Phosphine Resistance in *Tribolium castaneum* and *Rhyzopertha dominica* From Stored Wheat in Oklahoma

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ABSTRACT Phosphine gas, or hydrogen phosphide (PH_3), is the most common insecticide applied to durable stored products worldwide and is routinely used in the United States for treatment of bulk-stored cereal grains and other durable stored products. Research from the late 1980s revealed low frequencies of resistance to various residual grain protectant insecticides and to phosphine in grain insect species collected in Oklahoma. The present work, which used the same previously established discriminating dose bioassays for phosphine toxicity as in the earlier study, evaluated adults of nine different populations of red flour beetle, *Tribolium castaneum* (Herbst), and five populations of lesser grain borer, *Rhyzopertha dominica* (F.) collected from different geographic locations in Oklahoma. One additional population for each species was a laboratory susceptible strain. Discriminating dose assays determined eight out of the nine *T. castaneum* populations, and all five populations of *R. dominica*, contained phosphine-resistant individuals, and highest resistance frequencies were 94 and 98%, respectively. Dose–response bioassays and logit analyses determined that LC_{99} values were ≈3 ppm for susceptible and 377 ppm for resistant *T. castaneum*, and ≈2 ppm for susceptible and 3,430 ppm for resistant *R. dominica*. The most resistant *T. castaneum* population was 119-fold more resistant than the susceptible strain and the most resistant *R. dominica* population was over 1,500-fold more resistant. Results suggest a substantial increase in phosphine resistance in these major stored-wheat pests in the past 21 yr, and these levels of resistance to phosphine approach those reported for other stored-grain pest species in other countries.

KEY WORDS fumigation, stored-product, red flour beetle, lesser grain borer, phosphine resistance

The United States is among the world's leading producers of wheat. Oklahoma is a major producer of winter wheat in the United States and in 2010 was ranked the third largest producer of this type of wheat among U.S. states with production of 3.3 million tonnes (121 million bushels) (National Agricultural Statistics Service [NASS] 2012). Production levels this high are associated with grain pest management practices to protect the enormous wheat investment in storage from losses caused by stored-wheat insect pests. Moreover, Oklahoma is a high-risk state for grain storage because of the longer storage period for grain and the relatively high ambient temperatures (Cuperus et al. 1990, Hagstrum and Flinn 1992). The main method used for controlling insect infestations in stored wheat in Oklahoma is fumigation using phosphine gas, hydrogen phosphide (PH_3), and all wheat

stored in Oklahoma is fumigated at least once a year (Cuperus et al. 1986, Flinn et al. 2003). Commercial storage facilities in Oklahoma use fumigation as the primary management tool and on average fumigate 2.6 times per year (Cuperus et al. 1990).

In many grain elevators in the United States, phosphine is currently the only economically viable product for stored-wheat insect pest management (Hagstrum et al. 1999). This scenario has resulted in the frequent use of phosphine, especially in the southern Unite States. The common use of phosphine globally is because of government regulation of pesticides that led to the loss of older fumigants (carbon tetrachloride, carbon disulfide, ethylene dichloride, and ethylene dibromide), the phasing out of methyl bromide, the declining use of residual contact insecticides stemming from harmful residues they leave in food, and the lack of alternative fumigants that are as convenient to use and cost-effective as phosphine (e.g., Collins et al. 2001, Fields and White 2002, Nayak et al. 2003, Phillips and Throne 2010). Attributes that contribute to widespread use of phosphine are that it is relatively inexpensive, easy to apply, leaves minimal residues, and can be used in a wide range of storage types and commodities (Nayak and Collins 2008). These attributes plus the fact that wheat storage in southern United States is risky have made phosphine the

method of choice for the management of stored-wheat insect pests in Oklahoma.

