Phosphine Resistance in Red Flour Beetle and Indianmeal Moth Populations in California Almond Storage Facilities

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Call for Expanded Sampling!!!

• More extensive sampling that covers many geographic locations is needed to get better understanding of phosphine resistance in the Central Valley.

• We request for your help to enable us collect insect samples from new locations.
Outline

• Background
• Objectives
• Materials and Methods
• Results
• Future Research
• Relevance of Results
Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)

Red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)
California produces nearly all the almonds in the United States (952,540 metric tons) (NASS 2013).

In 2012, California almond production was valued at $4.4 billion (NASS 2013).

Fumigation is the method of choice for postharvest pest management in almonds.
Fumigation is the most effective tool available

- Phosphine is most widely used fumigant for disinfesting almonds during storage
- Sulfuryl fluoride use is increasing
  - Eggs of many stored-product pests require higher concentrations and longer exposure period for proper control
  - Residue concerns
Phosphine

• Phosphine is a colorless, flammable, extremely toxic gas, with a disagreeable, garlic-like odor

• Advantages of PH$_3$
  – low cost, easy application, nearly residue free, can be used in a wide range of storage types and commodities

• Disadvantages of PH$_3$
  – Metal corrosive
  – Development of resistance in stored-product insects
PH$_3$ Resistance in Stored-Product Insect Pests

- PH$_3$ resistance being reported worldwide
- PH$_3$ resistance present in Oklahoma

Zettler and Cuperus (1990)

Pesticide Resistance in Tribolium castaneum (Coleoptera: Tenebrionidae) and Rhyzopertha dominica (Coleoptera: Bostrichidae) in Wheat

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Stored-Product Insects Research and Development Laboratory, USDA-ARS, Savannah, Georgia 31403

ABSTRACT Eight strains of red flour beetle, Tribolium castaneum (Herbst), and 21 of the lesser grain borer, Rhyzopertha dominica (F.), collected from wheat stored on farms in Oklahoma were tested for resistance to malathion, chlorpyrifos-methyl, and phosphine. Results of discriminating dose tests of the red flour beetle indicated that all strains were resistant to malathion, one strain was resistant to phosphine, and none was resistant to chlorpyrifos-methyl. Similar discriminating dose tests for the lesser grain borer indicated that all strains were resistant to malathion, dichlorvos, and to chlorpyrifos-methyl, and 8 of 21 strains were resistant to phosphine.

KEY WORDS Insecta, Tribolium castaneum, Rhyzopertha dominica, fumigant

Opit et al. (2012)

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

G. P. OPIT, T. W. PHILLIPS, M. J. AIKINS, AND M. M. HASAN

ABSTRACT Phosphine gas, or hydrogen phosphate (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 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$_{50}$ values were $\leq$3 ppm for susceptible and 377 ppm for resistant T. castaneum, and $\leq$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
### PH$_3$ Resistance in Lesser Grain Borer and Red Flour Beetle in Oklahoma

<table>
<thead>
<tr>
<th>Zettler and Cuperus 1990</th>
<th>Opit et al. 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LGB</strong></td>
<td><strong>LGB</strong></td>
</tr>
<tr>
<td><strong>RFB</strong></td>
<td><strong>RFB</strong></td>
</tr>
<tr>
<td>• 67% of the populations tested had detectable resistance</td>
<td>• 100% of the populations tested had detectable resistance</td>
</tr>
<tr>
<td>• Only 8% had resistance frequencies ≥ 90%</td>
<td>• 60% had resistance frequencies ≥ 90%</td>
</tr>
<tr>
<td>• Highest resistance frequency was 6%</td>
<td>• Most resistant population was 1,519 times more resistant than susceptible strain</td>
</tr>
</tbody>
</table>

**Zettler and Cuperus did not determine the levels of resistance**

• 89% of the populations tested had detectable resistance

• 11.1% had resistance frequencies ≥ 90%

• Most resistant population was 119 times more resistant than susceptible strain
• Zettler and Cuperus (1990) recommended regular monitoring of Oklahoma stored-product insects for \( PH_3 \) resistance

• Opit et al. (2012) recommended determining presence and levels of \( PH_3 \) resistance in different pest species and different geographic regions of the U.S.
What is PH$_3$ Resistance Management?

Strategic adoption and implementation of commercially viable, practical, and scientifically-based management strategies

- To maintain the efficacy of PH$_3$
- To maintain its cost-effectiveness
- To extend the useful life of PH$_3$
In a situation where resistance has already evolved, tactics that eliminate resistant insects are essential for practical resistance management.

