

Levels of phosphine resistance in rusty grain beetles, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), from stored wheat in Oklahoma

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Abstract

Phosphine gas, or hydrogen phosphide (PH₃), is the most common insecticide applied to durable stored products worldwide and is routinely used in the U.S. for treatment of bulk-stored cereal grains and other durable commodities. Research from the late 1980s revealed low frequencies of resistance to various residual grain protectant insecticides and to PH₃ in grain insect species collected in Oklahoma. Recent research has found resistance in 13 populations of the rusty grain beetle (RGB), *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), collected from wheat storage facilities in Oklahoma. In the present study, we conducted dose-response tests on eight of the aforementioned populations of *C. ferrugineus* with detectable PH₃ resistance to determine concentrations needed to kill 99% of the individuals in each of the resistant populations and the concentration required to kill a similar percentage of individuals in a laboratory susceptible strain. Probit analyses of dose-response data determined that the LC₉₉ values for the susceptible strain and the most resistant population, DK Farm 20, were 7.3 and 968.6 ppm, respectively. The LC₉₉ values for the resistant populations ranged from 97.2 to 968.6 ppm. We found that the K Bin 47, K Bin 61, K Bin 77, K Bin 88, H Bin 2, H Bin 3, JE and DK Farm 20 populations were 21.5-, 7.3-, 44.1-, 32.4-, 16.6-, 5.0-, 87.7- and 133.5-fold, respectively, more resistant to PH₃ than the susceptible strain. This study shows levels of PH₃ resistance in some *C. ferrugineus* populations in Oklahoma and the need to conduct resistance tests in stored-product insect pests in all wheat growing regions of the United States in order to develop PH₃ resistance management strategies for these pests.

Keywords: fumigation, stored-product pest, phosphine resistance, level of resistance, dose-response

1. Introduction

Oklahoma produced 2.87 million metric tons (105.4 million bushels) of winter wheat (*Triticum aestivum* L.) worth \$738 million in 2013 (NASS, 2014). Due to the comparatively warmer temperatures in Oklahoma, stored-product insect pests pose a significant risk to wheat in storage. Several stored-product insect pests, including the rusty grain beetle (RGB), *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), infest wheat in storage in Oklahoma and worldwide. Even though *C. ferrugineus* is a secondary pest and does not usually contribute to insect damaged kernels (IDK), grain with high infestations of this insect usually receives a lower market price compared to un-infested grain (Flinn et al., 2010). Phosphine gas or Hydrogen phosphide (PH₃) is the method of choice for fumigating stored grain to manage stored-grain insect pests in Oklahoma. Commercial grain storage facilities in Oklahoma heavily rely on PH₃ for insect control and fumigate each lot or parcel of grain up to 3 times per year (Cuperus et al., 1990).

Governmental regulation of pesticides has significantly contributed to the frequent and widespread use of PH₃. These regulations led to the loss of older fumigants, the greatly diminished use of methyl bromide, and reduced use of residual contact insecticides because of harmful residues they leave in food. There are few alternative fumigants that are cost-effective, easy to apply, leave nearly no residues, and can be used in a wide range of storage types and commodities like PH₃ (Collins et al., 2001; Nayak et al., 2003; Phillips and Throne, 2010).

Heavy dependence on PH₃ has led to the development of strong resistance in *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) (Opit et al., 2012). A recent study by Konemann et al., (2013), showed PH₃ resistance in 13 *C. ferrugineus* populations collected from farm storage bins and commercial elevators in four counties in Oklahoma, USA. Based on a discriminating dose assessment, they demonstrated that PH₃ resistance was present in all of these populations. The resistance frequencies, i.e., percentage survival of insects exposed to the discriminating dose bioassay, ranged from 16-93%. In this study, our objective was to conduct PH₃ dose-response tests on adults of eight out of 13 of the aforementioned *C. ferrugineus* populations to determine their level of resistance.

2. Materials and Methods

2.1. Insects

Levels of PH₃ resistance were determined in eight populations of *C. ferrugineus* in our laboratory cultures, namely, JE, DK farm 20, H Bin 2, H Bin 3, K Bin 47, K Bin 61, K Bin 77 and K Bin 88 (Konemann et al., 2013) (Table 1). Cultures of these populations have been maintained in laboratory since 2013 at 28 ± 1°C and 65 ± 5% r.h. The susceptible strain was obtained from laboratory cultures maintained at the Center for Grain and Animal Health Research (CGAHR), Manhattan, KS.

Table 1 Survival of adults from the laboratory susceptible strain and 13 field-collected populations of *C. ferrugineus* after 20-h exposure to a PH₃ discriminating dose of 56.15 ppm.

