOSPHINE RESISTANCE IN STORED-PRODUCT INSECT PESTS: MANAGEMENT AND FITNESS COST

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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 for continued effective use of phosphine. Therefore, this study investigated the efficacies of two grain insecticides, namely, spinosad and chlorpyrifos-methyl + deltamethrin against highly phosphine-resistant *R. dominica* and *T. castaneum*. Observations showed that both spinosad and chlorpyrifos-methyl + deltamethrin caused 83-100% mortality in resistant *R. dominica* and caused total progeny production suppression for all post-treatment storage periods: 2, 84, 168, 252, and 336 d. However, in resistant *T. castaneum*, the highest mortality caused by spinosad was only 3% for all storage periods. Chlorpyrifos-methyl + deltamethrin was effective against resistant *T. castaneum* only in treated wheat stored for 2 and 84 d where it caused 93-99% mortality. However, chlorpyrifos-methyl + deltamethrin achieved total suppression of progeny production in *T. castaneum* at all storage periods. Spinosad was not as effective as chlorpyrifos-methyl + deltamethrin at suppressing progeny production of resistant *T. castaneum*. Experiments were also conducted to measure population growth and developmental rates of phosphine-resistant and -susceptible populations in a phosphine-free environment in order to assess the fitness effects caused by phosphine resistance in these two species. Three resistant *R. dominica* populations tested exhibited lower population growth and developmental rates than the susceptible population indicating fitness cost in resistant insects. However, the only resistant *T. castaneum* population tested exhibited a higher population growth and developmental rates than the susceptible population indicating fitness benefit in resistant insects. This means phosphine resistance development in *R. dominica* populations where resistance has not developed can be slowed by infrequent use of phosphine, whereas it can be mitigated by suspending phosphine use for extended periods of time in resistant populations. However, the same is not true for *T. castaneum*. For both scenarios, the most appropriate option is to eliminate the resistant individuals, for example, by using spinosad or chlorpyrifos-methyl + deltamethrin. These two insecticides can be used in a phosphine resistance management strategy for *R. dominica* and *T. castaneum* in the U.S.

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CHAPTER I

INTRODUCTION

Wheat is a major crop grown in Oklahoma. In 2012, Oklahoma produced 4.2 million tons (155 million bushels) of winter wheat (*Triticum aestivum* L.) worth $1.2 billion and ranked number two in the U.S. for production of this crop (National Agricultural Statistics Service [NASS] 2013). Wheat storage time in Oklahoma on average ranges from 6 to 12 months and storage occurs under relatively high temperature conditions thereby increasing the risk of serious infestation by stored-product insect pests (Cuperus et al. 1990). Therefore, insect pest management is important in mitigating economic losses associated with storage. In Oklahoma, phosphine (hydrogen phosphide or PH_3) fumigation is the preferred method for the control of insect infestations, and stored wheat in commercial storage facilities is fumigated on average three times a year (Cuperus et al. 1990). Given that methyl bromide has been phased out and there are no alternatives with the combined advantages of phosphine, it is critical that the effectiveness of phosphine be maintained. Some of the advantages of phosphine include

low cost, easy application, lack of residues, and it can be applied in many types of grain storage structures and on many commodities (Chaudhry 2000, Collins et al. 2001, Nayak and Collins 2008).

The popularity of phosphine has had the unintended effect of resistance development in stored-product insect pests. Repeated inefficient fumigations, including fumigating leaky structures, has over the years resulted in the development of resistant insect populations (Taylor 1989, Chaudhry 2000, Benhalima et al. 2004). Resistance to phosphine started to be documented in stored-product insects worldwide in the early 1970s (Champ and Dyte 1976). In the U.S., low levels of resistance to phosphine in stored-grain insects collected in Oklahoma were first reported in the 1980s (Zettler and Cuperus 1990). Recently strong phosphine resistance was found in, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), the lesser grain borer, and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle, which are key insect pests of stored grains in Oklahoma (Opit et al. 2012a). A population of *T. castaneum* that was 119 times more resistant to phosphine compared to the susceptible population and three populations of *R. dominica* that were 254, 910, and 1,519 times more resistant than the susceptible population were found in insects collected from commercial grain storage structures in Oklahoma (Opit et al. 2012a).

The occurrence of phosphine-resistant pest populations presents challenges to the continued effective use of this fumigant. As previously mentioned, phosphine is an important tool for the management of stored-grain insect pests. Phosphine causes respiratory stress in the target insect by disrupting the mitochondrial electron transport chain (Chefurka et al. 1976, Chaudhry 1997). The mechanisms for phosphine resistance

within resistant insects include active respiratory exclusion of phosphine resulting in less uptake of the gas, a lower respiration rate in resistant insects compared to their susceptible counterparts, and phosphine detoxification (Price 1984, Chaudhry and Price 1990, Chaudhry and Price 1992, Pimental et al. 2007). Given the discovery of strong resistance to phosphine in stored-product insect pests in the U.S., it is important to develop a phosphine resistance management strategy to ensure the continued effective use of this fumigant and to maintain its cost-effectiveness.

Development of a phosphine resistance management strategy, involves finding ways to maintain a large percentage of susceptible stored-product insect pests in grain storage structures (Opit et al. 2012b). In scenarios where phosphine resistance has already been detected, strategies are implemented to eliminate the resistant insect populations by use of alternative treatment methods which have a different mode of action than that of phosphine (Opit et al. 2012b). Spinosad is a potential alternative insecticide for the elimination of phosphine-resistant insects. Spinosad is a proven effective grain protectant that provides long-term protection against various species of stored-product insect pests on different grain varieties (Fang et al. 2002a, 2002b; Nayak et al. 2005, Subramanyam 2006, Vayias et al. 2010). Spinosad is labeled for use at an application rate of 1 ppm (Hertlein et al. 2011). Another alternative insecticide is a mixture of chlorpyrifos-methyl (3 ppm) (an organophosphate) and deltamethrin (0.5 ppm) (a pyrethroid) labeled for use on stored wheat at an application rate of 3.5 ppm (Subramanyam 2007, 2012).

Long-term frequent use of an insecticide exposes greater numbers of insects to selection pressure thereby increasing the rate of resistance development (Tabashnik 1990). This suggests that withholding the use of phosphine for an extended period may

reduce the selection pressure (Opit et al. 2012b). Phosphine resistance development in insect populations where resistance has not developed can be slowed by infrequent use of phosphine, whereas it cannot be mitigated by suspending phosphine use for extended periods of time in resistant populations with fitness benefit (Pimental et al. 2007, Sousa et al. 2009, Opit et al. 2012b). However, to determine if this assertion is true, a fitness cost or benefit associated with phosphine resistance needs to be determined when phosphine resistance is present within an insect population. Withholding phosphine use for long periods of time does not always mitigate phosphine resistance in resistant populations, for example in insects where resistance confers a fitness benefit (Jagadeesan et al. 2012). Where insecticide resistance confers a fitness benefit, it is likely that resistance will stabilize and become widespread rendering the insecticide ineffective (Arnaud et al. 2002). Insecticide resistance management seeks to maintain a large percentage of susceptible insects in target populations and avoid scenarios of widespread resistance (Opit et al. 2012b). This can be accomplished by identifying ways to prevent resistance from developing rapidly in susceptible populations and methods to eliminate resistant populations where they occur.

An important concern in grain processing and storage facilities is detection and monitoring of insect pest populations which facilitates making correct management decisions and accurate evaluation of the effectiveness of the integrated pest management programs for these facilities (Barak et al. 1990, Campbell et al. 2002). Insect traps have been developed commercially for the detection and monitoring of stored-product insects such as *T. castaneum* which is a common pest in grain processing and storage facilities (Mullen 1992). However, insect pests have low response to these commercially available

traps (Semeao et al. 2011, Duehl et al. 2011, Campbell 2012). Low trap catches give less accurate information on infestation levels in monitored facilities. Investigating the effectiveness of traps to monitor insect pests is vital for the identification of the most effective traps that provide more accurate population estimate data that improve pest management decision making.

Objectives

As previously stated, resistance to phosphine in stored-product insect pests is a threat to grain storage in the U.S. Detection of strong phosphine resistance in key stored-grain pests, namely, *R. dominica* and *T. castaneum*, in Oklahoma necessitates the development of a phosphine resistance management strategy for the U.S. to ensure continued effective use of phosphine. Knowledge of the fitness effects associated with insecticide resistance in insect populations and identification of tools that mitigate resistance development are necessary for developing resistance management. In addition, identification of effective tools that facilitate detection and monitoring of insect infestations in grain processing and storage facilities is essential for successful integrated pest management in monitored facilities (Barak et al. 1990, Campbell et al. 2002). Given the importance of a phosphine resistance management strategy to Oklahoma and wheat-growing regions of the U.S. and the significance of monitoring pest populations to integrated pest management in grain processing and storage facilities, relevant studies were conducted to address these issues.

The objectives are:

Objective 1:

Evaluate efficacies of the grain protectant insecticides spinosad and chlorpyrifos-methyl + deltamethrin against phosphine-resistant and -susceptible *R. dominica* and *T. castaneum* collected from Oklahoma.

Objective 2:

Measure population growth rates and developmental rates of phosphine-resistant *R. dominica* and *T. castaneum* collected from Oklahoma to determine whether phosphine resistance confers a fitness cost or benefit in a phosphine-free environment.

Objective 3:

Compare effectiveness of three types of traps used to monitor *T. castaneum* in grain processing and food storage facilities.

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CHAPTER II

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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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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Abstract

Highly phosphine-resistant populations of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) have recently been detected in Oklahoma grain storage facilities. These findings necessitate development of a phosphine resistance management strategy to ensure continued effective use of phosphine. This study determined 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 post-treatment were assessed. It was determined 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 previously mentioned 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 U.S.

Introduction

Oklahoma produced 4.2 million tons (155 million bushels) of winter wheat (*Triticum aestivum* L.) worth $1.2 billion in 2012 (National Agricultural Statistics Service [NASS] 2013). In Oklahoma, phosphine gas (hydrogen phosphide or PH_3) is the preferred method to fumigate stored grain to manage stored-product insect pests. Stored wheat in commercial grain storage facilities in Oklahoma is fumigated using phosphine on average 3 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 2009-11, 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 detected in Oklahoma (Opit et al. 2012a). It is likely that resistant populations of these pest species occur in other parts of the U.S. 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 fumigants 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 (e.g., Collins et al. 2001, Fields and White 2002, Nayak et al. 2003, Phillips and Throne 2010).

Phosphine kills insects by causing respiratory stress due to 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, Schlipalius 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, Pimental et al. 2010, Opit et al. 2012a). In order to effectively use phosphine in the future, a phosphine resistance management strategy for the U.S. needs to be developed in order 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: Actinobacteridae) (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 (GABA) 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 due to neuromuscular exhaustion (Salgado 1998). Spinosad was registered by the U.S. Environmental Protection Agency (E.P.A.) for use on stored grains in 2005, but it has not yet been made commercially available due to 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 which acts as an acetylcholinesterase inhibitor causing hyperexcitation leading to paralysis of insect neurons (O'Brien 1966), and deltamethrin is a pyrethroid which 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 a different manner than phosphine suggests 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, 2002b; 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. Additionally, 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-res and Tc-res were started using insects collected from concrete silos in Garfield Co., OK in 2009. Cultures of Tc-sus and Rd-sus were started using insects obtained from laboratory cultures maintained at the Center for Grain and Animal Health Research (CGAHR) 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% 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 (Storcide™ II; 253 AI Conc.; 21.6% chlorpyrifos-methyl and 3.7% deltamethrin; Bayer CropScience) applied at a label rate 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 Storcide™ II were diluted using distilled water and these solutions were used to treat wheat.