These can be either higher doses of the current material (e.g. phosphine), alternative chemicals or physical methods such as heat disinestation.
Pre-requisites for PH$_3$ Resistance Management Strategies

We need to know

- The geographic distribution of PH$_3$ resistance for several stored-product insect pest species in almond storage facilities
- Determine the resistance frequencies of field collected insect populations
- Determine the levels of resistance in populations with high resistance frequencies
Why Is It Important to Determine the Frequencies and Levels of PH$_3$ Resistance?

- To be able to develop PH$_3$ resistance management strategies to mitigate resistance development and spread

- PH$_3$ resistance monitoring will serve as a useful model for monitoring resistance of other postharvest insecticides in future
Objectives

1. **Determine** resistance frequencies **in** red flour beetle adults collected from almond storage facilities in California

2. **Determine the** levels **of** phosphine resistance **in** red flour beetle adults collected from almond storage facilities in California
Objectives

3. Establish **discriminating doses** against:
   - Eggs of red flour beetle
   - Eggs and larvae of Indianmeal moth

4. Determine the **levels** of phosphine resistance in RFB eggs and IMM eggs and larvae in populations collected from the Central Valley
Objective 1

Determine resistance frequencies in red flour beetle adults collected from almond storage facilities in California
• **Discriminating dose**
  - Baseline lethal dose data established with reference strains of known susceptibility using a discriminating dose bioassay (20-h fumigation at 25°C). The upper limit of the 95% confidence interval of the LD$_{99}$ for the referenced strain is the discriminating dose for that insect species.

• **Resistance Frequency**
  - The percentage of insects in each sample exposed to a discriminating dose of phosphine that survive.
Source of Insects

- RFB
- IMM
Materials and Methods

- FAO Protocol #16 was used to determine whether the samples had detectable resistance and the frequency of resistance (presence of resistance).
- FAO Protocol #16: RFB adults exposed to 30 ppm phosphine for 20 hours, at 25°C and ≈70% RH. Mortality determined after 2 wk.
- Presence of unaffected insects at the end of the test means there is detectable resistance.
- FAO Protocol #16 recommends 2 replications.
Glass vials with insects

3.92-liter fumigation jars

Tedlar bags for holding phosphine gas

Dosing 3.92-liter fumigation jars
A GC-FPD for measuring phosphine concentrations
Counting live/dead insects to determine resistance frequencies

Insects held for 14 days at 25 ± 1°C and 70 ± 5% RH

Fumigation jars held for 20 hours at 25 ± 1°C

Counting live/dead insects to determine resistance frequencies
### Results

<table>
<thead>
<tr>
<th>Red Flour Beetle Populations</th>
<th>% Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rep 1</td>
</tr>
<tr>
<td>Box B</td>
<td>48</td>
</tr>
<tr>
<td>Box BM</td>
<td>88</td>
</tr>
<tr>
<td>Box BN</td>
<td>94</td>
</tr>
<tr>
<td>Box BR</td>
<td>42</td>
</tr>
<tr>
<td>Box E1</td>
<td>0</td>
</tr>
<tr>
<td>Box E2</td>
<td>0</td>
</tr>
<tr>
<td>Box F</td>
<td>0</td>
</tr>
<tr>
<td>Box I</td>
<td>0</td>
</tr>
<tr>
<td>Susceptible</td>
<td>0</td>
</tr>
</tbody>
</table>

4 field populations had no detectable resistance, i.e., completely susceptible.
Resistance Frequencies in Red Flour Beetle Populations with Detectable Resistance

Red Flour Beetle Field Populations - Adult

Suceptible | Box B | Box BR | Box BM | Box BN
---|---|---|---|---
Rep 1 | Rep 2 | Rep 3

Objective 2

Determine the levels of phosphine resistance in red flour beetle adults collected from almond storage facilities in California
Materials and Methods

• Four populations that had detectable resistance were used
• Different ranges of concentrations of phosphine were used for the different populations
• Exposure period was 3 days at 25°C
• Mortality assessments were conducted after 5 days

<table>
<thead>
<tr>
<th>PH₃ concentrations tested (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sus.</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>0.0</td>
</tr>
<tr>
<td>1.9</td>
</tr>
<tr>
<td>2.4</td>
</tr>
<tr>
<td>3.3</td>
</tr>
<tr>
<td>5.4</td>
</tr>
<tr>
<td>7.1</td>
</tr>
<tr>
<td>7.7</td>
</tr>
<tr>
<td>-</td>
</tr>
</tbody>
</table>
Glass vials prepared for fumigation. Each vial was covered with a piece of paper towel to allow fumigant penetration while preventing beetles from escaping.
Materials and Methods

