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

S. G. Gautam and G. P. Opit

Department of Entomology and Plant Pathology
Oklahoma State University
Stillwater, OK

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

Insects

Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)

Red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

Background

- **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.**

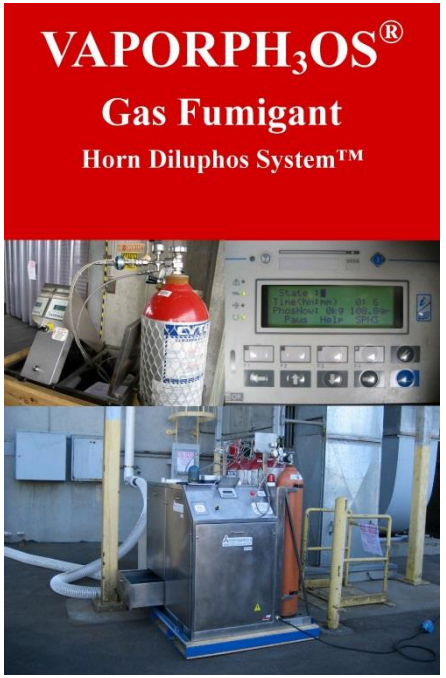
Postharvest Pest Management

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

J. LARRY ZETTLER AND GERRIT W. CUPERUS[1]

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

J. Econ. Entomol. 83(5): 1677–1681 (1990)

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, dichlorovs, 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)

FORUM

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

G. P. OPIT,[1] T. W. PHILLIPS,[2] M. J. AIKINS,[2] AND M. M. HASAN[2,3]

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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

PH_3 Resistance in Lesser Grain Borer and Red Flour Beetle in Oklahoma

Zettler and Cuperus 1990		Opit et al. 2012	
LGB	**RFB**	**LGB**	**RFB**
• 67% of the populations tested had detectable resistance • Only 8% had resistance frequencies ≥ 90%	• 13% of the populations tested had detectable resistance • Highest resistance frequency was 6%	• 100% of the populations tested had detectable resistance • 60% had resistance frequencies ≥ 90% • Most resistant population was 1,519 times more resistant than susceptible strain	• 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 did not determine the levels of resistance			

- **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**

PH_3 Resistance Management

- **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 disinfestation**

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

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

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

Counting live/dead insects to determine resistance frequencies

Results

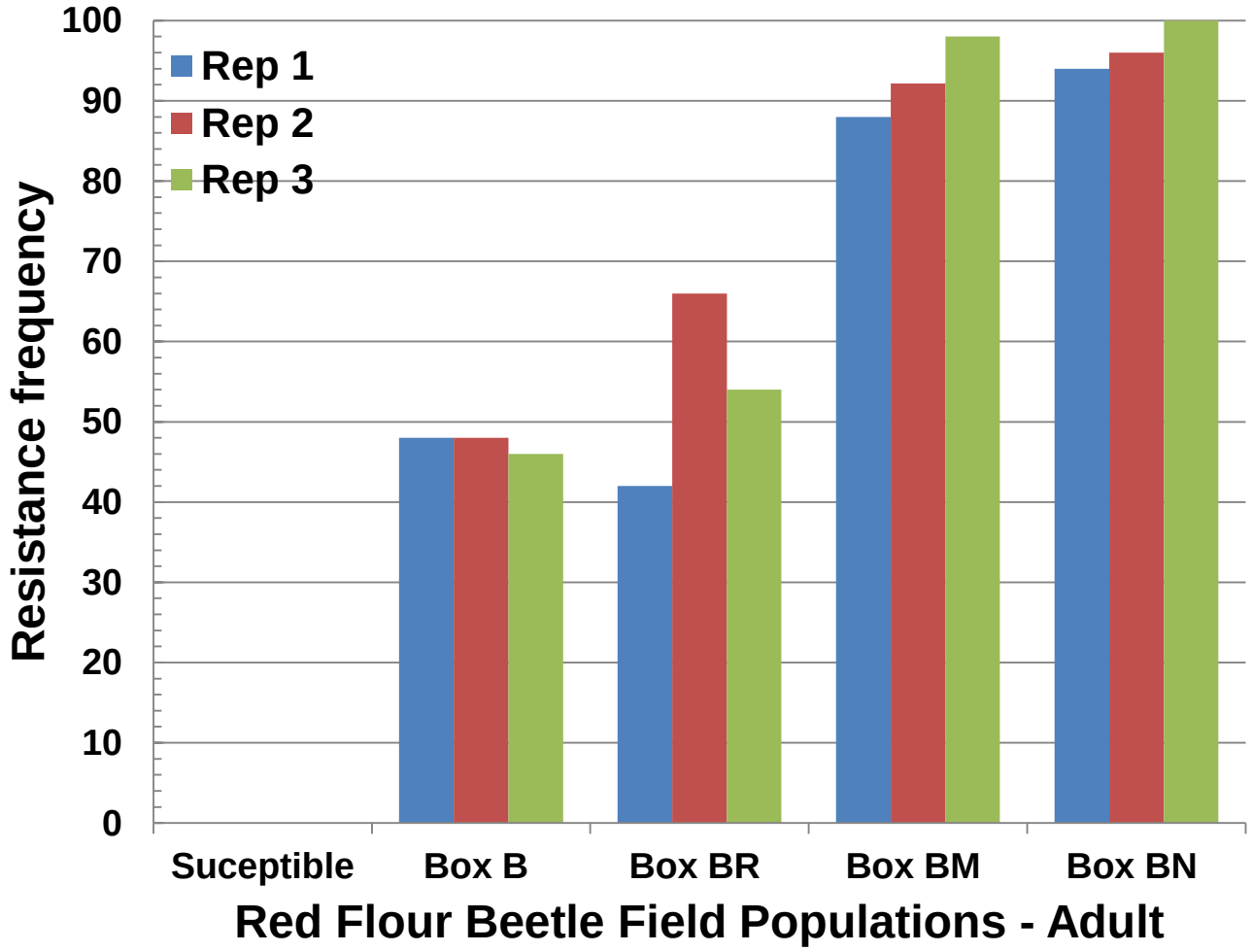
Red Flour Beetle Populations	% Survival		
	Rep 1	Rep 2	Rep 3
Box B	48	48	46
Box BM	**88**	**92**	**98**
Box BN	**94**	**96**	**100**
Box BR	42	66	54
Box E1	0	0	0
Box E2	0	0	0
Box F	0	0	0
Box I	0	0	0
Susceptible	0	0	0