Insecticide Applications. 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 2011). 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 2011).

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 sealed. Each jar was then turned end over end 10 times and then rotated a full revolution 10 times (Bonjour and Opit 2011). Jars were left for 2 h and then they were turned and rotated as previously mentioned (Bonjour and Opit 2011). 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 post-treatment storage periods of 2, 84, 168, 252, and 336 d (referred to as storage periods hereafter). These storage periods corresponded to spinosad- or chlorpyrifos-methyl + deltamethrin mixture-treated wheat that was stored for 2, 84, 168, 252, and 336 d post-treatment before use; wheat for the control treatment was also stored for the same storage periods. Prior to using treated wheat after each storage period, the 3.8-liter jars were rotated end over end 10 times before removing wheat for the experiment. For each storage period, a total of 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 (for example, three 3.8-liter jars were treated with spinosad 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 utilized for each *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 using an electric blender (Hamilton Beach 909 Clamshell Commercial Blender, HamiltonBeach/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 approximately 2-3 mo old and were obtained from laboratory colonies. Jars were randomly placed in a plastic box containing a saturated solution of sodium nitrite ($NaNO_2$) 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 re-evaluated after 24 h for recovery. Jars were then held for an additional 6 wk at the incubator conditions mentioned above after which the number of progeny was counted. Environmental conditions in the incubator were monitored using a temperature and relative humidity sensor (HOBO® U12, Onset Computer Corporation, Bourne, MA) and a digital thermometer (Mini-alarm thermometer with probe, Fisher Scientific 15-007-32).

Data Analyses. Control mortality did not exceed 3% in all cases and treatment mortalities were corrected using Abbott's formula (Abbott 1925). All statistical procedures were accomplished using Statistical Analysis System software (SAS Institute 2010). The mortality data were analyzed separately for each species using General Linear Model (GLM) procedure for a three-way analysis of variance (ANOVA) for treatment (type of insecticide), storage period, and resistance status as main effects. Percent mortality data were transformed using the arcsine square-root transformation to stabilize variances. Untransformed means and standard errors are reported to simplify interpretation. Least significant difference (LSD) test was used to determine differences

among mean adult mortalities. Despite storage period being a quantitative independent variable, regression analyses were not conducted. This was because regression equations were not particularly meaningful as responses from the experiments usually were either minimal or not in a pattern that were easily described by regression equations. In progeny production (number of progeny) data analyses, the control treatment was included. For *R. dominica* progeny production data, only the control treatment data were analyzed, because spinosad and chlorpyrifos + deltamethrin resulted in total suppression of progeny production. Spinosad and chlorpyrifos + deltamethrin were considered effective if the insecticides attained adult mortality and progeny production suppression of at least 80%.

Results

***Rhyzopertha 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). Spinosad (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 + deltamethrin declined from 100 to 83% from the first to last storage periods in the phosphine-resistant *R. dominica* and 100 to 84% in phosphine-susceptible insects of this species (Fig. 1B). Both spinosad and chlorpyrifos-methyl + 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 ± 46) was numerically higher than in the resistant *R. dominica* (91 ± 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).

Tribolium castaneum. For mortality counts, only treatment (type of insecticide), storage period, and treatment × storage period were significant at $P < 0.05$, i.e. resistance status had no effect on mortality (Fig. 3; 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. 3A). Chlorpyrifos-methyl (3 ppm) + 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. 3B). 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 + deltamethrin resulted in total suppression of progeny production in both phosphine-resistant and -susceptible *T. castaneum* population at all storage periods. Spinosad caused total progeny suppression of phosphine-susceptible *T. castaneum* in the 168-, 252-, and 336-d storage periods (Fig. 4A). In the 2- and 84-d storage periods, phosphine-susceptible *T. castaneum* progeny production declined with storage time from 15 to 2 (Fig. 4A). 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. 4A). In the control treatment, phosphine-resistant *T. castaneum* produced significantly more progeny than their susceptible counterparts at all storage periods except 252 d (Fig. 4B; 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. 4B). The number of progeny in the former, for all storage periods, ranged from 147 to 207 and in the latter 59 to 118 (Fig. 4B).

Discussion

Phosphine-resistant and -susceptible *R. dominica* and *T. castaneum* can be effectively controlled 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 using spinosad (1 ppm). These 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). In the present study, spinosad caused high mortality and complete progeny suppression of phosphine-resistant and -susceptible *R. dominica* at all the five post-treatment storage periods (2, 84, 168, 252, and 336 d). Effectiveness of spinosad showed no significant decline over time in both phosphine-resistant and -susceptible *R. dominica*. A field study conducted in Oklahoma using hard red winter wheat in small grain storage bins (4.6 tons or 169 bushels) demonstrated that stored wheat treated with 1 ppm of spinosad completely controlled *R. dominica* adults and progeny production for all the post-treatment storage periods (28, 84, 182, 252, 336, and 672 d) (Bonjour et al. 2006).

Based on the data within this study, 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. castaneum*, 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. Lastly, Subramanyam et al. (2007, 2012) have reported low progeny production by *T. castaneum* on wheat treated with spinosad; progeny production was assessed after 56 d 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 present study, it was determined that the effectiveness of chlorpyrifos-methyl + deltamethrin against adults of both phosphine-resistant and -susceptible *R. dominica* declined significantly as post-treatment 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 which 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 present 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 post-treatment storage period increased is most likely due to 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 where chlorpyrifos-methyl was applied at 3 ppm is effective against *T. castaneum* in stored wheat. Arthur (1994) suggested that chlorpyrifos-methyl is

more effective against *T. castaneum* compared to 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 present 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 this study in the efficacy of chlorpyrifos methyl + deltamethrin as the post-treatment period increased may be due to degradation of chlopyrifos-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 using 3 ppm of chlorpyrifos-methyl and 0.5 ppm of deltamethrin. This study expanded on the previous study by demonstrating 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 this insecticide mixture is highly effective against the immature stages.

If *T. castaneum* is the key target pest of insecticide applications, this study suggests 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 84 d post-treatment whereas it

was effective against *R. dominica* for 336 d post-treatment. This study demonstrated 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 using alternative fumigants or grain protectant insecticides will have greater success if the alternative insecticides have different modes of action and there is no cross-resistance.

In both *R. dominica* and *T. castaneum*, there was 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-susceptible 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 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 needs further investigation to provide confirmation of a fitness cost and/or benefit. Knowledge of the fitness effects when phosphine resistance is present can aide in the development of phosphine resistance management strategies (Opit et al. 2012b).

The goal of a phosphine resistance management strategy is to maintain a level of susceptibility within an insect population to phosphine so that a high level of mortality

can be attained each time phosphine is used for fumigation. The findings within this study show that grain protectants insecticides such as spinosad and chlorpyrifos-methyl + deltamethrin can be effective tools for the elimination of the 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 non-chemical control measures such as grain protectants, heat, aeration, sanitation, and other integrated pest management tools (Opit et al. 2012b).

Based on this study, it can be concluded 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 suggests that wheat infested by phosphine-resistant *R. dominica* can be treated using chlorpyrifos-methyl + deltamethrin mixture 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 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 U.S.

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Table 1. ANOVA results for main effects and interactions for mortality of phosphine- resistant and -susceptible populations of *R. dominica* and *T. castaneum*.

Source		*R. dominica*		*T. castaneum*	
	df	*F*	*P*	*F*	*P*
Resistance status	1	27.4	<0.0001	0.5	0.4999
Treatment	1	93.2	<0.0001	791.7	<0.0001
Resistance status × treatment	1	1.0	0.3343	0.7	0.4089
Storage period	4	31.4	<0.0001	51.0	<0.0001
Resistance status × storage period	4	1.0	0.4172	1.3	0.2969
Treatment × storage period	4	22.0	<0.0001	44.3	<0.0001
Resistance status × treatment × storage period	4	2.7	0.0432	0.4	0.8139

Table 2. ANOVA results for main effects and interactions for progeny production of phosphine-resistant and -susceptible populations of *R. dominica* and *T. castaneum*.

Source	*R. dominica*			*T. castaneum*		
	df	*F*	*P*	df	*F*	*P*
Resistance status	1	237.6	<0.0001	1	74.2	<0.0001
Treatment	-	-	-	2	355.7	<0.0001
Resistance status × treatment	-	-	-	2	43.7	<0.0001
Storage period	4	35.3	<0.0002	4	1.6	0.1845
Resistance status × storage period	4	13.3	<0.0003	4	2.6	0.0460
Treatment × time period	-	-	-	8	1.4	0.2210
Resistance status × treatment × storage period	-	-	-	8	2.5	0.0223

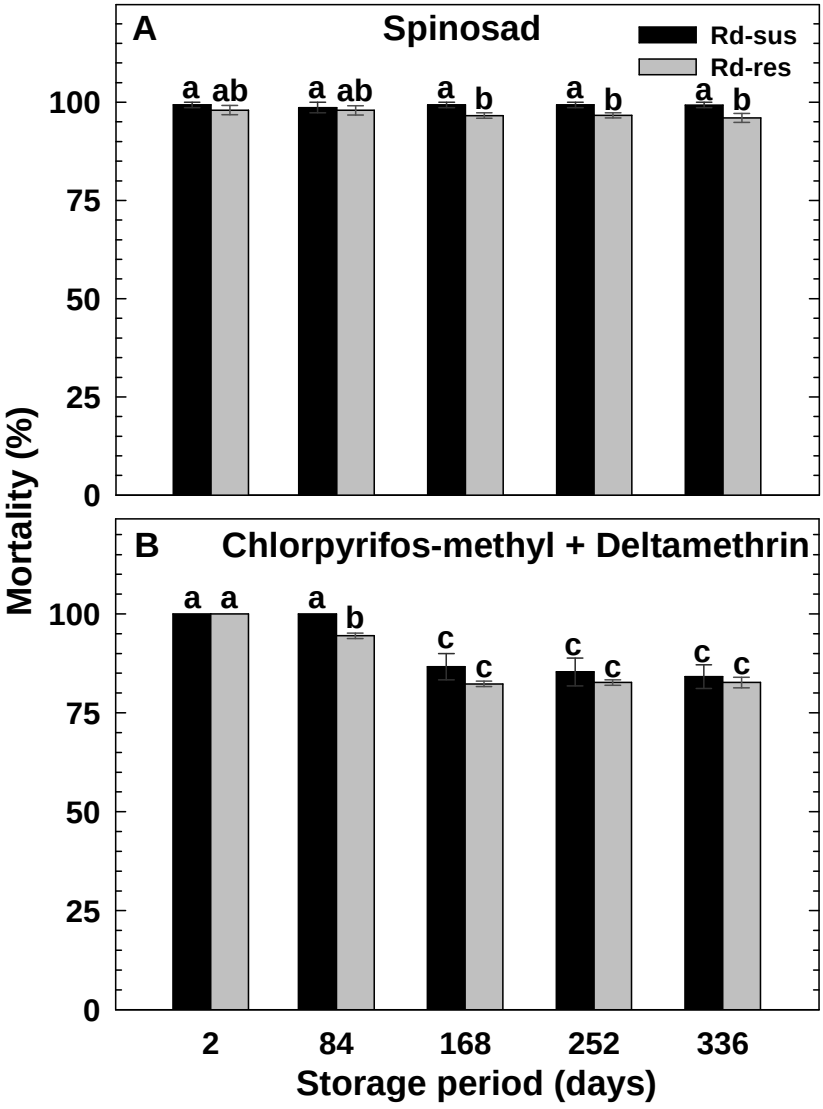

Fig. 1. Mortality (%) (mean ± SE) of *R. dominica* adults on wheat treated with spinosad (A) and chlorpyrifos-methyl + deltamethrin (B) (for each insecticide, means with the same letter are not significantly different). Storage periods correspond to the duration wheat was stored post-treatment before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