Population	Percentage of individuals surviving (%)		
	Replicate 1	Replicate 2	Replicate 3
DK Farm	44	32	31
H Bin 7	18	20	38
H Bin 11	31	28	38
H Bin 14	10	32	6
H Bin 71	35	40	43
DK Farm 20	100	94	84
JE	100	92	82
K Bin 47	36	44	32
K Bin 61	40	20	36
K Bin 77	31	48	56
K Bin 88	72	61	65
H Bin 2	17	22	24
H Bin 3	10	16	24
Susceptible (CGAHR)	0	0	0

2.2. Preparation of Insects

For each of the eight *C. ferrugineus* field populations, and each PH₃ concentration, 50 mixed-sex adults were placed in each of three cylindrical glass vials (6.5 cm in height × 2.9 cm in diameter) with a small quantity of diet (0.5 g of oats). In addition, 50 susceptible insects were also placed in each of another three cylindrical glass vials. The mouth of each glass vial was covered with a piece of paper towel and secured using Teflon[®] tape.

2.3. Fumigation chambers

Each fumigation chamber consisted of a 3.92-liter glass jar (S-12758M, Uline, Waukegan, IL) along with a plastisol[®] lined metal lid (S-18023, Uline, Waukegan, IL). The lid was equipped with a port in the center, which was fitted with a rubber injection septum for the introduction and sampling of the fumigant. A double layer of thread seal tape was applied to the outside of the lid after the lid was screwed on and to the outside edge of the rubber septum to prevent gas leakage. Prior to the placement of vials containing insects, two drops of water were added to each jar to maintain 70 ± 5% r.h. After placing the vials containing insects in each jar and prior to injection of the gas, a volume of air 1.5-times the amount of gas to be added was removed using a gas-tight syringe (100 ml, Hamilton 1100 SL SYR, Hamilton Inc., Reno, NV).

In the determination of dose-responses of the CGAHR, JE, DK Farm 20, K Bin 47, K Bin 61, K Bin 77, K Bin 88, H Bin 2, and H Bin 3 populations, the range of PH₃ concentrations tested were 2.0-20.7, 29.2-597.8, 29.1-599.7, 3.3-254.1, 3.3-254.1, 3.3-333.1, 29.2-484.2, 3.3-333.1, and 3.3-254.1 ppm, respectively. These concentrations were achieved by injecting pre-calculated volumes of 10,000 ppm PH₃ gas into 3.92-liter fumigation jars (S-12758M, Uline, Waukegan, IL). Each concentration was replicated three times.

2.4. Phosphine concentration measurement

The concentration of PH₃ gas in each jar was measured at the start and end of the 3-d exposure period using a gas chromatographic-flame photometric detector (GC-FPD) method. Prior to taking a gas sample from each jar, gas in the jar was evenly mixed by pumping 3 times with a 500-ml syringe. The GC-FPD was calibrated using 200 ppm PH₃ gas (Matheson Tri-gas) before sampling jar concentrations. The concentrations were established using a standard curve based on 10, 20, 30, 40 and 50 µl of 200 ppm PH₃. For establishing the standard curve, gas samples corresponding to each of the above volumes were withdrawn from the 200 ppm Tedlar[®] bag using a calibrated gas-tight syringe (50 µl, Hamilton 1705 TLL SYR, Hamilton Inc., Reno, NV) and injected into the on-column injector of the GC-FPD. After calibrating the GC-FPD, the concentration in each fumigation jar was measured by removing 30-µl gas samples using the same 50-µl syringe and analyzing using the GC-FPD method.

2.5. Post fumigation procedure

After the sampling of the start concentrations, jars were placed in an incubator maintained at 25 ± 1°C for 3 d then removed and the end concentrations were sampled. Average concentration of PH₃ in each jar was then determined. All vials within each jar were removed and kept in a plastic box (42.9 × 29.2 × 23.5 cm) in an incubator and maintained at 25 ± 1°C and 70 ± 5% r.h. for 5 d. After 5 d, insects were removed from the vials and counted as live or dead.

2.6. Data analysis

The experimental design was a completely randomized design with three replications. Responses of each resistant *C. ferrugineus* population to PH₃ was subjected to probit analysis using PoloPlus (Leora Software, Petaluma, CA) (LeOra Software, 2005) to determine the LC₅₀, LC₉₅, and LC₉₉ values and their 95% confidence intervals (CIs). A concentration ratio test to compare LCs required to kill specified percentages of individuals of each resistant population to that required to kill a similar percentage of individuals of the lab susceptible strain was also conducted (Robertson et al., 2007) to determine the levels of resistance.