As with other control agents, long-term heavy reliance on phosphine under circumstances where treatments are inadequate leads to selection of resistance in pest populations (Benhalima et al. 2004). Most of the wheat storage facilities in Oklahoma are not gastight, and such leakiness is probably the main cause of under-dosing (low gas concentration and short exposure times) and consequent survival of insect pests. The practice of over-dosing structures to compensate for the inability to adequately seal these structures before fumigation is common in Oklahoma and could also contribute to an increase in resistance. Indeed, clear evidence of phosphine resistance was found in several populations of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle, and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), the lesser grain borer obtained from 10 counties in Oklahoma (Zettler and Cuperus 1990). Based on this 21-yr-old study, 1 out of 8 populations of *T. castaneum* and 8 out of 12 populations of *R. dominica* tested with a discriminating-dose assay to adults (Champ 1968) were resistant to phosphine. *R. dominica* is the most prevalent internal-infesting insect pest and *T. castaneum* is one of the most prevalent external-infesting pests in sampled grain in Oklahoma (Cuperus et al. 1990). Zettler and Cuperus (1990) also reported phosphine control failures and suggested that these could have been due either to phosphine resistance or to inefficient fumigation. From the time of their study, use of phosphine in Oklahoma has continued with no concerted effort to periodically document whether the resistance or the phosphine failures reported were increasing over time. Given that fumigation practices in Oklahoma have not changed and some populations of *R. dominica* tested 21 yr ago showed extremely high frequencies of resistance (92%) (Zettler and Cuperus 1990), it is likely that phosphine resistance frequency has increased. To date, no published studies have used dose–response tests to examine the levels of resistance of field-collected *T. castaneum* and *R. dominica* in Oklahoma or elsewhere in the United States.

The resistance found by Zettler and Cuperus (1990), as mentioned above, was likely a result of repeated ineffective fumigations in situations in which phosphine gas was rapidly lost because of leakage (Halliday et al. 1983, Tyler et al. 1983) and/or because of overdosing of leaky structures. Similar factors have contributed to an increase in resistance globally. A global survey by the Food and Agriculture Organization (FAO) in 1972–1973 indicated that ≈10% of stored-product insect populations sampled in different countries contained phosphine-resistant individuals (Champ and Dyte 1976). Phosphine resistance in stored-product insect pests has become a major problem in many countries, with very high levels of resistance found in some parts of Asia and Africa (Mills 1983, Taylor and Halliday 1986, Taylor 1989, Zettler 1997, Sayaboc et al. 1998, Rajendran 1999), and more recently in Australia (Collins et al. 2001, Nayak et al.

2010, Emery et al. 2011) and South America (Lorini et al. 2007, Pimentel et al. 2010).

Based on data from their survey, Zettler and Cuperus (1990) recommended regular phosphine resistance monitoring of stored-product insects in Oklahoma as a tool for ensuring sustainability of phosphine use. However, no scientific resistance monitoring has been conducted since that study 21 yr ago. Therefore, the objectives of the current study were to conduct a follow-up assessment of phosphine resistance in Oklahoma and to determine the levels of resistance found in different populations of *T. castaneum* and *R. dominica* collected from different geographic locations in Oklahoma.

Materials and Methods

Insects. The experiments for establishing resistance in adults of *T. castaneum* and *R. dominica* to phosphine were conducted in 2010 and 2011 at the Department of Entomology at Kansas State University, Manhattan, KS. The nine *T. castaneum* and five *R. dominica* populations used in the studies were started using insects obtained by sampling steel bins and concrete grain silos in six Oklahoma counties using WBII pitfall probe traps (Trécé Incorporated, Salinas, CA) (Toews et al. 2005) in 2009. Four *T. castaneum* populations were started using insects obtained from four different steels bins in Payne Co.; subsequently referred to as Payne 1Tc, Payne 2Tc, Payne 3Tc, and Payne 4Tc. One population each was started using insects obtained from concrete silos in Garfield Co. (Garfield Tc); Tulsa Co. (Tulsa Tc); Kingfisher Co. (Kingfisher Tc); Texas Co. (Texas Tc); and Logan Co. (Logan Tc). In the case of *R. dominica*, three populations were started using insects obtained from three different steels bins in Payne Co. (Payne 1Rd, Payne 2Rd, and Payne 3Rd) and one each from concrete silos in Garfield Co. (Garfield Rd) and Logan Co. (Logan Rd). The distance between Oklahoma State University (OSU) (Payne Co.) and the furthest location beetles were sampled (Texas Co.) is ≈440 km. The four steel bins are 13.6-tonne (500-bushel) bins located at the Stored Product Research and Education Center, OSU (Stillwater, OK) and the concrete silos from grain elevators. Sample sizes used to start insect cultures ranged from 50 to 400 adults. The insecticide-susceptible laboratory reference strains of *T. castaneum* (Susceptible Tc) and *R. dominica* (Susceptible Rd) were 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 the 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 all *R. dominica* and *T. castaneum* populations that were used in this study were deposited in the K. C. Emerson Entomology

Museum at Oklahoma State University under lot numbers 121–126 and 127–136, respectively.