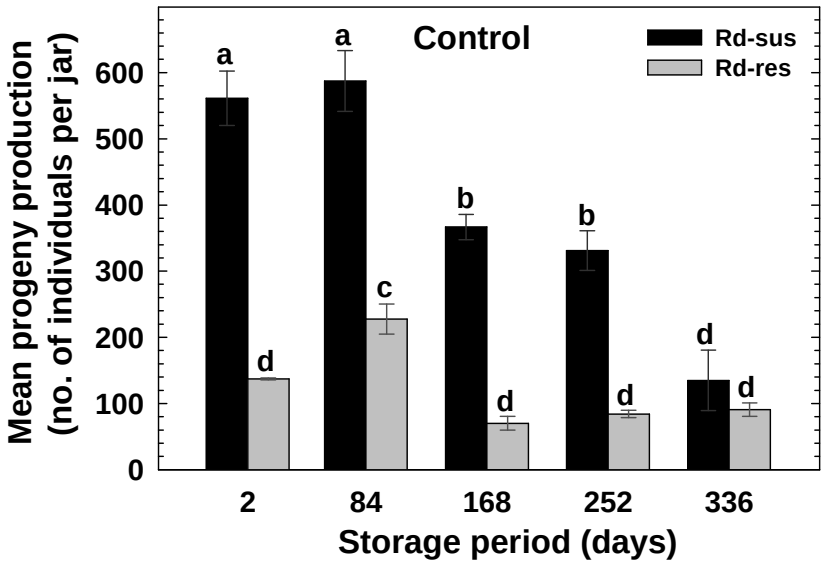

Fig. 2. Progeny production (no. of individuals per jar ± SE) of *R. dominica* in the control treatment (means with the same letter are not significantly different). Storage periods correspond to the duration wheat was stored post-treatment before use.

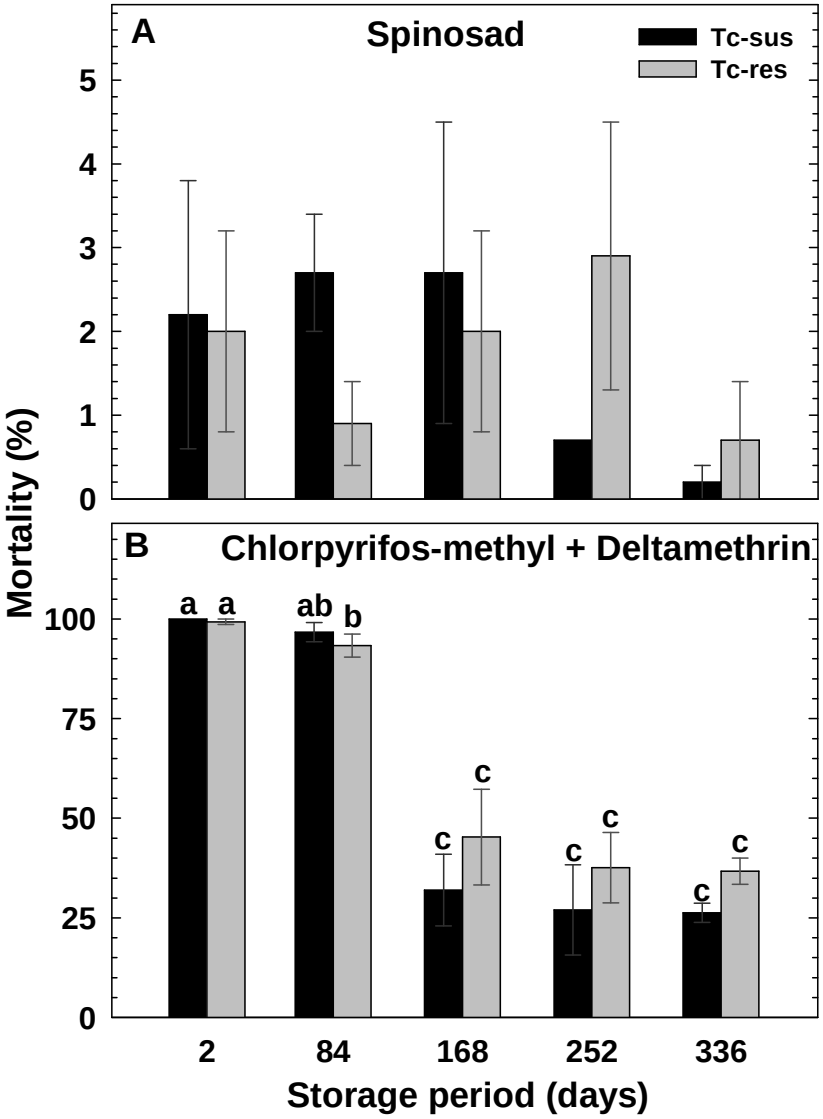

Fig. 3. Mortality (%) (mean ± SE) of *T. castaneum* adults on wheat treated with spinosad (A) and chlorpyrifos-methyl + deltamethrin (B) (for each insecticide, means with the same letter are not significantly different; where no letters exist, no significant differences were noted). Storage periods correspond to the duration wheat was stored post-treatment before use.

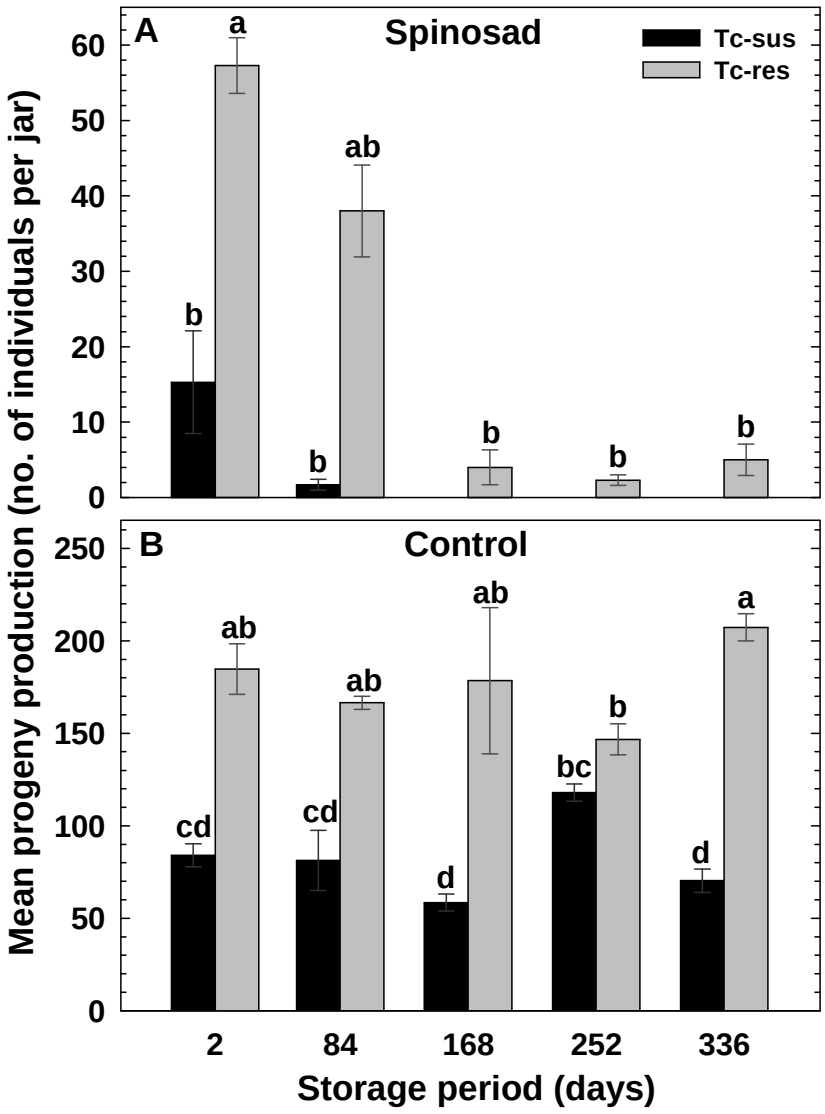

Fig. 4. Progeny production (no. of individuals per jar ± SE) of *T. castaneum* on wheat treated with spinosad (A) and in the control treatment (B) (means with the same letter are not significantly different). Storage periods correspond to the duration wheat was stored post-treatment before use.

CHAPTER III

ASSESSMENT OF FITNESS EFFECTS ASSOCIATED WITH PHOSPHINE RESISTANCE IN *RHYZOPERTHA DOMINICA* (COLEOPTERA: BOSTRICHIDAE) AND *TRIBOLIUM CASTANEUM* (COLEOPTERA: TENEBRIONIDAE)

(To be submitted to Journal of Economic Entomology, 2013)

Assessment of Fitness Effects Associated with Phosphine Resistance in *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Abstract

Strong phosphine resistance was reported in Oklahoma populations of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) in 2012. For continued effective use of phosphine, resistance management has to be implemented in the U.S. Knowledge of the fitness effects of phosphine resistance in a phosphine-free environment is important for resistance management. Therefore, the goal of this study was to determine if there are fitness effects caused by phosphine resistance in populations of *R. dominica* and *T. castaneum*. The population growth and developmental rates of phosphine-resistant and -susceptible populations of these two species were measured in a phosphine-free environment. Three resistant *R. dominica* populations tested exhibited lower population growth and developmental rates compared to the susceptible population, whereas the only resistant *T. castaneum* population tested exhibited a higher population growth and developmental rates compared to the susceptible population. These data indicate that for *R. dominica* and *T. castaneum*, there is a fitness cost and a fitness benefit, respectively, associated with phosphine resistance genes in these two species. This means phosphine resistance development in *R. dominica* populations where resistance has not developed can be slowed by infrequent use of phosphine, whereas it can be mitigated by suspending phosphine use for extended periods of time in populations with resistance. However, withholding phosphine use for long periods of time may not mitigate phosphine resistance in *T. castaneum*.

KEY WORDS Stored product, lesser grain borer, red flour beetle, fitness cost, phosphine resistance management

Introduction

A recent study by Opit et al. (2012a) found high levels of phosphine (hydrogen phosphide or PH_3) resistance in *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) collected from commercial grain storage facilities in Oklahoma. Because phosphine is greatly relied upon for integrated management of stored-grain pests in Oklahoma and the U.S., occurrence of phosphine resistance in pest populations presents challenges to its continued effective use. In order to maintain the cost-effectiveness of phosphine and to extend its useful life, it is important to develop a phosphine resistance management strategy for stored-product insect pests in the U.S.

The goal of a phosphine resistance management strategy is to maintain a high proportion of susceptible insects in populations found in grain storage facilities so that high levels of mortality are attained each time phosphine is used for fumigation (Opit et al. 2012b). Phosphine resistance management seeks to delay the development of resistance to phosphine where resistance has not yet occurred and, if resistance has already occurred, to eliminate resistant insect populations (Opit et al. 2012b). In a phosphine resistance management strategy, phosphine is applied as infrequently as possible in order to delay resistance development; however, this is most likely to happen if insects possessing resistance genes suffer a fitness cost (Pimental et al. 2007, Sousa et al. 2009).

Resistance to insecticides has a genetic basis and insects possessing resistance genes usually bear some adaptive costs whereby resources needed for physiological

processes such as development and reproduction are allocated for protection against the insecticide (Coustau et al. 2000, Berticat et al. 2002). Fitness costs associated with insecticide resistance may be in the form of decreased reproductive potential of insecticide-resistant individual in absence of the insecticide (selecting agent), suggesting that the number of resistant individuals will decline in the absence of the application of the insecticide (Coustau et al. 2000, Haubruge and Arnaud 2001, Foster et al. 2003). However, this is not always the case because insecticide use can result in selection of fitness modifier genes or selection of less costly genes which would confer a fitness benefit to resistant individuals (Coustau et al. 2000, Berticat et al. 2002), or there may be cases resulting in neutral effect where possessing resistance genes would neither have a fitness cost or fitness benefit (Heather 1982, Fragoso et al. 2005, Guedes et al. 2006).