- Vials with insects
- Vials inside fumigation jars
- Introducing phosphine into 3.92-liter fumigation jars
- Fumigation jars held for 3 days at 25 ± 1°C
Beetles held for 5 days at 25 ± 1°C and 70 ± 5% RH

Counting dead and live insects to determine the levels of phosphine resistance

- PH$_3$ dose-response data were subjected to probit analyses using PoloPlus.
- Lethal concentration ratio tests were also conducted to determine the levels of phosphine resistance in field populations.
# Probit Analyses of Mortality for Susceptible and Phosphine-Resistant Populations of RFB

<table>
<thead>
<tr>
<th>Sample</th>
<th>LC$_{50}$ (95% CI)</th>
<th>LC$_{95}$ (95% CI)</th>
<th>LC$_{99}$ (95% CI)</th>
<th>X$^2$ (df) [H$^*$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>3.39 (3.2 – 3.5)</td>
<td>5.9 (5.5 – 6.2)</td>
<td>7.4 (6.8 – 8.0)</td>
<td>12.9 (16) [0.8]</td>
</tr>
<tr>
<td>Box B</td>
<td>8.6 (7.9 – 9.3)</td>
<td>29.9 (26.5 – 34.5)</td>
<td>50.2 (41.5 – 63.4)</td>
<td>16.9 (19) [0.89]</td>
</tr>
<tr>
<td>Box BM</td>
<td>34.4 (29.9 – 38.9)</td>
<td>157.3 (129.5 – 202.3)</td>
<td>295.2 (226.0 – 421.3)</td>
<td>20.9 (16) [1.3]</td>
</tr>
<tr>
<td>Box BN</td>
<td>39.4 (34.4 – 44.8)</td>
<td>187.1 (152.7 – 243.7)</td>
<td>356.9 (270.4 – 515.8)</td>
<td>21.5 (16) [1.35]</td>
</tr>
<tr>
<td>Box BR</td>
<td>10.5 (9.7 – 11.3)</td>
<td>33.5 (29.3 – 39.5)</td>
<td>54.3 (45.4 – 67.6)</td>
<td>17.1 (19) [0.9]</td>
</tr>
</tbody>
</table>
Levels of Resistance in Comparison to the Susceptible Population

<table>
<thead>
<tr>
<th>Samples compared</th>
<th>LC50 (95% CI)</th>
<th>LC95 (95% CI)</th>
<th>LC99 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Box B vs Susceptible</td>
<td>2.5 (2.3 – 2.8)</td>
<td>5.1 (4.3 – 6.1)</td>
<td>6.8 (5.4 – 8.6)</td>
</tr>
<tr>
<td>Box BR vs Susceptible</td>
<td>3.1 (2.8 – 3.4)</td>
<td>5.7 (4.8 – 6.7)</td>
<td>7.4 (5.9 – 9.2)</td>
</tr>
<tr>
<td>Box BM vs Susceptible</td>
<td>10.1 (9.0 – 11.4)</td>
<td>26.8 (22.1 – 32.5)</td>
<td>40.1 (30.7 – 52.4)</td>
</tr>
<tr>
<td>Box BN vs Susceptible</td>
<td>11.6 (10.4 – 12.9)</td>
<td>31.9 (26.1 – 38.9)</td>
<td>48.5 (36.9 – 63.7)</td>
</tr>
</tbody>
</table>
Label Rate of Phosphine for Use in Nuts, Dates, or Dried Fruits

Aluminum phosphide tablets - used for fumigation of nuts, dates, or dried fruits in storage boxes or bulk fumigations is 20 - 40 tablets per 1,000 ft$^3$

1 tablet in 1,000 ft$^3$ produces a concentration of 25 parts per million (ppm) i.e. recommended fumigation is 500 - 1,000 ppm
Theoretically, Number of Aluminum Phosphide Tablets Required to Kill Resistant RFB Adults

<table>
<thead>
<tr>
<th>RFB Populations</th>
<th>LC$_{99}$ (95% CI) (ppm)</th>
<th>Number of tablets/1,000 ft$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Box B</td>
<td>50.2 (41.5 – 63.4)</td>
<td>2 (2-3)</td>
</tr>
<tr>
<td>Box BM</td>
<td>295.2 (226.0 – 421.3)</td>
<td>12 (9 - 17)</td>
</tr>
<tr>
<td>Box BN</td>
<td>356.9 (270.4 – 515.8)</td>
<td>14 (11 - 17)</td>
</tr>
<tr>
<td>Box BR</td>
<td>54.3 (45.4 – 67.6)</td>
<td>2 (2-3)</td>
</tr>
</tbody>
</table>

- But, reactivity of the tablets, retention of PH$_3$ in storage structures, and commodity absorption needs to be considered when fumigating.