Resistant

Completely susceptible

4 field populations had no detectable resistance, i.e., completely susceptible

Resistance Frequencies in Red Flour Beetle Populations with Detectable Resistance



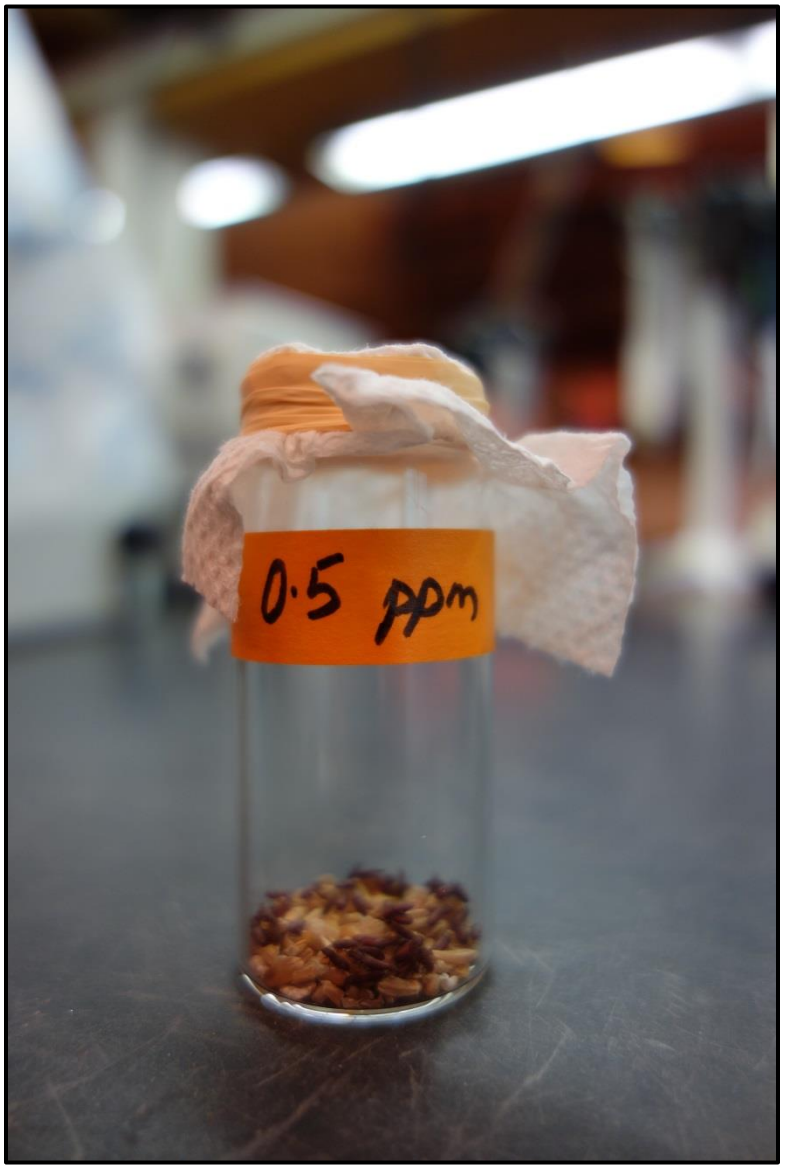
Objective 2

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

PH_3 concentrations tested (ppm)				
Sus.	Box B	Box BM	Box BN	Box BR
0.0	0.0	0.0	0.0	0.0
1.9	3.4	17.7	17.7	3.4
2.4	7.0	34.0	34.0	7.0
3.3	8.3	55.2	55.2	8.3
5.4	16.4	110.6	110.6	16.4
7.1	31.7	139.5	139.5	31.7
7.7	55.2	212.2	212.2	55.2
-	110.6	273.1	273.1	110.6



Glass vials prepared for fumigation. Each vial was covered with a piece of paper towel to allow fumigant penetration while preventing beetles from escaping.