Few studies have investigated potential fitness costs associated with phosphine resistance (Pimental et al. 2007, Sousa et al. 2009, Jagadeesan et al. 2012). Sousa et al. (2009) showed there was a fitness cost to possessing resistance genes in some *R. dominica* and *T. castaneum* populations from Brazil, but they also found one phosphine-resistant *R. dominica* population that showed there was a fitness benefit to possessing resistance genes. Some studies have reported lack of association between phosphine resistance and fitness cost in highly phosphine-resistant *R. dominica* (Schlipalius et al. 2008). A study by Jagadeesan et al. (2012) reported a weak fitness cost in a single pair inter-strain cross between the phosphine-susceptible and strongly-phosphine resistant strains of *T. castaneum*. However, this weak resistance was not consistent because the genetic background of the weakly resistant strain may have suppressed the cost based on

the fact that no association was observed when a strongly resistant strain was crossed with a weak resistant strain.

In the Opit et al. (2012a) study, highly phosphine-resistant populations of *R. dominica* and *T. castaneum* were found. The resistant *T. castaneum* population was 119 times more resistant than the susceptible population and the three resistant *R. dominica* populations were 254, 910, and 1,519 times more resistant than the susceptible population. In a study on the efficacies of spinosad or chlorpyrifos-methyl + deltamethrin mixture on phosphine-resistant *R. dominica* that was 1,519 times more resistant than the susceptible strain, Bajracharya et al. (2013) found that the number of progeny produced by the phosphine-susceptible insects was significantly higher than that by the phosphine-resistant insects. They found the converse was the case for the phosphine-resistant *T. castaneum* that was 119 times more resistant than the susceptible strain. Their data suggested that there may be a fitness cost to possessing resistance genes in the phosphine-resistant *R. dominica* populations and a fitness benefit in the phosphine-resistant *T. castaneum* population, in insects collected from Oklahoma commercial grain storage facilities (Bajracharya et al. 2013). Therefore, the objective of this study was to conduct demographic studies that measured population growth rates and developmental rates to determine whether phosphine resistance genes confer a fitness cost or benefit in the phosphine-resistant *R. dominica* and *T. castaneum* populations found in Oklahoma.

Materials and Methods

Insects. One phosphine-resistant population of *T. castaneum* and three phosphine-resistant populations of *R. dominica* were used for this study. In addition, one phosphine-

susceptible strain of each species was also used; altogether six beetle populations were utilized. The phosphine-resistant population of *T. castaneum* is 119 times more resistant compared to the susceptible population and the three populations of *R. dominica* are 254, 910, and 1,519 times more resistant than the susceptible population (Opit et al. 2012a). For this study, only those populations of *T. castaneum* and *R. dominica* with resistance ≥ 100 times that of their susceptible counterparts, i.e. populations with strong resistance were used (Opit et al. 2012a). The three phosphine-resistant *R. dominica* and one phosphine-resistant *T. castaneum* populations were put into colony by using insects obtained from steel bins and concrete grain silos from three Oklahoma counties in 2009 (Opit et al. 2012a). These will be referred to as Rd-res-G, Rd-res-L, and Rd-res-P for the phosphine-resistant *R. dominica* populations and Tc-res-G for the phosphine-resistant *T. castaneum* population. The phosphine-susceptible *T. castaneum* and *R. dominica* will subsequently be referred to as Tc-sus and Rd-sus, respectively. Cultures of Tc-sus and Rd-sus were put into colony by using insects obtained from laboratory cultures maintained at the Center for Grain and Animal Health Research (CGAHR) of the USDA Agricultural Research Service, Manhattan, KS. Cultures of these susceptible strains have been maintained since 1958 and 1972, respectively. *R. dominica* were reared on a mixture of 95% whole-wheat kernels and 5% Brewer's yeast (wt/wt) at 28°C and 65% RH and *T. castaneum* were reared on a mixture of 95% all-purpose wheat flour and 5% Brewer's yeast at 28°C and 65% RH. Voucher specimens of Rd-res-P, Rd-res-G, Rd-res-L, Rd-sus, Tc-res-G, 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 121, 122, 125, 126, 129, and 136, respectively. In this study, non-sexed adult insects (1- to 3-wk-old) of

each population were obtained from laboratory cultures. All of the adult insects were removed from the rearing medium, and the medium was held for 3 wk to obtain 1- to 3-wk-old non-sexed adult insects.

Population Growth. The experiment was conducted in a phosphine-free environment in 300-ml (10 oz.) glass jars that contained appropriate diet for the insects. For *R. dominica,* 200 g of a mixture of 95% whole-wheat kernels and 5% Brewer's yeast was used, and for *T. castaneum,* 150 g of a mixture of 95% all-purpose wheat flour and 5% Brewer's yeast was used. Twenty non-sexed adult insects (1- to 3-wk-old) of each population were placed in each glass jar. For jars receiving *R. dominica*, 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,* jar lids were fitted with two pieces of filter paper. Experimental jars were held at 30 ± 1°C and 75 ± 5% RH. Jars were randomly placed in a plastic box containing a saturated solution of sodium chloride (NaCl) below perforated false floors to maintain 75 ± 5% RH (Greenspan 1977). The box was placed in an incubator maintained at 30 ± 1°C and 24 h of darkness. Environmental conditions in the incubator were monitored using a temperature and relative humidity sensor (HOBO® U12, Onset Computer Corporation, Bourne, MA) and a digital thermometer (Mini-alarm thermometer with probe, Fisher Scientific 15-007-32). The number of live adult insects in each jar was recorded at 0, 35, 50, 65, 80, 95, and 110 d from the start of the experiment. For example, 20 insects were placed in each glass jar with diet at the beginning of the experiment. After 35 d, the diet in each jar was sieved using U.S. Standard #40 (0.425-mm openings) and #14 (1.41-mm openings) sieves (Seedburo Equipment Company, Chicago, IL), and the number of live adult insects was

counted. All of the insects and diet were then placed back into the jar and held for an additional 15 d at the incubator conditions mentioned above. After 50 d, the number of live adult insects in each jar was again determined as previously described. This procedure was repeated after 65, 80, 95, and 110 d from the start of the experiment. After the 110-d insect count, the cumulative number of insects for each jar was calculated. The experiment had 12 replications, with 4 replications conducted in each of three time blocks.

Developmental Rates. The experiment was conducted in a phosphine-free environment in plastic Petri dishes (150-mm diameter x 15-mm height) (VWR, Radnor, PA) which had their inner walls coated with Fluon® (polytetrafluoroethylene; Northern Products, Woonsocket, RI) to prevent insects from escaping. For *R. dominica*, the diet was 35 g of a mixture of 95% whole-wheat kernels and 5% Brewer's yeast, and for *T. castaneum*, 35 g of a mixture of 95% all-purpose wheat flour and 5% Brewer's yeast. Each Petri dish containing diet of the respective species was infested with thirty 1- to 3-wk-old non-sexed adult insects and maintained at 30 ± 1°C and 75 ± 5% RH. Petri dishes were randomly placed in a plastic box containing a saturated solution of NaCl below perforated false floors to maintain 75 ± 5% RH (Greenspan 1977). The box was placed in an incubator maintained at 30 ± 1°C and 24 h of darkness. Environmental conditions in the incubator were monitored using a temperature and relative humidity sensor (HOBO® U12, Onset Computer Corporation, Bourne, MA) and a digital thermometer (Mini-alarm thermometer with probe, Fisher Scientific 15-007-32). All parental insects were removed from Petri dishes after 13 d to allow relative standardization of progeny development. After removal of the parental insects, the diet containing the developing progeny was

maintained at incubator conditions mentioned above until adult emergence. On alternate days, starting after the first emergence from each Petri dish, the number of adult progeny was counted and removed from the Petri dishes. Daily and cumulative emergences were recorded for each Petri dish. The experiment had 12 replications, with 4 replications conducted in each of three time blocks.

Data Analyses. Individual population growth rate experiment data sets were subjected to non-linear regression analysis using TableCurve 2D (Systat Software, Inc. 2002). Selection of an equation to describe the data was based on the coefficient of determination (R^2), lack-of-fit *P*-values, and whether the curve had a shape that was reasonable for describing the data. Comparisons of slopes were conducted using the General Linear Model procedure (SAS Institute, Cary, NC) to determine whether there were significant differences in the population growth of the phosphine-resistant and -susceptible populations of each species. For non-linear regression equations describing the relationship between cumulative population and time (d) for four populations of *R. dominica* and two populations of *T. castaneum*, the parameter *A* indicates the minimum value of *y* (the expected *y*-value when $x = 0$) and the parameter *B* indicates how quickly the values of *y* changes (*y* and *x* represent the cumulative population and time, respectively, in the non-linear regression equations) (Table 1). The parameter *B* gives the slope estimates. Comparisons of slopes were conducted to determine whether there were significant differences in the population growth of the phosphine-resistant and -susceptible populations of each species.

Non-linear regressions of daily emergence and cumulative emergence data were conducted using TableCurve 2D (Systat Software, Inc. 2002). Selection of an equation to

describe the data was based on criteria already mentioned above. To determine whether there were differences in emergence rates for the four populations of *R. dominica* and two populations of *T. castaneum*, the cumulative emergence data were used. For non-linear regression equations describing the relationship between cumulative emergence and time elapsed (d) after first adult emergence for four populations of *R. dominica* and two populations of *T. castaneum*, the parameter *A* estimates the total number of adults that emerged, the parameter *B* estimates the time required for half the insects in a given population to emerge as adults, and the parameter *C* is a growth rate parameter that specifies width or steepness of the curves (Table 2). Estimates of *B* parameters for cumulative emergence data were used to determine whether significant differences existed among times required for half of the insects to emerge as adults, starting from when first emergence was observed. The values of *B* parameters were compared with the use of 85% confidence intervals (Payton et al. 2003). For the non-linear regression equation describing the relationship between daily emergence and time elapsed (d) after first adult emergence for the four populations of *R. dominica*, the parameter *A* indicates the height of the curve's peak and estimates the total number of adults that emerged during peak emergence, the parameter *B* indicates the position of the center of the peak and estimates the time required for half the insects in a given population to emerge as adults, and the parameter *C* is the standard deviation (controls the width of the bell) (Table 3). For the non-linear regression equation describing the relationship between daily emergence and time elapsed (d) after first adult emergence for the two populations of *T. castaneum*, the parameter *A* gives the estimated number of insects in the initial time period and the parameter *B* estimates the rate at which the daily emergence is declining

(Table 3). Analyses of the daily emergence data were not conducted because the cumulative emergence data comprised the daily emergence data. Additionally, the daily emergence data for *T. castaneum* indicated that emergence should have been monitored at much shorter intervals of 1 or 2 h instead of 48 h. Another dataset involving field-collected strains of *T. castaneum* where emergence data were taken every 2 h supports this assertion (J.E.T., unpublished data).