- Absorption by in-shell almonds fumigated at a rate of 2 g/m$^3$ (1,428 ppm) for 7 days was 100\% (Reddy et al. 2007).
Summary

- 50% of the RFB populations studied were completely susceptible.
- 25% had resistance frequencies below 80%.
- Only 25% had resistance frequencies above 80% (88-100).
- Highest level of resistance in RFB adults was 48. This resistant strain required 48 times more phosphine to kill than the susceptible strain (356 ppm for 3 days).
Objective 3

Establish discriminating doses against eggs of red flour beetle and eggs and larvae of Indianmeal moth
Why Eggs?

• For many insect pest species, eggs are the most fumigant-tolerant life stage
• Delayed egg hatch as a result of exposure to phosphine has been reported in red flour beetle
• For developing effective PH$_3$ resistance management strategies, information on the resistance of the most fumigant tolerant life stage is essential
• Resistance frequencies and levels of resistance in populations are usually different for different life stages
FAO Protocol #16

- FAO Protocol #16 does not have discriminating doses for eggs of red flour beetle and eggs and larvae of Indianmeal moth (based on susceptible strains)

**Discriminating dose**

Baseline lethal dose data established with reference strains of known susceptibility using a discriminating dose bioassay (20-h fumigation at 25°C). The upper limit of the 95% confidence interval of the LD$_{99}$ for the referenced strain is the discriminating dose for that insect species.
• Tests conducted using 30 ppm for 20 hours against RFB eggs
  – Found less than 20% mortality
• Tests were then conducted for a range of PH$_3$ concentrations from 30 – 300 ppm for 20 hours against RFB and IMM eggs.
  – 100% mortality was not achieved
• FAO Protocol #16
  • For RFB and IMM eggs – 3-day fumigation, count after 2 weeks
  • For IMM larvae – 20-hour fumigation, mortality count after 2 weeks
Materials and Methods

PH$_3$ concentrations tested (ppm)

<table>
<thead>
<tr>
<th></th>
<th>Exposure period was 3 days</th>
<th>Exposure period was 20 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RFB Eggs</td>
<td>IMM Eggs</td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>10.0</td>
<td>10.0</td>
<td>5.5</td>
</tr>
<tr>
<td>17.5</td>
<td>17.5</td>
<td>8.5</td>
</tr>
<tr>
<td>28.1</td>
<td>28.1</td>
<td>18.5</td>
</tr>
<tr>
<td>40.6</td>
<td>40.6</td>
<td>25.1</td>
</tr>
<tr>
<td>57.8</td>
<td>57.8</td>
<td>40.5</td>
</tr>
<tr>
<td>74.7</td>
<td>74.7</td>
<td>61.8</td>
</tr>
<tr>
<td>110.6</td>
<td>110.6</td>
<td>110.6</td>
</tr>
</tbody>
</table>

Exposure period was 3 days

Exposure period was 20 hours

RFB Eggs

IMM Eggs

IMM Larvae

Vials for fumigation
Materials and Methods

- The experiments were conducted at 25°C.
- Mortality assessments for eggs were made on the basis of hatched/unhatched eggs.
- For IMM larvae, morbid or dead larvae were counted as dead.
- Mortality data were subjected to probit analyses using PoloPlus (LeOra Software, Petaluma, CA).
Results

Probit analyses for establishing discriminating doses of phosphine against RFB eggs and IMM eggs

<table>
<thead>
<tr>
<th>Species</th>
<th>LC$_{50}$ (95% CI)</th>
<th>LC$_{95}$ (95% CI)</th>
<th>LC$_{99}$ (95% CI)</th>
<th>X$^2$ (df) [H$^*$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFB eggs</td>
<td>19.2 (16.7 – 21.6)</td>
<td>38.2 (32.2 – 50.0)</td>
<td>50.8 (40.7 – 73.6)</td>
<td>98 (19) [5.5]</td>
</tr>
<tr>
<td>IMM Eggs</td>
<td>20.4 (17.7 – 22.9)</td>
<td>53.8 (45.6 – 67.3)</td>
<td>80.3 (64.6 – 109.8)</td>
<td>57 (19) [3.0]</td>
</tr>
</tbody>
</table>

