CHENG C. C. AND H.S. DUCOF,F 1989: High-dose mode of death in *Tribolium*. Entomologia Experimentalis et Applicata **51:** 189–197.

Collins P.J., Daglish G.J., Nayak M.K., Ebert P.R., Schlipalius D.I., Chen, W., Pavic J., Lambkin T.A., Kopittke R.A. and B.W. Bridgeman, 2000: Combating resistance to phosphine in Australia. In E. J. Donahaye, S. Navarro and J. G. Leesch (Eds.), Proceedings of the International Conference for Controlled Atmosphere and Fumigation in Stored Products, Fresno CA, pp 593–607.

FAO, 1975: Recommended methods for detection and measurement of resistance of agricultural pests to pesticides - tentative method for adults of some major pest species of stored cereals, with methyl-bromide and phosphine - FAO Method No 16. FAO Plant Protection Bulletin 23: 12–25.

FRIENDSHIP C.A.R., HALLIDAY D. AND A.H. HARRIS 1986: Factors causing resistance to phosphine in insect pests of stored produce V. Howe (Ed.), Proceedings of GASGA Seminar on Fumigation Technology in Developing Countries, Tropical Development and Research Institute, London, pp. 141-149

HALLIDAY W.R., ARTHUR F.R AND J.L. ZETTLER 1983: Resistance status of red flour beetle (Coleoptera: Tenebrionidae) infesting stored peanuts in the Southeastern United states. Journal of Economic Entomology **81:** 74-77.

HASAN M.M. AND CH. REICHMUTH, 2004: http://onlinelibrary.wiley.com/advanced/search/results?searchRowCriteria%5B0%5D.fieldName=author&start=1&resultsPerPage=20&searchRowCriteria%5B0%5D.queryString=%22M.%20M.%20Hasan%22Relative toxicity of phosphine against the bean bruchid Acanthoscelides obtectus (Say) (Col., Bruchidae). Journal Applied Entomology **128:** 332-336.

JAGADEESAN R., COLLINS P.J., DAGLISH G.J., EBERT P.R., AND D.I. SCHLIPALIUS, 2012: Phosphine Resistance in the Rust Red Flour Beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae): Inheritance, Gene Interactions and Fitness Costs. PLoS ONE **7(2):** 31582. doi:10.1371/journal.pone.0031582

Kaur R., Subbarayalu M., Jagadeesan R., Daglish G.J., Nayak M.K., Naik H.R., Ramasamy S., Subramanian C., Ebert P.R. and D.I. Schlipalius, 2015: Phosphine resistance in India is characterised by a dihydrolipoamide dehydrogenase variant that is otherwise unobserved in eukaryotes. Heredity **115:** 188–194.

LORINI I., COLLINS P.J., DAGLISH G.J., NAYAK M.K. AND H. PAVIC, 2007: Detection and characterization of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae*)*. Pest Management Science **3:** 358–364.

MILLS K. A. 1983: Resistance to the fumigant hydrogen phosphide in some stored-product species associated with repeated inadequate treatments. Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie **4(1/3):** 98-101.

NAYAK M.K., HOLLOWAY J.C., EMERY R.N., PAVIC H., BARTLET J. AND P.J. COLLINS, 2013. Strong resistance to phosphine in the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) Coleoptera: Laemophloeidae): its characterisation, a rapid assay for diagnosis and its distribution in Australia. Pest Management Science **69:** 48-53.

OPIT G.P., PHILLIPS T. W., AIKINS M.J. AND M. M. HASAN, 2012. Phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica* from stored wheat in Oklahoma. Journal of Economic Entomology 105: 1107–1114.

PRICE L. A. AND K. A. MILLS, 1988: The toxicity of phosphine to the immature stages of resistant and susceptible strains of some common stored product beetles, and implications for their control. Journal of Stored Product Research **24:** 51-59.

RAHIM M. AND Z. SULAIMAN, 1999: Survey of resistance of stored grain pests to phosphine in Malaysia. Proceeding of International Conference on Plant Protection in the Tropics **5:** 226–229.

RAHIM M., FARIDAH, M.E. AND M. RASALI, 2004. Current status of phosphine resistance in stored grain insects in Malaysia. Journal of Tropical Agriculture and Food Science **32(1):** 101–107

REICHMUTH CH. 1986. A quick test to determine phosphine resistance in toredproducts

research. GASGA Newsletter **15:** 14–15.

SAS. 2002. User's Guide, v. 8. SAS Institute, Cary, NC.