Results

Population Growth Rates. Population growth rates for all the four populations (three phosphine-resistant populations and one phosphine-susceptible population) of *R. dominica* were significantly different ($P < 0.05$) (Fig. 1A; Table 1). The slopes (*B*) for Rd-sus, Rd-res-G, Rd-res-L and Rd-res-P were 0.43 ± 0.03, 0.35 ± 0.03, 0.24 ± 0.02, and 0.18 ± 0.03, respectively (Fig. 1A; Table 1). The population growth rate was lower in the phosphine-resistant populations of *R. dominica*, when compared with the phosphine-susceptible population. Population growth rates for the four populations in decreasing order were Rd-sus > Rd-res-G > Rd-res-L > Rd-res-P. After 110 d, values for cumulative population per jar for Rd-sus, Rd-res-G, Rd-res-L and Rd-res-P were 4,691 ± 461, 4,167 ± 562, 3,025 ± 473, and 2,195 ± 487 insects per jar, respectively.

For *T. castaneum*, population growth rates for the phosphine-resistant and -susceptible populations were significantly different ($P < 0.05$) (Fig. 1 B; Table 1). The slopes (*B*) for Tc-res-G and Tc-sus were 250.21 ± 7.63 and 172.60 ± 6.14, respectively (Fig. 1B; Table 1). The phosphine-resistant population of *T. castaneum* exhibited a higher population growth rate compared to the phosphine-susceptible population. After 110 d,

values for cumulative population per jar for Tc-res-G and Tc-sus were 2,512 ± 69 and 1,802 ± 48 insects per jar, respectively.

Developmental rates. *R. dominica*. The period between removal of parental adults from Petri dishes to emergence of the first adult beetles was 16 d. The parameter *B* from non-linear regression of cumulative emergence and time elapsed after first adult emergence estimates the time required for half the insects in a given population to emerge as adults, i.e. estimates how fast insects in a given population are developing from egg to adult (emergence) (Table 2). The parameter *A* estimates the total number of adults that emerged in each Petri dish. Cumulative emergence-related parameter *B* values were used to determine whether significant differences existed among developmental times of the four *R. dominica* populations. Emergence was significantly faster in the phosphine-susceptible population than in all the three phosphine-resistant populations (Fig. 2A; Table 2). However, there were no significant differences in the developmental times of insects in the three phosphine-resistant populations (Table 2). The total numbers of Rd-sus, Rd-res-G, Rd-res-L, and Rd-res-P adults that emerged were 666 ± 78, 647 ± 65, 632 ± 47, and 552 ± 73 per dish, respectively. Daily emergence data show that for the first 12 d after the first emergence was observed, there were numerically more susceptible than resistant *R. dominica* emerging (Fig. 3A; Table 3). Additionally, emergence was completed faster in the susceptible population than in the resistant *R. dominica* populations (Fig. 3A; Table 3). The faster emergence for susceptible insects is supported by both cumulative emergence and daily emergence data.

T. castaneum. The period between removal of parental adults from Petri dishes to emergence of the first adult beetles was 14 d. *T. castaneum* emergence was significantly

faster in the phosphine-resistant population compared to the susceptible population (Fig. 2B; Table 2). The numbers of Tc-sus and Tc-res-G adults that emerged were 659 ± 15 and 715 ± 24 per dish, respectively. Daily emergence data show that for the first 7 d after the first emergence was observed, there were numerically more resistant than susceptible *T. castaneum* emerging (Fig. 3B; Table 3). Additionally, emergence was completed faster in the resistant than susceptible population (Fig. 3B; Table 3). The faster emergence for resistant insects is supported by both cumulative emergence and daily emergence data.

Discussion

Based on data obtained, resistant *R. dominica* populations exhibited lower population growth and developmental rates compared to the susceptible population indicating a fitness cost associated with phosphine resistance. In resistant *T. castaneum*, higher population growth and developmental rates compared to the susceptible population were found and indicated a fitness benefit associated with phosphine resistance in this species. Therefore, in field populations of *R. dominica* and *T. castaneum* collected from Oklahoma, there is a fitness cost and a fitness benefit, respectively, associated with phosphine resistance.

Occurrence of fitness cost in resistant insects in an insecticide-free environment has been investigated in other studies as well (White and Bell 1990, Fragoso et al. 2005, Pimental et al. 2007, Sousa et al. 2009). Sousa et al. (2009) found that population growth and developmental rates were lower in some phosphine-resistant populations of *R. dominica* from Brazil than in phosphine-susceptible population, indicating that there is a fitness cost associated with phosphine resistance. However, they also found one

phosphine-resistant *R. dominica* population where there was a fitness benefit associated with phosphine resistance. A study by Pimental et al. (2007) found the existence of fitness costs associated with phosphine resistance in the resistant populations of *R. dominica* from Brazil.

From a phosphine resistance management perspective, where there is a fitness cost to having resistance genes, apparently resistance in a population can decline if phosphine is withheld from being used against the resistant population for a long enough time (Pimental et al. 2007, Sousa et al. 2009). The same is not true for resistant populations where phosphine resistance genes confer a fitness benefit (Schlipalius et al. 2008, Jagadeesan et al. 2012). For the management of phosphine-resistant individuals where phosphine resistance confers a fitness benefit, grain protectant insecticides such as spinosad and a mixture of chlorpyrifos-methyl + deltamethrin can be used to eliminate those resistant individuals (Bajracharya et al. 2013). Based on the present study, phosphine resistance development in *R. dominica* populations where resistance has not developed can be slowed by infrequent use of phosphine, whereas it can be mitigated by suspending phosphine use for extended periods of time in populations with resistance. However, withholding phosphine use for long periods of time may not mitigate phosphine resistance in *T. castaneum*.

The long term and frequent use of an insecticide exposes more insects to selection pressure thereby increasing the chances of resistance development (Tabashnik 1990), whereas withholding use of the insecticide for an extended period will probably reduce the selection pressure and slow resistance development. However, it cannot always be assumed that resistance frequency will decline in the absence of selection pressure. In

order to determine if suspended or infrequent use of a pesticide is a feasible resistance management strategy, it is important to establish whether insecticide resistance confers a fitness cost or benefit (Fragoso et al. 2005, Sousa et al. 2009). Therefore, fitness studies are important in designing appropriate insecticide resistance management plans. Suspending phosphine use for extended periods of time can mitigate phosphine resistance development in resistant populations in which phosphine resistance is associated with fitness cost (Pimental et al. 2007, Sousa et al. 2009). However, for resistant populations in which phosphine resistance confers a fitness benefit, suspension of phosphine use may not be an effective management strategy (Pimental et al. 2007, Sousa et al. 2009, Jagadeesan et al. 2012).

The real danger to insecticide resistance conferring fitness benefit is in the development and stabilization of resistance, i.e. occurrence of widespread resistance. Stability of resistance frequency has been observed in some insects in the absence of the insecticide (Beeman and Nanis 1986, Collins et al. 2001, Haubruge and Arnaud 2001). According to Arnaud et al. (2002), the widespread occurrence of malathion resistance and the almost complete replacement of malathion-susceptible population by the resistant *T. castaneum* population may have been favored by increased fecundity of resistant females. A similar trend was observed in malathion-resistant house flies, *Musca domestica* (L.) (Diptera: Muscidae) with increased female fecundity and resistance stability (Keiding 1967). These examples support the earlier assertion that it cannot always be assumed resistance frequency will decline in the absence of selection pressure. Because phosphine-resistant *T. castaneum* in the present study have a fitness benefit from having

phosphine resistance genes, it is possible that the phosphine resistance in this case could stabilize over time.

When insecticide resistance does not confer a fitness cost, it could be a result of selection of fitness modifier genes that improve the fitness of resistance genes by suppressing their fitness disadvantage in the absence of insecticides or selection of less costly genes which would confer a fitness benefit to resistant individuals (Roush and McKenzie 1987, Coustau et al. 2000, Raymond et al. 2001). The higher population growth and developmental rates observed in our phosphine-resistant *T. castaneum* population may be the effect of possessing such modifier genes or the less costly genes which give the resistant population a reproductive advantage compared to the susceptible population. The cost of insecticide resistance in insects is frequently associated with the cost of maintaining the defensive mechanism for the insecticide (Coustau et al. 2000, Guedes et al. 2006). Energy allocation to insecticide resistance mechanisms can diminish the reproductive capability of the resistant population in the absence of the selecting agent (Coustau et al. 2000, Berticat et al. 2002).

Demographic studies conducted within this study measured population growth rates and developmental rates in order to determine fitness effects of phosphine resistance in *R. dominica* and *T. castaneum* populations found in Oklahoma. Demographic studies are important in providing preliminary information on potential fitness costs associated with insecticide resistance because they transfer individual effects on population-level responses (Heather 1982, Stark and Wennergren 1995, Sousa et al. 2009).

Based on these data for *R. dominica* and *T. castaneum* populations from Oklahoma grain storage facilities, phosphine resistance confers a fitness cost in *R. dominica* but confers a fitness benefit in *T. castaneum*. This may mean that withholding phosphine use for long periods of time can lower phosphine resistance in the *R. dominica* populations whereas doing the same may not mitigate phosphine resistance in the *T. castaneum* populations. This information is vital for the development of phosphine resistance management strategies in the U.S. Additionally, these results indicate that the genetic basis for phosphine resistance in *R. dominica* and *T. castaneum* populations from Oklahoma may be different. Research to investigate the genetic basis of phosphine-resistance in Oklahoma populations of these two species needs to be conducted.

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Table 1. Parameters for non-linear regression equations describing the relationship between cumulative population and time (d) for four populations of *R. dominica* and two populations of *T. castaneum*.

Species	Model	Population	Parameter estimates (± SE)		*F*	R^2
			A	*B*		
R. dominica	$y = A + Bx^2$	Rd-sus	− 49.88 ± 213.26	0.43 ± 0.03a	174.09	0.68
		Rd-res-G	− 307.30 ± 221.77	0.35 ± 0.03b	106.41	0.56
		Rd-res-L	− 314.42 ± 156.02*	0.24 ± 0.02c	98.23	0.55
		Rd-res-P	− 172.29 ± 167.10	0.18 ± 0.03d	47.57	0.37
T. castaneum	$y = A + Bx^{0.5}$	Tc-sus	37.70 ± 48.42	172.60 ± 6.14a	789.64	0.91
		Tc-res-G	− 28.48 ± 60.15	250.21 ± 7.63b	1075.16	0.93

The parameter *A* indicates the minimum value of *y* (the expected *y*-value when $x = 0$). The parameter *B* indicates how quickly the values of *y* changes. The parameter *B* gives the slope estimates. Comparisons of slopes were conducted to determine whether there were significant differences in the population growth of the phosphine-resistant and -susceptible populations of each species. In all cases $df_{error} = 82$. Parameter *A* was only significant where there is an asterisk (*); in all cases for each species, all values of *B* were significant ($P < 0.05$). Lack-of-fit *P* values for Rd-sus, Rd-res-G, Rd-res-L, Rd-res-P, Tc-sus, and Tc-res-G were 0.05596, 0.85305, 0.11749, 0.87271, 0.01486, and 0.00002, respectively. For each species, the values of *B* with different letters are significantly different.

Table 2. Parameters for non-linear regression equations describing the relationship between cumulative emergence and time elapsed (d) after first adult emergence for four populations of *R. dominica* and two populations of *T. castaneum*.