Frequency of Phosphine Resistance. FAO Method No. 16 (Food and Agriculture Organization 1975) was used on mixed-sex adult insects, 1–6 wk old, with the following modifications. For each of the *T. castaneum* susceptible and field populations, 10 insects were placed in each of 10 cylindrical glass vials (4.5 cm in height × 1.2 cm in diameter), and then five glass vials, bundled together using a rubber band, were placed in each of two 3.8-liter glass jars before the introduction of phosphine fumigant ($n = 2$). The vials had lids with tops made of U.S. Standard #40 mesh screen with 0.42-mm openings to permit fumigant entry and prevent beetles from escaping. A small quantity of diet (0.5 g of cracked wheat) was added to each vial. Lids were screwed on tightly after insects and diet had been placed in the vials. The 3.8-liter jars acted as fumigation chambers. The jars were airtight and equipped with a port in the center of the metal screw-on lid that was fitted with a rubber injection septum that was used for the introduction and sampling of the fumigant. Before the lid was screwed onto the glass jar, a rubber gasket was placed in it, and a thin layer of vacuum grease applied for a tight seal between the metal lid and the top edge of the jar to increase gas-tightness. Insects were also placed in another two 3.8-liter jars as previously described but fumigant was not added to these jars. Tests to determine the frequency of phosphine resistance in insects from field populations were completed by the F_3 generation.

Laboratory fumigation methods and gas chromatographic-flame photometric detector (GC-FPD) quantification of the average applied concentration of phosphine in jars was done according to the methods described by Sekhon et al. (2010). The quantity of phosphine gas (10,000 ppm, in N_2; Matheson Tri-Gas) required to attain 30 ppm of phosphine in a 3.8-liter jar was calculated (1 mg/liter of phosphine = 714.18 ppm or one ppm = 0.0014 mg/liter). The gas was introduced separately into each of the two jars with *T. castaneum* through the rubber septum by using a gas-tight syringe after first removing an equivalent volume of air from the jar using a syringe. Two drops of water were added to each jar using a syringe to maintain ≈70% RH inside the jars. Jars were then placed in an incubator maintained at 25°C. The jars were opened 20 h later, and all vials were removed and held at 25°C and 70% RH. The concentration of phosphine in each jar was measured at the start and end of the 20-h period using quantitative GC-FPD methods, and an average concentration for the exposure period was calculated for each fumigation chamber. Beetles were removed from the vials and counted as live, moribund, or dead after 2 wk. Moribund and dead beetles were placed in a 9-cm petri dish containing a piece of filter paper moistened with 0.5 ml of water. These insects were then reevaluated after 24 h for recovery. A similar protocol was followed for *R. dominica* susceptible and field populations except the concentration of phosphine gas in each fumigation chamber was 20

ppm—the discriminating dose for *R. dominica* (Food and Agriculture Organization 1975).

Level of Phosphine Resistance. *Selection of Field Populations for Testing.* We considered populations of *T. castaneum* and *R. dominica* that had ≥80% survival in both replications, based on the FAO Method No. 16 (Food and Agriculture Organization 1975) above, to have high frequencies of resistance, and these populations were tested in dose–response studies. Concentrations of phosphine required to kill 50, 95, and 99% of the sample beetle population (LC_{50}, LC_{95}, and LC_{99}) for each of the selected populations (Garfield Tc, Payne 1Rd, Logan Rd, and Garfield Rd) were determined as a means of establishing their level of resistance.

Susceptible Strains. In the determination of the level of mortality in the susceptible *T. castaneum* and *R. dominica* strains, phosphine concentrations evaluated were 0.0, 0.48, 1.20, 1.89, and 3.04 ppm. For *T. castaneum*, groups of 50 mixed-sex adults were placed in each of 15 cylindrical plastic vials (6 cm in height × 3.5 cm in diameter) and one vial was placed in each of fifteen 3.8-liter glass jars before the introduction of the fumigant. The vials were placed in the jars with no lids, and the neck of the vial was covered with Fluon (polytetrafluoroethylene; Northern Products, Woonsocket, RI) to prevent beetles from escaping. A small quantity of diet (0.5 g of cracked wheat) was added to each vial. Three 3.8-liter jars were allocated to each of five aforementioned phosphine concentrations ($n = 3$). The jars acted as fumigation chambers and their constituent parts and use have already been described above. For susceptible *R. dominica*, a similar protocol was used and a vial containing 50 insects was placed in each of the jars with the susceptible *T. castaneum.* Jars were then maintained at 27.5°C and ≈70% RH internally. The jars were opened 3 d later, and all vials were removed and held at 27.5°C and ≈70% RH. Mortality was assessed 5 d later.