Discriminating dose for RFB eggs - **73.6 ppm** for 3 days

Discriminating dose for IMM eggs - **109.8 ppm** for 3 days
**Probit analyses for establishing discriminating dose of phosphine against IMM larvae**

<table>
<thead>
<tr>
<th>Species</th>
<th>LC$_{50}$ (95% CI)</th>
<th>LC$_{95}$ (95% CI)</th>
<th>LC$_{99}$ (95% CI)</th>
<th>X$^2$ (df) [H$^*$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMM larvae</td>
<td>11.4 (9.7 – 12.9)</td>
<td>40.7 (33.6 – 52.5)</td>
<td>69.1 (53.4 – 98.3)</td>
<td>42.1 (19) [2.2]</td>
</tr>
</tbody>
</table>

Discriminating dose for IMM larvae - 98.3 ppm for 20 hours
Summary

• The discriminating dose for RFB eggs based on 3-day fumigation is 73.6 ppm

• The discriminating dose for IMM eggs based on 3-day fumigation is 109.8 ppm

• The discriminating dose for IMM larvae based on 20-h fumigation is 98.3 ppm
• 2 field populations
• A discriminating dose of 98.3 ppm was tested for 20 hours at 25°C.

<table>
<thead>
<tr>
<th>Populations</th>
<th>% Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep 1</td>
<td></td>
</tr>
<tr>
<td>Sus.</td>
<td>0</td>
</tr>
<tr>
<td>Box E</td>
<td>90</td>
</tr>
<tr>
<td>Box F</td>
<td>78</td>
</tr>
</tbody>
</table>
Saw Toothed Grain Beetle

- 3 populations of STGB from almond storage facilities in California were tested
- The discriminating dose for STGB adults is 37.5 ppm for 20 hours at 25°C (FAO Protocol #16)

<table>
<thead>
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<th>% Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rep 1</td>
</tr>
<tr>
<td>Sus.</td>
<td>0</td>
</tr>
<tr>
<td>Box A</td>
<td>4</td>
</tr>
<tr>
<td>Box BR</td>
<td>98</td>
</tr>
<tr>
<td>Box BF</td>
<td>100</td>
</tr>
</tbody>
</table>
Future Experiments

• Determine the phosphine resistance frequencies and levels of resistance in eggs of RFB and eggs and larvae of IMM.

• Determine the phosphine resistance frequencies and levels of resistance for another 3 RFB populations and 1 IMM population currently being reared in our laboratory – cultures were started using insects collected in 2014.

• Conduct tests using many samples from almond storage facilities from various geographic locations in the Central Valley.

• Use all data collected to develop PH$_3$ resistance management strategies for almond storage facilities in the Central Valley.
Insect samples received from the Central Valley

Insect samples mass reared in environmental chambers maintained at 28 ± 1°C and 65 ± 5 % RH
Overall Conclusions

• 25% of the RFB populations had high PH$_3$ resistance frequencies above 80% (88-100).

• 25% of the RFB populations collected from almond storage facilities had moderately high PH$_3$ resistance frequencies.

• 50% of the RFB populations studied were completely susceptible to PH$_3$.

• Discriminating doses found:
  • RFB eggs – 73.6 ppm for 72 h (3 days) at 25°C
  • IMM eggs – 109.8 ppm for 72 h (3 days) at 25°C
  • IMM larvae – 98.3 ppm for 20 h at 25°C
Relevance of Research Data

• For RFB populations studied, using phosphine at the recommended label dose and exposure should kill all resistant populations.

• The variation of PH$_3$ resistance observed in RFB populations calls for expanded sampling and more PH$_3$ resistance testing.

• High resistance frequencies in STGB populations tested necessitate that this species be included in future surveys of PH$_3$ resistance in stored-product insect pests in almond storage facilities.
Acknowledgements

• Dr. George Opit
• Mr. Ed Hosoda
• Mr. Charlie Konemann
• Ms. Kandara Shakya
• Mr. Nirajan Bhattarai
• Ms. Nisha Bajracharya
Call for Sampling New Locations!!!

• More extensive sampling that represents different geographic locations is needed to better address the issue of phosphine resistance in the Central Valley.

• Therefore, we call for your help to facilitate collection of samples from new locations.