3. Results and Discussion

We determined LC₅₀, LC₉₅, and LC₉₉ values and their 95% confidence intervals for each of the eight populations of *C. ferrugineus* (Table 2). Concentrations of PH₃ required to kill 50, 95 and 99% of individuals in the lab susceptible strain were compared with those required to kill similar percentages in each of the eight PH₃ resistant *C. ferrugineus* populations in order to determine the level of resistance of each population (Table 3). We found that the LC₉₉ for the lab susceptible strain was 7.3 ppm based on 3-d fumigation.

Table 2 Lethal concentrations of phosphine required to kill 50, 95, and 99% of individuals in the lab susceptible strain and each of the eight PH₃ resistant *C. ferrugineus* populations.

Samples	LC ₅₀ (95% CI)	LC ₉₅ (95% CI)	LC ₉₉ (95% CI)	X ² (df) [H*]
CGAHR	3.19 (2.89 – 3.48)	5.70 (4.99– 7.06)	7.25 (6.078– 9.75)	129.13 (21) [4.61]
JE	78.77 (67.84 – 90.68)	345.08 (277.65 – 456.60)	636.38 (478.02 – 928.97)	15.04 (13) [1.15]
DK Farm 20	88.97 (73.97 – 105.19)	481.26 (356.43– 732.85)	968.59 (649.76 -1729.66)	49.81 (16) [3.11]
H Bin 2	13.09 (10.24 – 16.75)	62.81 (43.78 – 105.97)	120.28 (76.01 – 239.27)	140.51 (28) [5.01]
H Bin 3	9.39 (8.22 – 11.02)	24.41 (18.66 – 38.2)	36.26 (25.65 – 65.33)	77.45 (19) [4.07]
K Bin 47	11.24 (9.96 – 12.71)	51.66 (41.96 – 66.84)	97.15 (74.21 – 136.41)	27.12 (22) [1.23]
K Bin 61	15.15 (12.99 – 17.78)	78.87 (60.53 – 110.57)	156.22 (111.34 – 242.48)	70.25 (28) [2.50]
K Bin 77	16.75 (13.65 – 20.45)	135.05 (96.86 – 209.21)	320.64 (207.26 – 579.96)	109.05 (31) [3.51]
K Bin 88	25.26 (17.31 – 31.92)	122.33 (91.47 – 203.92)	235.18 (153.32 – 522.90)	24.26 (13) [1.89]

As expected, all eight populations tested required higher PH₃ concentrations to achieve the same level of mortality compared to the susceptible laboratory strain. The LC₉₉ values required for eight PH₃-resistant populations ranged from 36.3-968.6 ppm. Based on probit analyses, LC₉₉ values for DK Farm 20 and JE populations were the highest and were 968.6 and 636.4 ppm, respectively. These concentrations were 133.5 and 87.7 times higher than that required to kill a similar percentage of individuals in the susceptible strain (Table 3).

Table 3 Comparisons of PH₃ concentrations required to kill 50, 95, and 99% of the individuals in the lab susceptible strain to those required to kill similar percentages in each of the eight PH₃ resistant *C. ferrugineus* populations.

Samples Compared	Lethal Concentration Ratios		
	LC ₅₀ (95% CI)	LC ₉₅ (95% CI)	LC ₉₉ (95% CI)
JE vs CGAHR	24.65 (21.65 – 28.06)	65.49 (48.43 – 75.55)	87.74 (65.18 – 118.09)
DK Farm 20 vs. CGAHR	27.84 (24.07 – 31.37)	84.36 (69.06 – 103.04)	133.54 (101.83 – 175.12)
H Bin 2 vs. CGAHR	4.06 (3.64 – 4.54)	11.01 (9.02 – 13.42)	16.62 (9.02 – 13.42)
H Bin 3 vs. CGAHR	2.92 (2.71 – 3.15)	4.27 (3.58 – 5.09)	5.00 (3.97 – 6.28)
K Bin 47 vs. CGAHR	4.75 (3.15 – 3.94)	13.81 (7.3 – 11.20)	21.49 (16.63 – 27.77)
K Bin 61 vs. CGAHR	3.18 (2.88 – 3.47)	5.70 (4.99 – 7.09)	7.26 (6.07 – 9.21)
K Bin 77 vs. CGAHR	5.25 (4.70 – 5.87)	23.66 (19.17 – 29.19)	44.12 (33.34 – 58.38)
K Bin 88 vs CGAHR	7.92 (6.53 – 9.61)	21.43 (16.59 – 27.68)	32.36 (22.01 – 47.57)