Field Populations. In the determination of the level of resistance of the Garfield Tc population of *T. castaneum*, phosphine concentrations evaluated were 0.0, 26.8, 45.5, 79.8, 104.9, 141.7, 166.4, 200.0, and 221.2 ppm. Groups of 50 mixed-sex adults were placed in vials, that were then placed in 3.8-liter jars as already described to achieve a replication of three ($n = 3$). In addition, 50 susceptible insects in vials were placed in each of the jars containing insects of the field population. The insects were fumigated for 3 d and mortality was assessed 5 d later.

The level of resistance of the Payne 1Rd, Logan Rd, and Garfield Rd populations of *R. dominica* were evaluated using concentrations of 0.0, 29.5, 48.4, 173.7, 279.3, 466.7, 546.7, and 640.7 ppm. The protocol used was similar to that for the Garfield Tc population of *T. castaneum*, except all the *R. dominica* populations were tested at one time, that is, each fumigation chamber contained insects from each of the three populations plus susceptible *R. dominica*. Tests to determine levels of phosphine resistance in insects from field populations were completed by the F_7 generation.

Table 1. Survival of adults from laboratory susceptible strains and field-collected populations of *T. castaneum* (30 ppm) and *R. dominica* (20 ppm) after 20-h exposure to discriminating doses of phosphine fumigant

Species	Population	% survival	
		Replicate 1	Replicate 2
T. castaneum	Payne 1Tc	31	34
	Payne 2Tc	34	38
	Payne 3Tc	2	18
	Payne 4Tc	26	18
	Garfield Tc	94	94
	Tulsa Tc	0	0
	Kingfisher Tc	20	24
	Texas Tc	16	28
	Logan Tc	64	46
	Susceptible Tc	0	0
R. dominica	Payne 1Rd	98	82
	Payne 2Rd	12	38
	Payne 3Rd	34	14
	Garfield Rd	98	96
	Logan Rd	90	98
	Susceptible Rd	0	0

Table 2. Logit analyses of mortality for laboratory susceptible strains and phosphine-resistant field populations of *R. dominica* and *T. castaneum*, after 3 d

Population	LC$_{50}$ (ppm) (95% CI)	LC$_{95}$ (ppm) (95% CI)	LC$_{99}$ (ppm) (95% CI)	Slope ± SE	Intercept ± SE	χ^2 (df) [H]^a
Susceptible Tc	1.35 (1.24–1.45)	2.34 (2.07–2.84)	3.18 (2.66–4.29)	12.31 ± 1.125	−1.593 ± 0.222	16.71 (10) [1.67]
Garfield Tc	27.90 (23.55–31.96)	148.09 (126.81–180.43)	377.49 (290.78–533.54)	4.062 ± 0.309	−5.872 ± 0.553	19.29 (22) [0.88]
Susceptible Rd	0.65 (0.60–0.71)	1.45 (1.28–1.70)	2.26 (1.70–2.90)	8.50 ± 0.682	1.556 ± 0.185	4.14 (10) [0.41]
Payne 1Rd	288.17 (260.36–311.39)	447.52 (400.17–548.55)	572.78 (485.32–790.58)	15.402 ± 1.597	−37.884 ± 3.994	46.87 (19) [2.47]
Logan Rd	62.24 (39.99–91.44)	591.71 (360.07–1377.69)	2054.4 (972.25–8002.3)	3.054 ± 0.190	−5.52 ± 0.402	106.80 (19) [5.62]
Garfield Rd	104.90 (44.97–167.69)	980.18 (556.39–3233.41)	3430.8 (1426.7–27142.0)	3.034 ± 0.230	−6.131 ± 0.558	138.72 (19) [7.30]

LC, lethal concn; Tc, *T. castaneum*; Rd, *R. dominica*.
^{a} Heterogeneity value (quotient of χ^2 and df).

Data Analysis. To determine the level of resistance of adult susceptible strains and field populations of *T. castaneum* and *R. dominica* to phosphine, their response to phosphine was subjected to logit analysis using PoloPlus (LeOra Software, Petaluma, CA) (LeOra Software 2005). A ratio test to compare LCs was also conducted (Robertson et al. 2007). Logit analyses were used instead of probit analyses because a logit transformation of the proportion kill resulted in a better fit to the data than use of a probit transformation (LeOra Software 2005). We ensured that slopes and intercepts differed from 0 when selecting equations to fit the data.