SCHLIPALIUS D.I., VALMAS N., TUCK A.G., JAGADEESAN R., MA L. AND R. KAUR, 2012: A core metabolic enzyme mediates resistance to phosphine gas. Science **338:** 807–810.

TAYLOR R.W.D. 1986: Response of field strains of some insect pests of stored products. (In) Proceedings GASGA Seminar on Fumigation Technology in Developing Countries, Tropical Development Research Institute, London pp 132–140.

TYLER P.S., TAYLOR R.W.D. AND D.P. FLEES, 1983: Insect resistance to phosphine fumigation in food warehouses in Bangladesh. International Pest Control **25:** 10–13.

## Phosphine resistance in Saw-toothed Grain Beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) in the United States

**Zhaorigetu Hubhachen[1], George Opit [1*], Sandipa G. Gautam[2], Charles Konemann[1], Ed Hosoda[3]**

[1]Department of Entomology and Plant Pathology, Oklahoma State University, 127 Noble Research Center, Stillwater, OK, 74078, U.S.A.

[2]Kearney Agricultural Research and Extension Center, 9240 S Riverbend Avenue, Parlier, CA, 93648

[3] Cardinal Professional Products, 57 Matmor Road, Woodland, CA, 95776

*Corresponding and presenting author: george.opit@okstate.edu

DOI 10.5073/jka.2018.463.136

**Abstract**

Sub-lethal dose application of phosphine ($PH_3$) that is mostly caused by leakage during fumigation has resulted in resistance in *Tribolium castaneum*, *Rhyzopertha dominica*, *Cryptolestes ferrugineus*, *Sitophilus oryzae*, and other stored-product insect pest species worldwide. However, $PH_3$ resistance in the saw-toothed grain beetle, *Oryzaephilus surinamensis,* has not been reported in any country. Additionally, the discriminating dose of $PH_3$ for eggs of *O. surinamensis* has not been estimated. In this study, the discriminating dose for eggs of the susceptible strain of *O. surinamensis* was estimated as 28.4 ppm applied for 3 d. Adults from 4 out of 14 field-collected populations showed detectable resistance to $PH_3$ whereas eggs in 9 out of 14 populations had detectable resistance. Resistance frequencies in both adults and eggs in Box BF, Box BR and OKWat populations were > 90%. Levels of resistance ($LC_{99}$) in these three populations were estimated using probit analysis. $LC_{99}$ values for adults of Box BF, Box BR, and OKWat populations were 320.5, 290.7 and 263 ppm, respectively, whereas those in eggs from the same populations were 1055.9, 1030.7, and 564.5 ppm, respectively, over 3-d fumigation. Resistance levels of adults and eggs of the most resistant population, Box BF, were 24.3- and 43.6-fold, respectively, higher than those of the lab-susceptible strain. The resistance levels in eggs from these three populations were > 3-fold higher than that in adults and this shows eggs of *O. surinamensis* are more tolerant to $PH_3$ than adults. These results indicate that it may not be practical to use $PH_3$ to control Box BF and Box BR populations. Therefore, it is important to develop alternative pest management strategies for controlling highly $PH_3$-resistant populations of stored-product insect pests.

**Keywords:** almond storage, resistance management, fumigation, resistance level

## 1. Introduction

The Central Valley of California produces ~ 840,000 metric tons/year of almonds valued at ~ $6.5 billion, and this accounts for nearly all almond production in the United States (National Agricultural Statistics Service [NASS], 2017). Such almond production levels are associated with a high level of risk from stored product insect pest infestation. Postharvest fumigation using phosphine is usually the method of choice for disinfestation of most stored agricultural commodities such as almonds (Johnson et al., 2012).

Phosphine or hydrogen phosphide ($PH_3$) is the most widely used fumigant for stored product insect pest control in the world because it is relatively inexpensive, easy to apply, and nearly no residue is left in the treated commodity. Sub-lethal dose application of $PH_3$ that is mostly caused by leakage during fumigation has resulted in resistance in *Tribolium castaneum* (Herbst) (Coleoptera: Tenerionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and other stored-product pest species worldwide (Zettler and Cuperus, 1990; Zettler and Keever, 1994; Rajendran, 1999; Collins et al., 2001; Cao et al., 2003; Benhalima et al., 2004; Pimental et al., 2010; Lorini et al., 2007; Opit et al., 2012; Ahmad et al., 2013; Nayak et al., 2013; Jittanun and Chongrattanameteekul, 2014; Chen et al., 2015; Koçak et al., 2015; Sağlam et al., 2015; Gautam et al., 2016; Afful et al., 2017; Cato et al., 2017; Konemann et al., 2017).