Phosphine resistance in stored-product insect pest species continues to be a widespread problem in many countries including China, India, Australia, South America, and the United States (Nayak et al., 2003; Opit et al., 2012; Nayak et al. 2013). These studies report high PH₃ resistance in *T. castaneum*, *R. dominica*, *Cryptolestes* spp. and *Liposcelis* spp and indicate that PH₃ resistance in stored-product insects is a growing problem globally. The study by Opit et al. (2012) reported strong PH₃ resistance (>100 times that of the susceptible strain) in *T. castaneum* and *R. dominica*. In this study, we report that the resistance of the most resistant *C. ferrugineus* population was 134 times that of the susceptible strain.

4. Conclusions

According to our data, LC₉₉ values for eight PH₃ resistant *C. ferrugineus* populations tested ranged from 36.3-968.6 ppm, based on 3-day fumigation. We also determined that based on LC₉₉, the resistant field-collected populations were 5 to 133.5 times more resistant than the susceptible laboratory strain. The most PH₃ resistant *C. ferrugineus* population was DK Farm 20

which was 133.5-fold more resistant than the susceptible strain. This was followed by population JE with resistance that was 87.7-fold. The levels of PH₃ resistance in DK, H Bin 7, H Bin 11, H Bin 14 and H Bin 71 populations of *C. ferrugineus* reported in Konemann et al. (2013) need to be determined. This study shows the need to conduct resistance tests in all major stored-product insect pests in all wheat-growing regions of the United States in order to develop PH₃ resistance management strategies for these pests in the United States.

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References

- Collins, P.J., Daghli, G.J., Nayak, M.K., Ebert, P.R., Schlipalius, D., Chen, W., Pavic, H., Lambkin, T.M., Kopittke, R., Bridgeman, B.W., Combating resistance to phosphine in Australia, 2001. In: Donahaye, E. J., Navarro, S., Leesch J. G., (Eds.), Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products, 29 October – 3 November 2000, Fresno, CA. Executive Printing Services., pp. 593-607.
- Cuperus, G.W., Noyes, R.T., Fargo, W.S., Clary, B.L., Arnold, D.C., Anderson, K., 1990. Management practices in a high-risk stored-wheat system in Oklahoma. *American Entomologist* 36, 129-134.
- Flinn, P.W., Hagstrum, D.W., Reed, C., Phillips, T.W., 2010. Insect population dynamics in commercial grain elevators. *Journal of Stored Product Research* 46, 43-47.
- Konemann, C., Opit, G. P., Shakya, K., Bajracharya, N.S., 2013. Phosphine resistance in *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidea) from stored wheat in Oklahoma. In: Athanassiou, C. G., Trematerra, P., Kavallieratos, N. G., Weintraub, P. G. (Eds), Proceedings of the International Organization for Biological and Integrated Control - West Palaearctic Region Section Working Group on Integrated Protection of Stored Products, 1-4 July 2013, Bordeaux, France. IOBC/WPRS Bulletin 82. pp. 363-366.
- LeOra Software. 2005. PoloPlus user's manual, version 2.0. LeOra Software, Petaluma, CA
- (NASS) National Agricultural Statistics Service. 2014. United States Department of Agriculture. (<http://usda.mannlib.cornell.edu/usda/current/CropProdSu/CropProdSu-01-10-2014.pdf>).
- Nayak, M.K., Collins, P.J., Pavic, H., Kopittke, R.A., 2003. Inhibition of egg development by phosphine in the cosmopolitan pest of stored products *Liposcelis bostrychophila* (Psocoptera: Liposcelididae). *Pest Management Science* 59, 1191-1196.
- Nayak M.K., Holloway, J.C., Emery, R.N., Pavic, H., Bartlet, J., Collins, P.J., 2013. Strong resistance to phosphine in the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae): its characterization, a rapid assay for diagnosis and its distribution in Australia. *Pest Management Science* 69, 48-53.

- Opit, G.P., Phillips, T.W., Aikens, M.J., Hassan M.M., 2012. Phosphine resistance in *Tribolium castaneum* and *Rhyssopertha dominica* from stored wheat in Oklahoma. *Journal of Economic Entomology* 105, 1107-1114.
- Phillips, T.W., Throne, J.E., 2010. Biorational approaches to managing stored-product insects. *Annual Review of Entomology* 55, 375-397.
- Robertson, J.L., Russell, R.M., Preisler, H.K., Savin, N.E., 2007. *Bioassays with Arthropods*, 2nd ed. CRC Press, Boca Raton, FL.