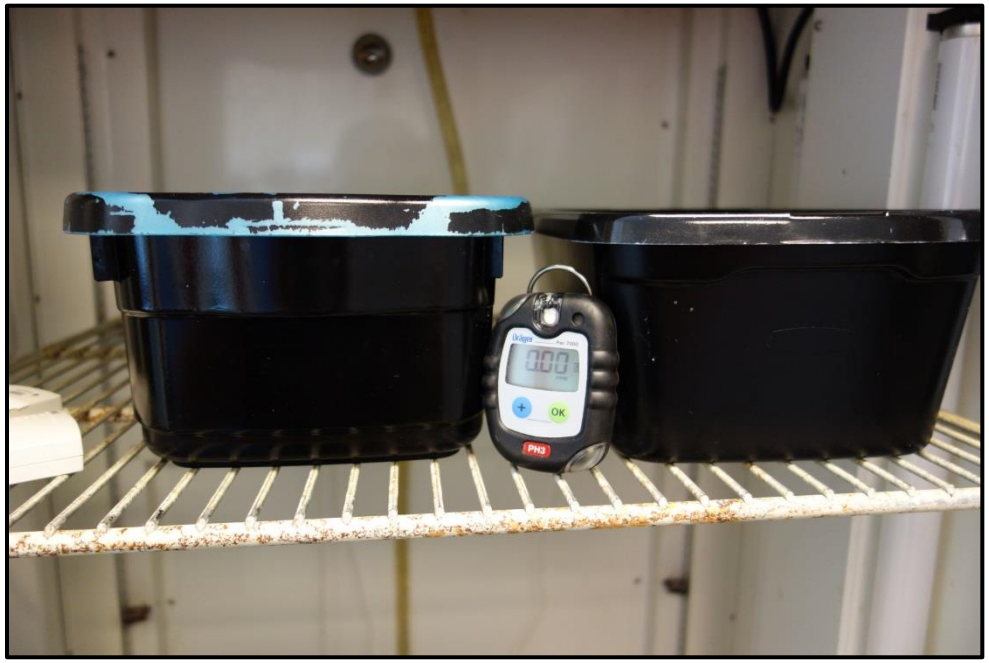
Materials and Methods

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

Fumigation room where all the phosphine fumigation experiments were conducted

- 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

Sample	LC_{50} (95% CI)	LC_{95} (95% CI)	LC_{99} (95% CI)	X^2 (df) [H*]
Susceptible	3.39 (3.2 – 3.5)	5.9 (5.5 – 6.2)	7.4 (6.8 – 8.0)	12.9 (16) [0.8]
Box B	8.6 (7.9 – 9.3)	29.9 (26.5 – 34.5)	50.2 (41.5 – 63.4)	16.9 (19) [0.89]
Box BM	34.4 (29.9 – 38.9)	157.3 (129.5 – 202.3)	295.2 (226.0 – 421.3)	20.9 (16) [1.3]
Box BN	39.4 (34.4 – 44.8)	187.1 (152.7 – 243.7)	356.9 (270.4 – 515.8)	21.5 (16) [1.35]
Box BR	10.5 (9.7 – 11.3)	33.5 (29.3 – 39.5)	54.3 (45.4 – 67.6)	17.1 (19) [0.9]