There are currently no published studies on $PH_3$ resistance in saw-toothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) in the United States and other countries worldwide despite $PH_3$ resistance having been reported in several species of storage pests including *T. castaneum*, *R. dominica*, *C. ferrugineus*, *S. oryzae* and other stored-product pest species globally. Therefore, the objectives of this study were to estimate the discriminating dose for eggs of a laboratory-susceptible strain of *O. surinamensis,* given that this information is lacking in literature, and to estimate the resistance frequencies of both adults and eggs of 14 *O. surinamensis* populations collected from almond storage and processing facilities in California and wheat storage facilities in Oklahoma. An additional objective was to estimate $PH_3$ resistance levels ($LC_{99}$) in adults and eggs of those populations found to have resistance frequencies ≥ 40%.

## 2. Materials and methods

Insects

A $PH_3$-susceptible laboratory strain (Susceptible *STGB*) of *O. surinamensis* maintained in culture since 1972 was obtained from the Center for Grain and Animal Health Research (CGAHR) of the USDA Agricultural Research Service, Manhattan, KS. Fourteen field-collected populations of *O. surinamensis* were used. Eleven out of 14 populations were collected from almond storage and processing facilities in the Central Valley of California during the period 2013–2016 whereas 3 populations were collected using probe traps from wheat storage bins in Oklahoma in 2015 and 2016. Each sample received from California was transferred to a glass jar, labeled, and kept in an incubator at 28 ± 1°C and 65 ± 5% RH for 2–3 weeks to allow for the immature stages to develop. Adult *O. surinamensis* were then transferred to laboratory diet comprising 95% oats and 5% Brewer's yeast (wt: wt) and kept in an incubator at the same conditions described above. Code names were given to different populations, for example "Box A *STGB*" representing an *O. surinamensis* population from facility "A"; the purpose of code names was to conceal identities of the facilities insects were obtained. Voucher specimens of 20 adult insects of each population preserved in 95% ethanol that were used in this study were deposited at K.C. Emerson Entomology Museum at Oklahoma State University under lot numbers: 163 (Box A *STGB* ), 164 (Box BF *STGB*), 165 (Box BR *STGB*), 166 (Box Q *STGB* ), 167 (Box S *STGB* ), 168 (Box U3 *STGB*), 169 (Box W *STGB*), 170 (Box X *STGB* ), 171 (Susceptible *STGB* ), 192 (OKBur *STGB*). 193 (OKSti *STGB*), 194 (OKWat *STGB*), 195 (Box 16A), 196 (Box 16B) and 197 (Box 16C).

In the estimation of discriminating dose for eggs of the lab susceptible strain, resistance frequencies of both adults and eggs of 14 field-collected populations, resistance levels of both adults and eggs of three populations with resistance frequencies ≥ 40% (resistance frequencies were > 80%), procedures in FAO Method No. 16 (FAO, 1975), Opit et al. (2012), Gautum et al. (2016), and Konemann et al. (2017) were used and are briefly described below**.**

Estimation of discriminating dose

Experimental procedures to estimate the discriminating dose for eggs of *O. surinamensis* laboratory strain were similar to those described by Gautam et al. (2016) and Konemann et al. (2017). To obtain eggs for fumigation, 200–300 *O. surinamensis* adults were transferred from lab culture into a glass jar containing ~ 20-g mixture of oats and wheat flour. Three jars were set up. After 3 d, *O. surinamensis* eggs were harvested by sifting contents of jars using U.S. Standard #40 and #70 (0.42- and 0.297-mm openings, respectively) pair of sieves (Seedburo Equipment Company, Des Plaines, IL). Fifty 0- to 3-d-old eggs were placed on a transparent piece of double-sided sticky tape that was attached to a piece of black filter paper. Each sticky tape with eggs was then transferred to a glass vial. The glass vials with eggs were then placed in fumigation jars as described by Gautam et al. (2016). Based on preliminary experiments, concentrations of $PH_3$ required ranged from 2.5–38.2 ppm with seven dose points over a 72-h (3-day) fumigation period at 25°C. After the fumigation, vials were removed from jars and kept in an incubator maintained at 28 ± 1°C and 65 ± 5% RH. Eggs that hatched were counted 10 d post fumigation.