Species	Model	Population	Parameter estimates (± SE)			*F*	R^2
			A	*B*	*C*		
R. dominica	*y = A/(1.0 + exp(-(x - B)/C))*	Rd-sus	704.71 ± 30.67	9.17 ± 0.54a	2.38 ± 0.46	131.92	0.64
		Rd-res-G	721.74 ± 27.20	13.02 ± 0.48b	2.95 ± 0.39	308.80	0.79
		Rd-res-L	679.89 ± 22.46	12.69 ± 0.42b	2.84 ± 0.35	365.19	0.82
		Rd-res-P	593.63 ± 29.03	11.94 ± 0.59b	2.55 ± 0.49	154.23	0.66
T. castaneum	*y = A/(1.0 + exp(-(x - B)/C))*	Tc-sus	709.40 ± 27.94	8.52 ± 0.64a	5.15 ± 0.59	250.69	0.76
		Tc-res-G	744.43 ± 14.11	1.77 ± 0.27b	2.75 ± 0.35	160.04	0.75

The parameter *A* estimates the total number of adults that emerged. The parameter *B* estimates the time required for half the insects in a given population to emerge as adults. Cumulative emergence-related parameter *B* values were used to determine whether significant differences existed among developmental times of the phosphine-resistant and -susceptible populations of each species. The parameter *C* is a growth rate parameter that specifies width or steepness of the curves. In all cases, all values of *A*, *B*, and *C* were significant ($P < 0.05$). Respective df_{error} and lack-of-fit *P* values for Rd-sus, Rd-res-G, Rd-res-L, Rd-res-P, Tc-sus, and Tc-res-G were 147 and 0.70752, 164 and 0.84676, 164 and 0.86144, 157 and 0.57766, 155 and 0.99855, and 109 and 0.81028, respectively. For each species, the values of *B* with the same letter are not significantly different.

Table 3. Parameters for non-linear regression equations describing the relationship between daily emergence and time elapsed (d) after first adult emergence for four populations of *R. dominica* and two populations of *T. castaneum*.

Species	Model	Population	Parameter estimates (± SE)			*F*	R^2
			A	*B*	*C*		
R. dominica	$y = A\ exp(-0.5((x - B)/C)^2)$	Rd-sus	150.02 ± 8.31	9.56 ± 0.23	3.53 ± 0.23	120.93	0.62
		Rd-res-G	126.17 ± 6.09	13.23 ± 0.23	4.13 ± 0.23	154.61	0.65
		Rd-res-L	118.55 ± 4.87	13.25 ± 0.20	4.32 ± 0.21	207.10	0.71
		Rd-res-P	122.05 ± 7.48	12.33 ± 0.25	3.52 ± 0.25	101.21	0.56
T. castaneum	$y = A + B\ ln(x)$	Tc-sus	115.89 ± 8.58	− 28.20 ± 3.47	-	65.98	0.30
		Tc-res-G	288.87 ± 11.95	− 107.50 ± 5.60	-	368.06	0.77

For the non-linear regression equation describing the daily emergence patterns for the four populations of *R. dominica*, the parameter *A* indicates the height of the curve's peak and estimates the total number of adults that emerged during peak emergence; the parameter *B* indicates the position of the center of the peak and estimates the time required for half the insects in a given population to emerge as adults; the parameter *C* is the standard deviation (controls the width of the bell). For the non-linear regression equation describing the daily emergence patterns for the two populations of *T. castaneum*, the parameter *A* gives the estimated number of insects in the initial time period and the parameter *B* estimates the rate at which the daily emergence is declining. In all cases, all values of *A*, *B*, and *C* were significant ($P < 0.05$). Respective df_{error} and lack-of-fit *P* values for Rd-sus, Rd-res-G, Rd-res-L, Rd-res-P, Tc-sus, and Tc-res-G were 149 and 0.93976, 164 and 0.94351, 166 and 0.64846, 157 and 0.66940, 156 and 0.04487, and 107 and 0.40189, respectively.

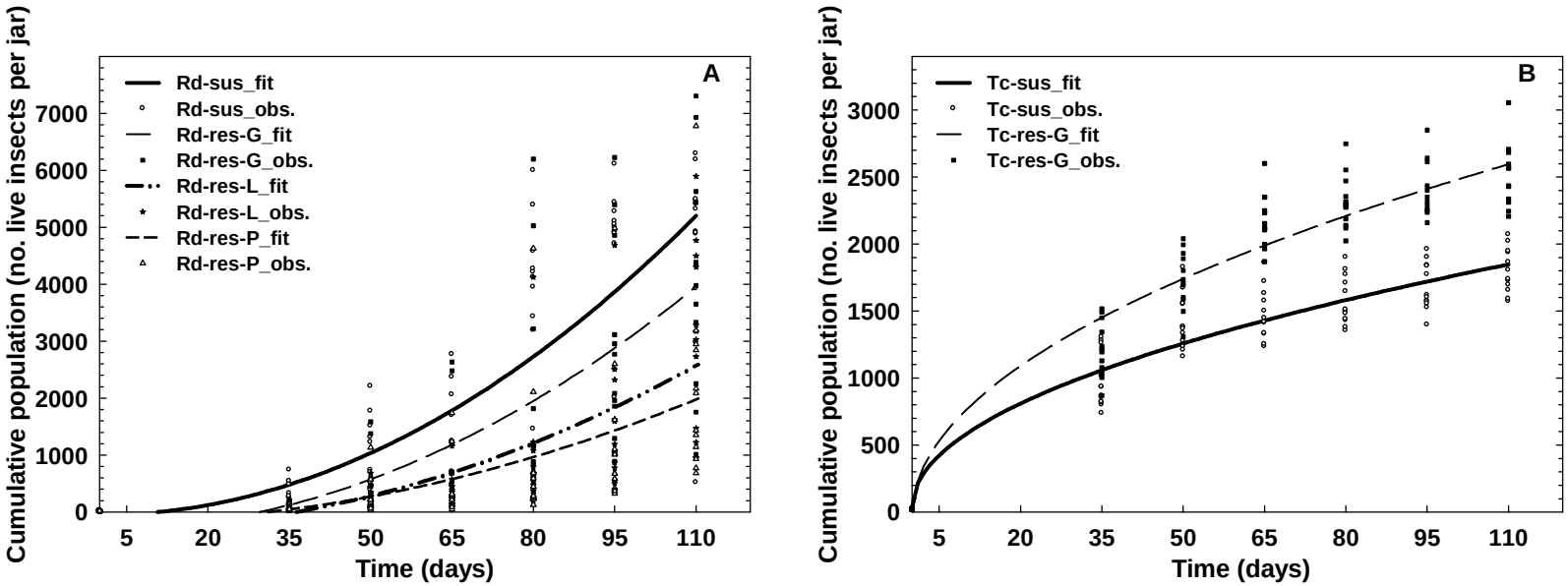

Fig. 1. Cumulative population growth of phosphine-resistant and -susceptible populations of *R. dominica* (A) and *T. castaneum* (B) (n = 12). Symbols represent the observed data. Summary of the non-linear regression analyses of the curves is presented in Table 1.

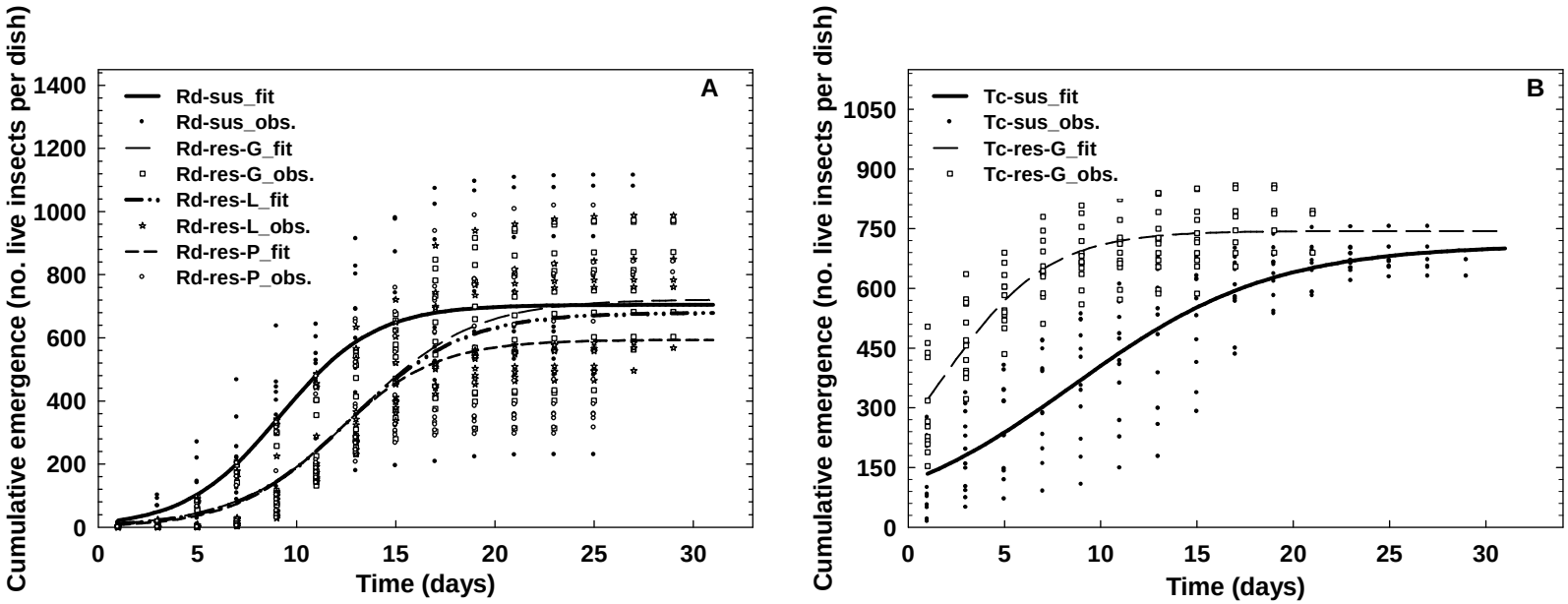

Fig. 2. Cumulative emergence of phosphine-resistant and -susceptible populations of *R. dominica* (A) and *T. castaneum* (B) (n = 12). Symbols represent the observed data. Summary of the non-linear regression analyses of the curves is presented in Table 2.

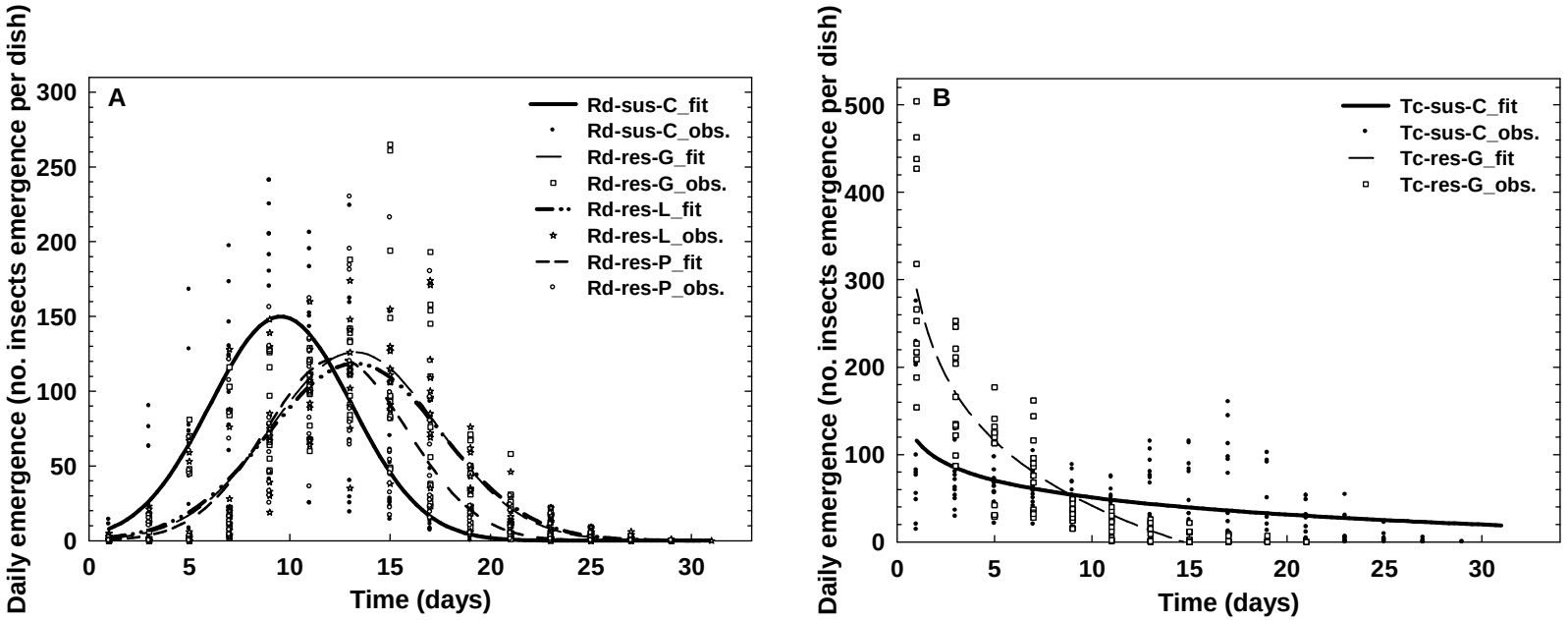

Fig. 3. Daily emergence of phosphine-resistant and -susceptible populations of *R. dominica* (A) and *T. castaneum* (B) (n = 12). Symbols represent the observed data. Summary of the non-linear regression analyses of the curves is presented in Table 3.