Results

We found clear evidence of resistance in eight out nine field populations of *T. castaneum* and in all field populations of *R. dominica* (Table 1). Resistance frequencies of field populations of *T. castaneum* ranged from 2 to 94% whereas those of *R. dominica* ranged from 12 to 98% (Table 1). Numerically, the Garfield Tc population of *T. castaneum* had the highest frequencies of resistance, 94% in both replicates one and two. The Tulsa Tc population of *T. castaneum* had no detectable resistance. Numerically, the Garfield Rd population of *R. dominica* had the highest frequencies of resistance, with 98 and 96%, respectively. High frequencies of resistance in *R. dominica* were also found in the Logan Rd population, with 90 and 98%, respectively, and in the Payne 1Rd population, with 82 and 98%, respectively (Table 1). Insect mortality in control jars that did not receive fumigant was extremely low and only one *T. castaneum* from the susceptible strain died. The same was true for *R. dominica* where only two insects died, one from the susceptible strain and one from the Payne 3Rd population.

Concentrations of phosphine required to kill 50, 95, and 99% of the Susceptible Tc strain and Garfield Tc population (Table 2) were compared (Table 3). In all

Table 3. Comparison of lethal concentrations (ppm) required to kill 50, 95, and 99% of *T. castaneum* and *R. dominica* adults of susceptible strains and field-collected populations, after 3 d

Populations compared	No. conc. groups	Lethal conc. ratios		
		LC_{50} (95% CI)	LC_{95} (95% CI)	LC_{99} (95% CI)
Garfield Tc vs Susceptible Tc	36	20.71 (17.62–24.35)	63.38 (51.65–77.78)	118.65 (84.40–166.79)
Payne 1Rd vs Susceptible Rd	33	442.80 (401.34–488.53)	309.80 (262.46–365.69)	253.59 (198.85–323.39)
Logan Rd vs Susceptible Rd	33	98.72 (82.62–117.95)	409.62 (307.77–545.18)	909.57 (587.46–1408.28)
Garfield Rd vs Susceptible Rd	33	161.19 (129.58–200.52)	678.55 (503.47–914.51)	1518.91 (943.28–2445.79)
Logan Rd vs Payne 1Rd	42	0.22 (0.19–0.26)	1.32 (1.02–1.72)	3.59 (2.38–5.40)
Garfield Rd vs Payne 1Rd	42	0.36 (0.30–0.45)	2.19 (1.66–2.89)	5.99 (3.82–9.40)
Garfield Rd vs Logan Rd	42	1.63 (1.27–2.11)	1.66 (1.15–2.38)	1.67 (0.94–2.98)

LC, lethal concn; Tc, *T. castaneum*; Rd, *R dominica*.
A 95% CI that includes one in any comparison means that the two lethal concentrations are not significantly different.

cases, the comparisons yielded 95% CIs that did not include 1, so the LC values in each case were different (Table 3). Therefore, in relation to LC_{99}, the Garfield Tc population was 119 times more resistant than the Susceptible Tc strain. Similarly, LC_{50}, LC_{95}, and LC_{99} values for the Payne 1Rd field-collected insects and the Susceptible Rd strain (Table 2) were compared, and the LC values in each case were different (Table 3). Therefore, in relation to LC_{99}, the Payne 1Rd population was 254 times more resistant than the Susceptible Rd strain. Similar comparisons involving the Logan Rd and Garfield Rd populations showed they were 910 and 1,519 times, respectively, more resistant than the Susceptible Rd strain (Table 3). Comparison of the Logan Rd and Payne 1Rd populations showed that the Logan Rd population was four times more resistant than the Payne 1Rd population. Garfield Rd beetles were six times more resistant than Payne 1Rd beetles (Table 3). Finally, a comparison of the Garfield Rd and Logan Rd populations showed their LC_{99} values did not differ (Table 3). However, the LC_{95} values differed and the Garfield Rd population was two times more resistant than the Logan Rd population. It is important to note that the significant differences found in all but a few of these comparisons (Table 3) existed despite the fact that heterogeneity values in most cases were >1 (Table 2), which implies the differences between the populations compared were quite pronounced.