Resistance frequencies

Discriminating doses of 37.5 ppm of $PH_3$ for 20 h for adults and 28.4 ppm of $PH_3$ for 3 d for eggs, respectively, at 25°C were applied to estimate $PH_3$ resistance frequencies in these life stages of 14 field-collected populations of *O. surinamensis*. Preparation of egg samples from 14 field-collected populations was conducted as described above. For *O. surinamensis* adults, for the laboratory susceptible strain and each of the 14 field-collected populations, 50 adult insects of each population were selected randomly and placed in individual glass vials that contained 0.5 g of oats diet. Vials containing insects were then placed in each of three fumigation jars. Insects were also placed in three additional fumigation jars and prepared as previously described but fumigant was not added

to these jars which served as the controls. Mortality assessments for eggs and adults were conducted 10 d and 14 d post fumigation, respectively.

Levels of resistance

The susceptible *O. surinamensis* (Susceptible *STGB*) and three field-collected populations, Box BF, Box BR and OKWat were tested in dose-response assays to estimate their levels of resistance for both adults and eggs. Populations Box BF, Box BR and OKWat had resistance frequencies > 40%. Concentrations of $PH_3$ used for dose-response tests for the Susceptible *STGB* strain have previously been described above whereas those for adults of Box BF, Box BR and OKWat were 24.4–354.1, 24.4–354.1 and 30.0–366.1 ppm, respectively. For eggs, these concentrations were 28.4–449.2, 28.4–449.2 and 42.4–420.0 ppm, respectively. Experimental set-up and fumigation procedures were similar to those described in Opit et al. (2012), Gautam et al. (2016) and Konemann et al. (2017). Fumigation period was 3 d and mortality assessments for adults and eggs were conducted 5 d and 10 d post-fumigation, respectively.

$PH_3$ concentration analysis

The concentration of $PH_3$ gas in each fumigation jar was measured at the beginning and at the end of the respective exposure periods using a gas chromatograph coupled with a flame photometric detector (GC-FPD) (Model 8610C, SRI Instruments, Torrance, CA). The concentrations were established using a standard curve based on 50, 40, 30, 20, and 10 µl of 200 ppm $PH_3$. The areas under the peak in microvolts (µV) were recorded along with the volume of $PH_3$ injected. $PH_3$ volumes were regressed against measured peak areas to generate a linear regression equation that had a coefficient of determination ($r^2$) value between 0.96 and 0.99 in all cases. Thirty microliters gas samples from each fumigation jar were analyzed using the GC-FPD and quantified using the regression equation generated from the standard curve.

Data analysis

The experimental designs for determining discriminating doses were completely randomized designs with three replications. $LC_{50}$ and $LC_{99}$ values and their 95% confidence intervals (CIs) of both adults and eggs were estimated by probit analysis using PoloPlus (LeOra Software, Petaluma, CA) (LeOra Software, 2005). The discriminating dose of eggs was the upper limit of the 95% CI of the $LC_{99}$ value at a given exposure period at 25°C. A ratio test to compare LCs was also conducted for eggs and adults of *O. surinamensis* (Robertson et al., 2007) to estimate the degree by which the field populations were more resistant to $PH_3$ than the susceptible laboratory strain. In order to ascertain that the value of the mean is within the limit at 95% probability, we calculated G-factor using the equation, $t^2\ V(b)/b^2$, where t = student's *t* test with error degrees of freedom, V(b) is the slope variance estimate given in the variance-covariance matrix, and b is the slope estimate. If a G-value is less than 0.5, it suggests that the value of the mean is within the limit at 95% probability.

## 3. Results and Discussion

Estimation of discriminating dose for eggs of *Oryzaephilus surinamensis*

Given that there was no previously published phosphine discriminating dose for eggs of *O. surinamensis*, we first estimated it in using a dose-response experiment and a laboratory susceptible strain of this species. $PH_3$ discriminating dose for eggs of *O. surinamensis* was estimated as 28.4 ppm over a 3-d fumigation period at 25°C (Table 1).

**Tab. 1** Estimation of $PH_3$ discriminating dose for eggs of the laboratory susceptible strain of saw-toothed grain beetle, *Oryzaephilus surinamensis* (Susceptible *STGB*) based on 3-d fumigation at 25°C.

| Population/Strain | N | Slope ± SE | $LC_{50}$ (95% CI) | $LC_{99}$ (95% CI) | $X^2$ (df) [H*] | G-factor |
|---|---|---|---|---|---|---|
| Susceptible *STGB* | 1200 | 9.8 ± 0.6 | 14.0 (13.3 – 14.7) | 24.2 (21.8 – 28.4) | 59.4 (17) [3.5] | 0.016 |

*Heterogeneity factor, chi-square value/ degrees of freedom (chi-square is significant < 0.05).