CHAPTER IV

COMPARING EFFECTIVENESS OF THREE TRAPS USED TO MONITOR *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA: TENEBRIONIDAE)

Abstract

Integrated management of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle is facilitated by use of insect traps for its detection and monitoring. *T. castaneum* is a major pest of grain processing and storage facilities. The goal of this study was to compare the effectiveness of three types of traps used to monitor *T. castaneum*, namely, ClimbUP® BG (Black Grip), Dome™, and Torios®. Three experimental sheds (2.5 m x 3 m) located at the Stored Product Research and Education Center (SPREC), Oklahoma State University, Stillwater, OK were used. The trap catches of ClimbUP® BG, which is a new type of trap, was compared with those of two commercially available traps, namely, Dome™ and Torios®. The ClimbUP® BG trap is used with a corn oil kairomone, the Dome™ trap with a kairomone and a pheromone lure, and the Torios® trap has a pheromone lure and a sticky surface. The experiment comprised 3 replications of a 3 x 3 Latin square design. In all cases, one type of trap was placed in each experimental shed containing 200 g of diet and 200 adult beetles. After 1 wk the numbers of beetles in traps were counted. Based on this data, Dome™ trap caught the highest number of *T. castaneum*. However, there was no significant difference in the numbers of *T. castaneum* caught in the ClimbUP® BG and Torios® traps. Future research needs to assess how inclusion of visual cues to these traps, in addition to kairomone and pheromone enhances their effectiveness.

KEY WORDS Red flour beetle, ClimbUP® BG trap, Dome™ trap, Torios® trap

Introduction

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a major external-feeding pest of stored grain and other grain-based products and is commonly found in grain processing and storage facilities (Arbogast et al. 2000, Campbell et al. 2010). Insect traps have been developed commercially for the detection and monitoring of *T. castaneum* and other stored-product insects inside grain processing and storage facilities (Barak and Burkholder 1985, Mullen 1992). Traps provide monitoring data that are useful for making appropriate management decisions and evaluating the effectiveness of integrated pest management programs for stored-product insects in grain processing and storage facilities (Campbell et al. 2002).

Several different types of traps for capturing stored-product insects that walk and crawl, e.g. *T. castaneum*, have been developed for monitoring insect infestation levels inside the grain processing, storage, and food facilities (Chambers 1990, Mullen 1992, Phillips 1997). These traps are usually positioned on the floor inside facilities and at locations where insects are likely captured when moving between foods sources (Campbell 2012). However, various studies have shown that trap catches for insects such as *T. castaneum* that have patchy distribution in grain processing and storage facilities, may depend on several factors such as location of traps, trap type and design, presence of food and shelter, temperature, and population density (Mullen 1992, Stejskal 1995, Campbell et al. 2002, Campbell and Arbogast 2004, Toews et al. 2005, Campbell 2012, Semeao et al. 2012).

Pheromones based traps for the stored-product insects have facilitated the development and availability of commercial lures for stored-product insects, and these have aided the improvement of monitoring populations of stored-product insects through the use of baited traps (Barak and Burkholder 1985, Chambers 1990, Phillips 1997). Traps developed for commercial use on *T. castaneum* use food baits such as kairomone and pheromone lures and are typically designed as pitfall-type traps (Barak and Burkholder 1985, Mullen 1992). Food-based kairomones are used as insect baits in traps due to their potential to attract insects as volatile chemical cues, and various studies have been conducted on the different materials that could be potential kairomones for attracting stored-product insects (Barak and Burkholder 1985, Subramanyam et al. 1992, Phillips et al. 1993).

Studies have demonstrated that pheromone and kairomone baited traps have been successfully used for monitoring populations of *T. castaneum* (Mullen 1992, Campbell et al. 2010) in commercial grain processing and storage facilities. However, it has been anecdotally reported that the response of *T. castaneum* to these commercially available traps is low, perhaps resulting in lower trap catches which could give less accurate information on infestation levels in facilities being monitored (Semeao et al. 2011, Campbell 2012).

As previously mentioned, trap type and design are important factors that influence trap catches. Examples of traps that have been developed for detection and monitoring of *T. castaneum* include ClimbUP® BG (Black Grip), Dome™ trap, and Torios® trap. Therefore, the objective of the present study was to compare the effectiveness of these

three types of traps to monitor *T. castaneum* in a simulated grain-based product storage or grain processing environment.

Materials and Methods

Insects. In this study, *T. castaneum* were 3- to 4-mo-old adults that were obtained from our laboratory cultures. *T. castaneum* were reared on a mixture of 95% all-purpose wheat flour and 5% Brewer's yeast at 28°C and 65% RH. Voucher specimens of *T. castaneum* that were used in this study were deposited in the K. C. Emerson Entomology Museum at Oklahoma State University under lot number 136.

Traps. ClimbUP® BG (Black Grip) trap is a new kind of trap produced by Susan McKnight, Inc, Memphis, TN (Fig. 1A). The exterior climbing surface of the ClimbUP® BG trap is made of rough black paper to facilitate insect climbing. The ClimbUP® BG is round with total diameter of 15.24 cm and height of 2.54 cm. The trap has a center well with a diameter of 9.55 cm. Approximately 3 drops of corn oil placed on 3.5-cm filter paper is used as kairomone in ClimbUP® BG trap. The oil-soaked filter paper was placed in the middle of the center well. The inner walls of the ClimbUP® BG trap were lubricated with a very thin dust-like film of commonly available talcum powder so that the beetles that climbed into the outside well of the trap could not climb out. The trap had a perforated lid with 6 holes that was used as a cover to prevent dirt from accumulating inside the trap. The perforated lid permitted escape of kairomone odor from the trap to the surrounding environment.

Dome™ trap (Trécé Inc., Adair, OK) consisted of a trap or catch reservoir based on a pitfall type design and a dome cover (Fig. 1B). Both the kairomone (≈3 drops onto a

supplied piece of filter paper) and pheromone lure marketed with the Dome™ trap were used with this trap (Fig. 1B). The pheromone lure was inserted into one of the slots on the dome cover. The lure was inserted such that the larger end of the lure pointed down into the catch reservoir. The kairomone-covered filter paper was placed at the bottom of the catch reservoir. After the lure and kairomone were placed in their respective positions, the dome cover and the catch reservoir was snapped together by aligning projections on the dome cover with indents on the base of the catch reservoir.

Torios® trap (Fuji Flavor Co. Ltd, Japan; marketed by Insects Limited, Westfield, IN) is a reusable pheromone trap for monitoring crawling stored-product insects (Fig. 1C). A pheromone lure and a sticky inner surface marketed with the Torios® trap were used with this trap (Fig. 1C). The lure was set at the center of the sticky surface and then the sticky surface with the lure was inserted into the bottom window of the trap and the bottom window was closed.

Experimental Sheds. The study was conducted in three experimental sheds (2.5 m x 3 m) located at the Stored Product Research and Education Center (SPREC), Oklahoma State University, Stillwater, OK. The inside of each shed was covered with new plastic (6 mm thick) (Blue Hawk, Poly-America, Grand Prairie, TX) every time a different type of trap was used during the experiment. This was done to ensure that pheromone from previous tests did not interfere with subsequent tests by adhering to surfaces of the sheds.

Trap Comparison. Trap catches of the ClimbUP® BG (Black Grip) trap were compared with those of two previously described commercial traps - Dome™ and Torios®.

Two hundred grams of all-purpose wheat flour and a similar amount of cracked wheat were evenly spread on the floor of each shed. Two hundred beetles were released in each shed, immediately after traps were placed in the sheds. The traps were placed on the floor and against the wall on the east side of each shed. Traps were placed at the center of the east side wall. For each replication, one type of trap was placed in each experimental shed. Traps were always placed in the same position. After 1 wk in the sheds, the number of beetles in each trap was counted. Plastic in each shed was then removed and the shed was properly cleaned to remove all the wheat flour, cracked wheat, and beetles. The sheds were left to "wash out" for 1 wk before they were again set up for sampling. The inside of each shed was then covered with new plastic.

Environmental conditions in each shed were monitored using a temperature and relative humidity sensor (HOBO® U12, Onset Computer Corporation, Bourne, MA) during the experimental period. The experiment was initiated in May 2012 and ended in September 2012. The average temperature and relative humidity recorded in the sheds were 31 ± 2°C and 52 ± 4%, respectively.

The experiment had a 3 x 3 Latin square design with three replications (squares). This involved double blocking - blocking for the shed and the sampling time when the traps were used to catch the beetles in the sheds. The order in which traps were placed in different sheds at different times for the first square is shown in Table 1. The order was

changed accordingly for the second and third squares (Table 1). Statistical analysis was accomplished using Statistical Analysis System software (SAS Institute 2010). PROC ANOVA was used for analysis of variance (ANOVA) to determine whether significant differences existed in trap catches among the three types of traps.

Results

Dome™ traps caught significantly more *T. castaneum* (49.6 ± 11.1) than ClimbUP® BG (28.4 ± 4.6) and Torios® (27.7 ± 6.6) traps (Table 2). There was no significant difference in the numbers of *T. castaneum* caught in the ClimbUP® BG and Torios® traps (Table 2). The ClimbUP® BG trap with only the kairomone performed half as well as the Dome™ trap with a lure + kairomone, which is commercially marketed for *T. castaneum* monitoring. The ClimbUP® BG trap with only the kairomone performed as well as the Torios® trap with a lure + sticky inner surface, which is commercially marketed for *T. castaneum* monitoring.

Discussion

Based on the results of this study, Dome™ traps were found to be comparatively more effective than the ClimbUP® BG and Torios® traps. Similar trap catches were found in the ClimbUP® BG and Torios® traps. The ClimbUP® BG trap had only the kairomone compared to the Dome™ trap that had a lure and kairomone and the Torios® trap that had a lure and sticky inner surface.

Pheromone- and kairomone-baited pitfall traps, such as the Dome trap, have been commonly used inside food facilities for monitoring *Tribolium* species and are effective for tracking population trends of the infesting pests (Campbell et al. 2010, Campbell

2012). Phillips et al. (1993) reported that *T. castaneum* was attracted to wheat-germ and corn oils. According to Barak and Burkholder (1985), pitfall traps baited with food-based oils (wheat-germ oil) resulted in higher trap captures compared to mineral oil baited pitfall traps. In the study conducted by Barak and Burkholder (1985), they combined a food attractant with a synthetic male-produced pheromone (wheat-germ oil + aggregation pheromone) to capture *T. castaneum*. Food-based baits may be effective as a short distance attractant and possibly help in the final steps of capture with the aid of a pheromone attractant (Barak and Burkholder 1985, Phillips et al. 1993).