Discussion

There is a trend to more resistant populations with higher frequencies of resistance in our study compared with a similar study done 21 yr ago (Zettler and Cuperus 1990). We found that eight out of nine (89%) of the *T. castaneum* populations had detectable phosphine resistance, whereas all the five (100%) *R. dominica* populations showed detectable phosphine resistance. Sampling from 10 Oklahoma counties, Zettler and Cuperus (1990) found that one out of eight populations (13%) of *T. castaneum* and eight out of 12 populations (67%) of *R. dominica* had detectable resistance to phosphine. In the current study, the ranges for frequencies of resistance were 2–94% for *T. castaneum* and 12–98% for *R. dominica.* In the Zettler and Cuperus (1990) study, the ranges of these frequencies

were 0–6% for *T. castaneum* and 0–92% for *R. dominica.* Only one of their *R. dominica* populations had a resistance frequency of ≥80%.

Phosphine resistance has been found in many stored-product insect species from many countries since the 1970s and the global survey conducted in 1973 found that phosphine resistance has been present in many countries for several decades (Champ and Dyte 1976). At the time of that survey, the most resistant populations were 12 times more resistant than the susceptible ones. High levels of phosphine resistance (at least 100 times) have since been found in Africa, Asia, Australia, and South America (Mills 1983, Tyler et al. 1983, Taylor and Halliday 1986, Taylor 1989, Zettler 1997, Sayaboc et al. 1998, Collins et al. 2001, Lorini et al. 2007, Pimentel et al. 2010, Nayak et al. 2010, Emery et al. 2011). And now, the current study shows high levels of resistance occur in U.S. populations of *T. castaneum* and *R. dominica* as well. Based on 99% mortality level (LC_{99}), Oklahoma populations of *T. castaneum* and *R. dominica* investigated in the current study had phosphine resistance levels that were up to 119 and 1,519 times, respectively, higher than those of susceptible strains. Although an evaluation of the frequency of phosphine resistance in *T. castaneum* and *R. dominica* was previously conducted with U.S. populations, that study did not determine level of resistance in dose–response tests. Therefore, ours is the first study to document the level of resistance in field populations of these species in the United States.

The levels of phosphine resistance in *T. castaneum* and *R. dominica* reported in the current study are based on a 72-h fumigation period with adult beetles only. Different levels of phosphine resistance can occur within a species. In *R. dominica,* for example, at least two levels of resistance appear to be present, that is, a "weak resistance" with resistant adults being ≈23 times more resistant than susceptible beetles when fumigated for 20 h, and a "strong resistance" with resistant adults being hundreds of times more resistant than susceptible insects when fumigated for 48 h (Collins et al. 2002). Resistance factors used for defining these two resistances are based on LC_{50}. Our data show that strong resistance, as defined by Collins et al. (2002) and based on adult bioassays, very likely exists

in the field populations of *R. dominica* from the wheat-growing region of the southern United States.

Concentration and/or duration can be increased during fumigation to enhance fumigant efficacy. The general recommendation for phosphine fumigation of stored grain in the United States is 200 ppm for 4 d or more at 20–30°C (Leesch et al. 1995, Phillips et al. 2012). It is likely that these conditions are adequate to control insects with weak resistance and susceptible insects. The LC_{99} of the resistant *T. castaneum* and *R. dominica* populations in the current study ranged from 377 to 3,431 ppm, with a 3-d exposure period. This most probably means the conditions required to kill 99% of the individuals in field populations where these insects were collected are much higher than what is currently recommended. Perhaps, this could be the reason for some of the fumigation failures that have been reported in Oklahoma (Zettler and Cuperus 1990). In Australia, phosphine label rate changes had to be implemented to ensure successful control of insects with strong phosphine resistance (Emery et al. 2011). In fact, another phosphine label rate change will likely soon occur in Australia as a result of the identification of *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) with extremely high levels of resistance, the highest level so far recorded for any stored grain pest in that country (Nayak et al. 2010, Emery et al. 2011). Label rate changes made in Australia are based on laboratory experiments and field trials (Nayak et al. 2010). Given the high levels of phosphine resistance in *R. dominica* reported in the current study, steps need to be taken to collect the necessary data to enable a change in the phosphine label rate in the United States.