Resistance frequencies

Adults from 4 out of 14 populations showed detectable resistance to phosphine whereas eggs in 9 out of 14 populations had detectable resistance to phosphine. The resistance frequencies in both adults and eggs in "Box BF", "Box BR" and OKWat *STGB* populations were > 80% (Table 2). These results suggest that in some almond storage and processing facilities in California, populations of *O. surinamensis* with strong phosphine resistance co-exist with populations of *T. castaneum* with strong phosphine resistance (Gautam et al. 2016).

**Tab. 2** Survival of adults from a laboratory susceptible strain (Susceptible STGB) and 14 field-collected populations of *Oryzaephilus surinamensis*. Data for adults are based on 20-hour exposure to a $PH_3$ discriminating dose of 37.5 ppm at 25°C and for eggs, a 3-day exposure to a discriminating dose of 28.4 ppm.

| Life Stage | Population | Percentage survival | | | |
|---|---|---|---|---|---|
| | | Rep. 1 | Rep. 2 | Rep. 3 | Mean ± SE |
| Adults | Box BF *STGB* | 100 | 94 | 80 | 91.3 ± 4.7 |
| | Box BR *STGB* | 98 | 98 | 100 | 98.7 ± 0.5 |
| | Box A *STGB* | 4 | 0 | 2 | 2.0 ± 0.9 |
| | Box Q *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | Box U3 *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | Box S *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | Box X *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | Box W *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | Box 16A *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | Box 16B *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | Box 16C *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | OKBur *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | OkSti *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | OKWat *STGB* | 100 | 98 | 98 | 98.7 ± 0.5 |
| | Susceptible *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| Eggs | Box BF *STGB* | 100 | 100 | 98 | 99.3 ± 0.5 |
| | Box BR *STGB* | 98 | 98 | 100 | 94.7 ± 1.4 |
| | Box A *STGB* | 2 | 0 | 0 | 0.7 ± 0.5 |
| | Box Q *STGB* | 4 | 6 | 2 | 4.0 ± 0.9 |
| | Box U3 *STGB* | 2 | 10 | 2 | 4.7 ± 2.1 |
| | Box S *STGB* | 2 | 8 | 0 | 3.3 ± 1.9 |
| | Box X *STGB* | 0 | 8 | 2 | 3.3 ± 1.9 |
| | Box W *STGB* | 0 | 4 | 0 | 1.3 ± 1.1 |
| | Box 16A *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | Box 16B *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | Box 16C *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | OKBur *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | OkSti *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |
| | OKWat *STGB* | 100 | 97 | 98 | 98.3 ± 0.7 |
| | Susceptible *STGB* | 0 | 0 | 0 | 0.0 ± 0.0 |

Levels of resistance

$LC_{99}$ values for adults of Box BF", "Box BR" and OKWat *STGB* populations were 320.5, 290.7 and 264.1ppm over 3-d fumigation, respectively, whereas those in eggs of the same populations were

1055.9, 1030.7 and 564.5ppm, respectively (Tables 3 and 5). The resistance levels of adults and eggs in the population with the highest resistance, Box BF were 24.3 and 43.6-fold, respectively, higher than that in the lab-susceptible strain (Table 4 and 6). The resistance levels in eggs of these two populations were >3-fold higher than that in adults. These data show that eggs of *O. surinamensis* have much higher levels of resistance than adults; similar results were reported by Gautam et al. (2016) for *T. castaneum*, also collected from almond storage and processing facilities.

**Tab. 3** Probit analyses of dose-response data for the susceptible and three phosphine-resistant populations of *Oryzaephilus surinamensis* adults. LC values are lethal concentrations of phosphine (ppm) over 3 d fumigation period at 25°C.

| Population/ Strain | N | Slope ± SE | $LC_{50}$ (95% CI) | $LC_{99}$ (95% CI) | $X^2$ (df) [H*] | G-factor |
|---|---|---|---|---|---|---|
| Susceptible | 1447 | 5.4 ± 0.3 | 4.8 (4.6 – 5.2) | 13.2 (11.5 – 15.9) | 49.6 (20) [2.5] | 0.013 |
| Box BF | 1155 | 6.0 ± 0.3 | 118.7 (107.6 – 129.7) | 290.8 (249.1 – 362.8) | 55.8 (19) [2.9] | 0.014 |
| Box BR | 1321 | 3.0 ± 0.2 | 52.8 (44.3 – 60.9) | 320.5 (249.9 – 456.9) | 49.2 (19) [2.6] | 0.014 |
| OKWat | 1000 | 3.5 ± 0.2 | 56.6 (52.0 – 61.5) | 264.1 (215.1 – 344.3) | 15.7(15) [1.1] | 0.019 |

*Heterogeneity factor, chi-square value/ degrees of freedom (chi-square is significant <0.05).