Campbell (2012) reported that there were more frequent *T. castaneum* encounters with traps baited with pheromone or pheromone and kairomone combination when compared to unbaited traps or traps with kairomone only. Duehl et al. (2011a) also reported low attraction to only food-based volatiles. Campbell et al. (2002) found higher trap catches in FLITeTRAK traps that used food oil and pheromone as attractants compared to Pherocon traps that used only a sticky surface. Phillips et al. (1993) observed that although food based attractants enhanced the response of insects, there was low response of insects to food only or pheromone only baited traps compared to the response to traps baited with both food and pheromone. This could explain why the Dome™ traps with both pheromone and kairomone caught more beetles than ClimbUP® BG traps with only kairomone and the Torios® traps with only pheromone.

Although a combination of pheromone and kairomone is supposed to increase trap catches, percentages of beetles caught are still relatively low. According to Campbell (2012), even under conditions where air was flowing, the average number of beetles encountering traps with the best combination of attractants was only 40% (Campbell

2012). Duehl et al. (2011a) also found that only approximately 50% of beetles responded to pheromone when used as an attractant in traps. In the present study, the Dome™, ClimbUP® BG, and the Torios® traps caught only 25, 14, and 14%, respectively of *T. castaneum*. These results indicate that the effectiveness of pheromone and kairomone baited traps need to be improved in order to provide more accurate information for pest management. Effective use of the chemical cues such as kairomone and pheromone could be improved by incorporating dark shapes (Semeao et al. 2011) and other visual cues such as light (Sheribha et al. 2010, Duehl et al. 2011b).

Based on this data, the Dome™ was more effective for monitoring *T. castaneum* than the ClimbUP® BG, and the Torios® traps. However, the effectiveness of all the three traps in terms of the percentage of beetles caught was quite low. Perhaps inclusion of visual cues, in addition to kairomone and pheromone, could be used to enhance the effectiveness of these traps. Future research is needed to assess these potential additions to traps.

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Table 1. Placement of traps in the sheds during different sampling times for the first, second, and third squares.

Square 1	Shed 1	Shed 2	Shed 3
Time 1	TORIOS®	Dome™	ClimbUP® BG
Time 2	ClimbUP® BG	TORIOS®	Dome™
Time 3	Dome™	ClimbUP® BG	TORIOS®
Square 2	**Shed 1**	**Shed 2**	**Shed 3**
Time 1	ClimbUP® BG	Dome™	TORIOS®
Time 2	TORIOS®	ClimbUP® BG	Dome™
Time 3	Dome™	TORIOS®	ClimbUP® BG
Square 3	**Shed 1**	**Shed 2**	**Shed 3**
Time 1	ClimbUP® BG	Dome™	TORIOS®
Time 2	TORIOS®	ClimbUP® BG	Dome™
Time 3	Dome™	TORIOS®	ClimbUP® BG

Table 2. Number of *T. castaneum* (mean ± SE) caught in traps.

Trap Type	Number of *T. castaneum* (± SE)
ClimbUP® BG	28.4 ± 4.6a
Dome™	49.6 ± 11.1b
Torios®	27.7 ± 6.6 a

Means followed by the same letter are not significantly different (P > 0.05).

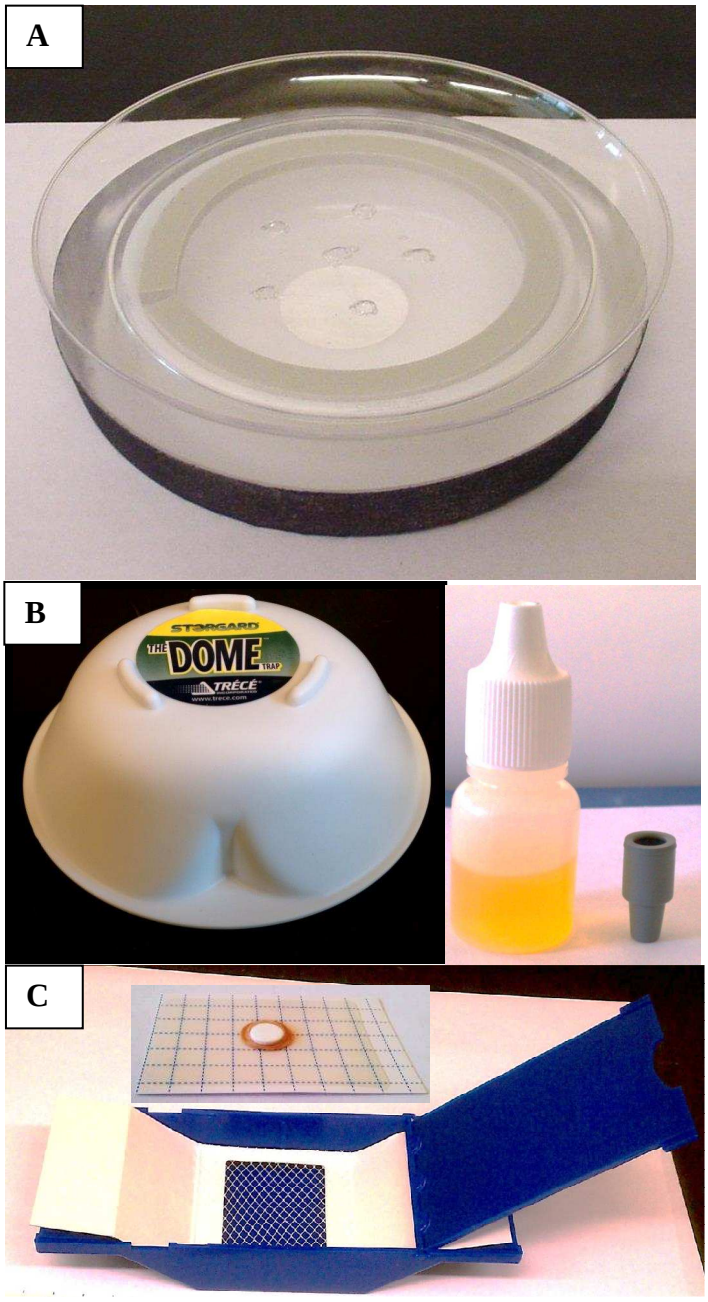

Fig. 1. Pictures of ClimbUP® BG trap (A), Dome™ trap with a lure + kairomone (B), and Torios® trap with a lure + sticky inner surface (C).

CHAPTER V

CONCLUSIONS

The presence of highly phosphine-resistant populations of *R. dominica* and *T. castaneum* in Oklahoma grain storage facilities has necessitated the development of a phosphine resistance management strategy to ensure continued effective use of phosphine. For implementation of effective phosphine resistance management strategy, identification of tools that mitigate resistance development and knowledge of the fitness effects associated with phosphine resistance in insect populations are important. Given the significance of a phosphine resistance management strategy to the U.S. and the importance of detection and monitoring of stored-product insect pests in grain processing and storage facilities to integrated management, relevant studies were conducted to address these issues. The first objective was to investigate the efficacies of the grain protectant insecticides spinosad and chlorpyrifos-methyl + deltamethrin against phosphine-resistant and -susceptible *R. dominica* and *T. castaneum* collected from Oklahoma. The second objective assessed the fitness effects associated with phosphine

resistance in the *R. dominica* and *T. castaneum* populations collected from Oklahoma. The third objective was to compare the effectiveness of three types of traps used to monitor *T. castaneum* in grain processing and food storage facilities.

It was determined that both spinosad and chlorpyrifos-methyl + deltamethrin mixture were effective against phosphine-resistant *R. dominica* and caused 83-100% mortality and also caused total progeny production suppression for all post-treatment storage periods: 2, 84, 168, 252, and 336 d. Spinosad was not effective against phosphine-resistant *T. castaneum*; the highest mortality attained was only 3% for all storage periods investigated. Chlorpyrifos-methyl + deltamethrin mixture was effective against phosphine-resistant *T. castaneum* only in treated wheat stored for 2 and 84 d where it caused 93-99% mortality. However, the mixture of chlorpyrifos-methyl + deltamethrin was effective and achieved total suppression of progeny production in *T. castaneum* at all storage periods. Spinosad was not as effective as chlorpyrifos-methyl + deltamethrin mixture at suppressing progeny production of phosphine-resistant *T. castaneum*.

Based on these data, both spinosad and chlorpyrifos-methyl + deltamethrin mixture can be used to eliminate phosphine-resistant *R. dominica*, whereas only chlorpyrifos-methyl + deltamethrin mixture can be used to eliminate phosphine-resistant *T. castaneum*. This suggests that wheat infested by phosphine-resistant *R. dominica* can be treated using spinosad or chlorpyrifos-methyl + deltamethrin mixture. Wheat infested by phosphine-resistant *T. castaneum* and empty storage structures infested by resistant insects of both species can be treated using chlorpyrifos-methyl + deltamethrin to eliminate these insect pests. Spinosad and chlorpyrifos-methyl + deltamethrin mixture are

grain protectant insecticides that can be effectively used for the management of phosphine-resistant *R. dominica* and *T. castaneum*. These two insecticides can be used in a phosphine resistance management strategy developed for stored-product insect pests in the U.S.

The goals of phosphine resistance management are to slow resistance development 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 periods of time to mitigate resistance, respectively. Knowing whether there is a fitness cost associated with phosphine resistance is important for the development of a resistance management strategy. Experiments were conducted to measure the population growth rates and developmental rates of phosphine-resistant and -susceptible populations of *R. dominica* and *T. castaneum* in a phosphine-free environment to determine the fitness effects caused by phosphine resistance in these two species. Based on this study, it was determined that three resistant *R. dominica* populations tested exhibited lower population growth rates and developmental rates compared to the susceptible population, whereas the resistant *T. castaneum* population tested exhibited a higher population growth rate and developmental rates compared to the susceptible population.

Data for *R. dominica* and *T. castaneum* indicate that there is a fitness cost and a fitness benefit, respectively, associated with phosphine resistance in these two species. This suggests that phosphine resistance development in *R. dominica* populations where resistance has not developed can be slowed by infrequent use of phosphine, whereas it can be mitigated by suspending phosphine use for extended periods of time in the phosphine-resistant *R. dominica* populations. Conversely, withholding phosphine use for

long periods of time may not mitigate phosphine resistance in the *T. castaneum* populations in Oklahoma. However, for both scenarios, the most appropriate option is to eliminate the phosphine-resistant individuals by using an alternative product such as spinosad or chlorpyrifos-methyl + deltamethrin mixture. These results also indicate that genes responsible for phosphine resistance in *R. dominica* and *T. castaneum* from Oklahoma are probably different and molecular studies need to be conducted to investigate the genetic basis for phosphine resistance in these two species.

The study comparing the effectiveness of three types of traps used to monitor *T. castaneum* in grain processing and food storage facilities namely, ClimbUP® BG, Dome™, and Torios® showed that Dome™ traps caught the highest number of *T. castaneum*. There was no significant difference in the numbers of *T. castaneum* caught in the ClimbUP® BG and Torios® traps. Interestingly, the ClimbUP® BG trap with only the kairomone performed half as well as the Dome™ trap with a lure + kairomone. The ClimbUP® BG trap with only the kairomone performed as well as the Torios® trap with a lure + sticky inner surface. Future research needs to be conducted to compare the ClimbUP® BG trap with a lure + kairomone, the Torios® trap with a lure + sticky surface + kairomone, and the Dome™ trap with a lure + kairomone. Based on published studies, research needs to be conducted to determine if trap captures in the three types of traps tested within this study can be enhanced by incorporation of visual cues such as light.

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