Phosphine gas is an important tool for the management of stored grain pests, and the occurrence of phosphine resistance in pest populations presents challenges to the continued effective use of this fumigant. The presence of highly phosphine resistant *T. castaneum* and *R. dominica* populations in Oklahoma could be an indication of the same in other parts of the United States and North America, and an indication that it could develop in areas where resistance may currently not exist but phosphine is being widely used. Our findings call for a survey of key stored-grain insect pest species across the United States to determine the presence and extent of phosphine resistance. In addition, serious consideration needs to be given to the development of a national resistance monitoring program for the U.S. Development of such a program could benefit heavily from the experience of Australia, the only country in the world with a national resistance monitoring program (Emery et al. 2011). However, this may have been possible in Australia because the grain industry is smaller, and better at developing a coordinated strategy than the larger more diverse U.S. grain industry.

In Australia, phosphine resistance monitoring is based on a resistance management strategy whose goal is "To ensure the long-term sustainability of phosphine through the strategic adoption and implementation of commercially viable, practical, scientifically-based management strategies" (Collins 2009). Management strategies that have been used to eradicate resistant insects from bulk grain in Australia include, longer exposure periods and/or higher concentrations (that have resulted from changes in the phosphine label rate), proper sealing of grain storage structures, replacement of phosphine application equipment, turning of grain, application of effective grain protectants, treating empty storage structures using residual pesticides, and sanitation (Emery et al. 2011). Other management strategies include combating highly phosphine resistant stored-product insect pests using sulfuryl fluoride (Profume) and monitoring pest populations by inspection, sampling, and resistance testing (Nayak et al. 2010). Where strong resistance exists, new fumigation protocols can be developed based on laboratory experiments and field trials to increase phosphine exposure periods and/or concentrations to enable the eradication of such insects (Collins et al. 2001, Nayak et al. 2010).

In relation to phosphine resistance management in India, Rajendran (1999) recommended revising phosphine dosage schedules, improving the sealing of stacks of gunny sacks during fumigation, fumigant monitoring, and the investigation of possible substitutes such as CO_2. Some of the phosphine resistance management components used in Australia and India that apply to bulk storage environments could also be used in the United States. Through the use of such management strategies, the susceptibility to phosphine of stored-product insect pests in the United States could be maintained. Increased adoption of integrated pest management (IPM) will be an important part of any phosphine resistance management strategy for the United States. This could involve development of adequate educational programs for wheat postharvest systems and adequate demonstration of IPM principles and practices in on-farm and commercial storage facilities (Cuperus et al. 1993).

Implementation of a phosphine resistance management strategy is dependent on the ability to quickly and accurately distinguish between insects with weak or no resistance and those with strong resistance. Currently, a discriminating concentration of phosphine of 71 ppm (0.1 mg/liter), with 48-h exposure is used in Australia to distinguish insects with strong resistance from those with weak resistance and from susceptible insects (Collins et al. 2002). However, key drawbacks of this distinguishing method are the long response time and low sensitivity (Collins et al. 2002).

In the current study, tests to determine levels of phosphine resistance in insects from field populations were completed by the F_7 generation. Given that 50–400 adults were used to start populations of field collected insects and F_7 generation insects were tested, founder effects in this case would probably be minimal. Founder effects are usually more pronounced in populations started using a very small number of individuals after many generations. The optimum size for a founder population, based on theoretical studies, is 200–1,000 (Bartlett 1985, Mackauer 1976, Waage et al. 1995). However, one laboratory

study showed that it is possible to start a culture from a single mating pair without significantly altering biological features of the population for at least 25 generations (Prezotti et al. 2004). One possible conclusion that may be drawn from the use of F_7 generation insects is that much higher resistance levels exist in Oklahoma field populations of *T. castaneum* and *R. dominica* because there are still high resistance levels after seven generations of no exposure to phosphine. For many resistant insects there are reproductive fitness consequences that result in them reproducing at lower rates than susceptible individuals when taken away from the selecting force, phosphine in this case.

Phosphine resistance in pest populations presents new problems for future research. As previously mentioned, long response time and low sensitivity are two major drawbacks of the traditional bioassay for determining phosphine resistance. These drawbacks can be solved by the development of either biochemical or molecular testing methods. Already, genomic methods have been used to identify the major genes responsible for phosphine resistance in *R. dominica* and *T. castaneum* (Schlipalius et al. 2008, Jagadeesan 2011). Future research should also determine the extent of different pest species and geographic locations that manifest phosphine resistance in North America, and should develop plans for managing or ameliorating phosphine resistance so this valuable fumigant can remain a viable pest management tool.

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