**Tab. 4** Comparison of lethal concentrations of phosphine (ppm) required to kill 50, 95, and 99% of insects in samples from three phosphine-resistant populations of *Oryzaephilus surinamensis* and those required to kill similar percentage from the lab susceptible population.

| Samples compared | Lethal concentration ratios | | |
|---|---|---|---|
| | $LC_{50}$ (95% CI) | $LC_{95}$ (95% CI) | $LC_{99}$ (95% CI) |
| Box BF vs Susceptible | 24.4 (22.9 – 25.9) | 22.7 (20.5 – 25.2) | 22.1 (19.2 – 25.2) |
| Box BR vs Susceptible | 10.9 (9.8 – 12.0) | 19.2 (16.7 – 22.1) | 24.3 (19.9 – 29.7) |
| OKWat vs Susceptible | 12.0 (10.6 – 12.6) | 17.9 (15.9 – 18.8) | 19.9 (18.2 – 22.6) |

**Tab. 5** Probit analyses of dose-response data for three phosphine-resistant populations of *Oryzaephilus surinamensis* eggs. LC values are lethal concentrations of phosphine (ppm) over a 3-d fumigation period at 25°C.

| Population | N | Slope ± SE | $LC_{50}$ (95% CI) | $LC_{99}$ (95% CI) | $X^2$ (df) [H*] | G-factor |
|---|---|---|---|---|---|---|
| Box BF | 1050 | 2.5 ± 0.2 | 122.2 (105.4 – 139.5) | 1055.9 (755.6 – 1706.0) | 44.0 (19) [2.3] | 0.015 |
| Box BR | 1050 | 2.3 ± 0.1 | 101.7 (85.2 – 118.5) | 1030.7 (714.9 – 1762.5) | 50.0 (19) [2.6] | 0.016 |
| OKWat | 1000 | 3.1 ± 0.2 | 98.0 (89.0 – 106.8) | 564.5 (473.3 – 704.7) | 19.6(18) 1.1 | 0.014 |

*Heterogeneity factor, chi-square value/ degrees of freedom (chi-square is significant <0.05).

**Tab. 6** Comparison of lethal concentrations of phosphine (ppm) required to kill 50, 95, and 99% of insects in samples from three phosphine-resistant populations of *Oryzaephilus surinamensis* eggs and those required to kill similar percentage from the lab susceptible population.

| Samples compared | Lethal concentration ratios | | |
|---|---|---|---|
| | $LC_{50}$ (95% CI) | $LC_{95}$ (95% CI) | $LC_{99}$ (95% CI) |
| Box BF vs Susceptible | 8.7 (7.9 – 9.6) | 27.2 (23.1 – 34.1) | 43.6 (34.7 – 60.1) |
| Box BR vs Susceptible | 7.3 (6.4 – 8.1) | 25.4 (21.1 – 32.9) | 42.6 (32.8 – 62.1) |
| OKWat vs Susceptible | 7.0 (6.7 – 7.3) | 16.4 (15.7 – 17.0) | 23.3 (21.7 – 24.8) |

## 4. Conclusions

Resistance frequencies in adults and eggs of 14 field-collected populations of *O. surinamensis* ranged between 0–100 for both stages but were highest in three populations, namely "Box BF", "Box BR" and OKWat *STGB*, where frequencies were > 80%. Resistance levels (based on $LC_{99}$) in adults of these 3 most resistant populations were 22.1-, 24.3- and 19.9-fold, respectively, higher than in the susceptible strain, whereas those in eggs were 43.6-, 42.6- and 23.3-fold higher than in the

susceptible strain. These results show that phosphine-resistant populations of *O. surinamensis* are found in both almond storage and processing facilities in California and in wheat storage facilities in Oklahoma. For the control of stored product insect pests, currently the almond industry in California recommends a dose is 500–1,000 ppm of $PH_3$ for a minimum of 3 d, but 5–7 d are highly recommended, at 20–30°C. Therefore, it may not be possible to use $PH_3$ to effectively control Box BF and Box BR populations given that the $LC_{99}$ values of eggs of these populations are 1055.9 and 1030.7 ppm, respectively. These data highlight the importance of developing alternative pest management strategies for controlling highly $PH_3$-resistant populations of stored product insect pests.

**Acknowledgements**

We thank the staff of the Cardinal Professional Products for their help with collecting insect samples that were used in this study. Thanks also go to Kandara Shakya and Friendly Yang for their excellent technical support. This work was funded by the Almond Board of California (ABC) and the Oklahoma Agricultural Experiment Station (Project No. OKL2949). Trade names or commercial products mentioned in this proceeding paper are solely for the purpose of providing specific information and does not imply recommendation or endorsement by Oklahoma State University, Kearney Agricultural Research and Extension Center, or ABC.

**References**

AFFUL, E., ELLIOTT, B., NAYAK, M. K., AND T.W. PHILLIPS, 2018. Phosphine resistance in North American field populations of the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Journal of Economic Entomology **111,** 463–469.

AHMAD, A., AHMED, M., NOURULLAH, ALI, G.M., ABBAS, M. AND S. ARIF, 2013. Monitoring of resistance against phosphine in stored grain insect pests in Sindh. Middle-East Journal of Scientific Research **16**, 1501–1507.

BENHALIMA, H., CHAUDHRY, M. Q., MILLS, K. A., AND N.R. PRICE, 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. Journal Stored Products Research **40,** 241–249.

CAO, Y., SON, Y., AND G.Y. SUN, 2003. A survey of psocid species infesting stored grain in China and resistance to phosphine in field populations of *Liposcelis entomophila* (Enderlein) (Psocoptera: Liposcelididae), pp. 662–667. *In* Credland, P.F.; D.M. Armitage, C.H. Bell, P.M. Cogan, E. Highley (Eds.), Proceedings of the 8th International Working Conference on Stored Product Protection, 22-26 July 2002, York, UK. CAB International, Wallingford, United Kingdom, (ISBN 0851996914).

CATO, A. J., ELLIOTT, B., NAYAK, M. K., AND T.W. PHILLIPS, 2017. Geographic variation in phosphine resistance among North American populations of the red flour beetle. Journal of Economic Entomology **110,** 1359–1365.

CHEN, Z., SCHLIPALIUS D., OPIT G., SUBRAMANYAM B., AND T.W. PHILLIPS, 2015. Diagnostic molecular markers for phosphine resistance in U.S. populations of *Tribolium castaneum* and *Rhyzopertha dominica*. PLoS One **10,** e0121343.

COLLINS, P. J., DAGLISH, G. J., NAYAK, M. K., EBERT, P. R., SCHLIPALIUS, D., CHEN, W., PAVIC, H., LAMBKIN, T. M., KOPITTKE, R., AND B.W. BRIDGEMAN, 2001. Combating resistance to phosphine in Australia, pp. 593-607. *In* E. J. Donahaye, S. Navarro, and J. G. Leesch (Eds.), Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 29 October-3 November 2000, Fresno, CA. Executive Printing Services, Clovis, CA.

FOOD AND AGRICULTURE ORGANIZATION, 1975. Tentative method for adults of some major pest species of stored cereals with methyl bromide and phosphine, FAO method no. 16. FAO Plant Protection Bulletin **23,** 12–25.

GAUTAM, S. G., OPIT, G. P., AND E. HOSODA, 2016. Phosphine resistance in *Tribolium castaneum* and *Plodia interpunctella* populations in California. Journal of Economic Entomology **109,** 2525-2533.

JITTANUN, C. AND W. CHONGRATTANAMETEEKUL, 2014. Phosphine resistance in Thai local strains of *Tribolium castaneum* (Herbst) and their response to synthetic pheromone. Kasetsart Journal of Natural Sciences **48,** 9–16.

JOHNSON, J. A., WALSE, S. S., AND J.S. GERIK, 2012. Status of alternatives for methyl bromide in the United States. Outlooks on Pest Management **23,** 53-56.

KOÇAK, E., SCHLIPALIUS, D., KAUR, R., TUCK, A., EBERT, P., COLLINS, P., AND YILMAZ, A., 2015. Determining phosphine resistance in rust red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) populations from Turkey. Türkish Journal of Entomology **39,** 129–136.

KONEMANN, C. E., HUBHACHEN, Z., OPIT, G. P., GAUTAM, S. AND N.S. BAJRACHARYA, 2017. Phosphine resistance in *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) collected from grain storage facilities in Oklahoma, USA. Journal of Economic Entomology **110,** 1377–1383.

LEORA SOFTWARE, 2005. PoloPlus user's manual, version 2.0. LeOra Software, Petaluma, CA.

LORINI, I., COLLINS, P. J., DAGLISH, G. J., NAYAK, M. K., AND H. PAVIC, 2007. Detection and characterization of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Pest Management Science **63,** 358–364.

(NASS) NATIONAL AGRICULTURAL STATISTICS SERVICE, 2017. United States Department of Agriculture. https://www.nass.usda.gov/Publications/Ag_Statistics/ (Accessed 1 April 2018).

NAYAK, M. K., HOLLOWAY, J. C., EMERY, R. N., PAVIC, H., BARTLET, J., AND P.J. COLLINS, 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., AIKINS, M. J., AND M.M. HASAN, 2012. Phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica* from Stored Wheat in Oklahoma. Journal of Economic Entomology **105,** 1107–1114.

PIMENTEL, M.A.G., FARONI, L. R. D'A., DA SILVA, F. H., BATISTA, M. D., AND R.N.C. GUEDES, 2010. Spread of phosphine resistance among Brazilian populations of three species of stored product insects. Neotropical Entomology **39,** 101–107.

RAJENDRAN, S., 1999. Phosphine resistance in stored grain insect pests in India, pp. 635-641. *In* Z. Jin, Q. Liang, Y. Liang, X. Tan, and L. Guan (eds.), Proceedings of the 7th International Working Conference on Stored-Product Protection, 14-19 October 1998, Beijing, China. Sichuan Publishing House of Science and Technology, Chengdu, China.

ROBERTSON, J. L., RUSSELL, R. M., PREISLER, H. K., AND N.E. SAVIN, 2007. Bioassays with arthropods, pp. 199, 2nd ed. CRC, Boca Raton, FL.

SAĞLAM, O., EDDE, P. A., AND T.W. PHILLIPS, 2015. Resistance of *Lasioderma serricorne* (Coleoptera: Anobiidae) to fumigation with phosphine. Journal of Economic Entomology **108,** 2489–2495

ZETTLER, J. L. AND G. W. CUPERUS, 1990. Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in wheat. Journal of Economic Entomology **83,** 1677–1681.

ZETTLER, J. L, AND D. W. KEEVER, 1994. Phosphine resistance in Cigarette beetle (Coleoptera: Anobiidae) associated with tobacco storage in the southeastern United States. Journal of Economic Entomology **87,** 546–550

## Molecular mechanisms of metabolic resistance in booklice (Psocoptera: Liposcelididae)

**Dan Dan Wie*, Ning Lang, Tian Xing Jing, Wie Dou, Jin Jun Wang**

College of Plant Protection, Southwest University
*Corresponding and presenting author: weidandande@163.com
DOI 10.5073/jka.2018.463.137

### Abstract

The psocids from the genus *Liposcelis* are also named booklice, which are stored-product insect pests. Recently, apparent insecticide resistances have been observed in booklice. Here, we mainly focus on mechanisms of metabolic resistance associated with the three major enzymes, Cytochrome P450 monooxygenases (P450s), Estrases (ESTs), and Gluthione-S-transferases (GSTs) in booklice. We developed four comprehensive transcriptomic databases for four booklice, and a large number of detoxification genes potentially involved in insecticide resistance were identified. Totally, 49, 68, 94 and 82 P450 genes, 31, 37, 35 and 23 GST genes, 21, 19, 34 and 19 EST genes were identified for *L. bostrychophila*, *L. entomophila*, *L. tricolor* and *L. decolor*, respectively. The large number of P450s and GSTs implied that Liposcelis species could potentially develop high level of insecticide resistance. The mRNA expression levels of detoxification genes showed that these genes expressed at all tested stages, but exhibited stage-specific patterns, with the higher expression in adults and elder nymphs. Additionally, mRNA abundances of P450 genes were relatively more abundant in adult females than in adult males. The research on different strains showed that the resistance strain of both *L. bostrychophila* and *L. entomophila* had significantly higher mRNA expression and enzyme activity of the detoxification enzymes than the sensitive strain. The above data indicated that detoxification genes might be associated with metabolism insecticides in psocids.

## "Remote Sensing, Predictable Storage of Agricultural Commodities and Advances in Hermetic Storage"

**Philippe Villers[1], Tom de Bruin[2], Patrick Plijter[3]**

[1]GrainPro, Inc., 200 Baker Avenue, Suite 309, Concord, MA 01742 United States, pvillers@grainpro.com, +978-371-7118
[2]GrainPro Philippines, Inc., Subic Bay Freeport Zone, Zambales, 2222 Philippines, tdb@grainpro.com, +63 47 22 7884
[3]GrainPro Philippines, Inc., Subic Bay Freeport Zone, Zambales, 2222 Philippines, patrick@grainpro.com, +63 91 7500 8365
DOI 10.5073/jka.2018.463.138