Levels of Resistance in Comparison to the Susceptible Population

Samples compared	Lethal concentration ratios		
	LC_{50} (95% CI)	LC_{95} (95% CI)	LC_{99} (95% CI)
Box B vs Susceptible	2.5 (2.3 – 2.8)	5.1 (4.3 – 6.1)	6.8 (5.4 – 8.6)
Box BR vs Susceptible	3.1 (2.8 – 3.4)	5.7 (4.8 – 6.7)	7.4 (5.9 – 9.2)
Box BM vs Susceptible	10.1 (9.0 – 11.4)	26.8 (22.1 – 32.5)	40.1 (30.7 – 52.4)
Box BN vs Susceptible	11.6 (10.4 – 12.9)	31.9 (26.1 - 38.9)	48.5 (36.9 – 63.7)

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

RFB Populations	LC_{99} (95% CI) (ppm)	Number of tablets/ 1,000 ft^3
Box B	50.2 (41.5 – 63.4)	2 (2-3)
Box BM	295.2 (226.0 – 421.3)	12 (9 - 17)
Box BN	356.9 (270.4 – 515.8)	14 (11 - 17)
Box BR	54.3 (45.4 – 67.6)	2 (2-3)

- 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)**

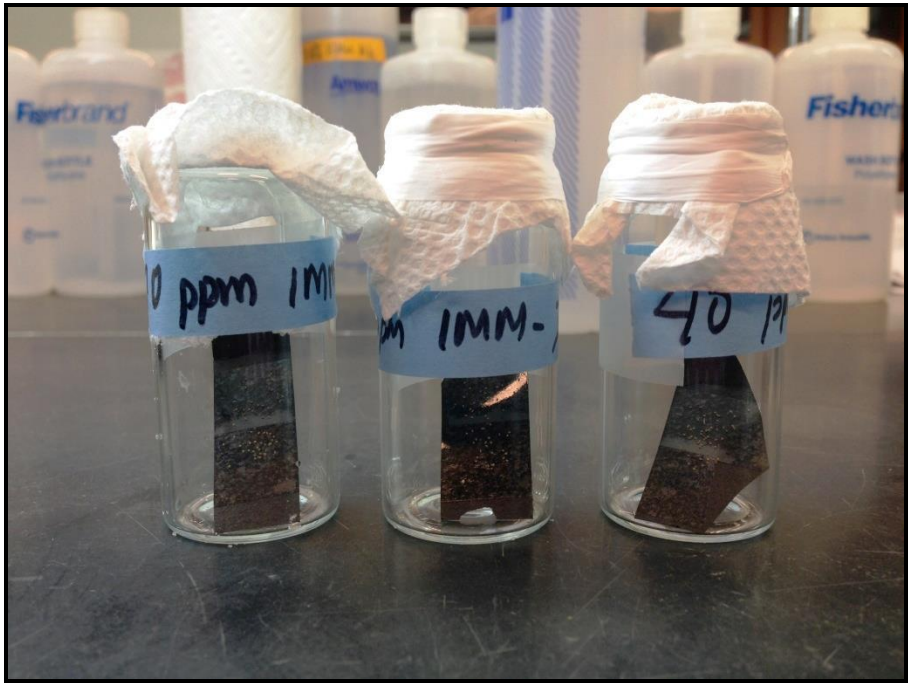
Discriminating dose

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.

Materials and Methods

- **Tests conducted using 30 ppm for 20 hours against RFB eggs**
  - **<u>Found less than 20% mortality</u>**
- **Tests were then conducted for a range of PH_3 concentrations from 30 – 300 ppm for 20 hours against RFB and IMM eggs.**
  - **<u>100% mortality was not achieved</u>**
- **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



Vials for fumigation

PH_3 concentrations tested (ppm)		
Exposure period was 3 days		Exposure period was 20 hours
RFB Eggs	IMM Eggs	IMM Larvae
0.0	0.0	0.0
10.0	10.0	5.5
17.5	17.5	8.5
28.1	28.1	18.5
40.6	40.6	25.1
57.8	57.8	40.5
74.7	74.7	61.8
110.6	110.6	110.6

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).**

Fumigation jars inside an environmental chamber

Results

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

Species	LC_{50} (95% CI)	LC_{95} (95% CI)	LC_{99} (95% CI)	X^2 (df) [H*]
RFB eggs	19.2 (16.7 – 21.6)	38.2 (32.2 – 50.0)	50.8 (40.7 – 73.6)	98 (19) [5.5]
IMM Eggs	20.4 (17.7 – 22.9)	53.8 (45.6 – 67.3)	80.3 (64.6 – 109.8)	57 (19) [3.0]

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

Species	LC_{50} (95% CI)	LC_{95} (95% CI)	LC_{99} (95% CI)	X^2 (df) [H*]
IMM larvae	11.4 (9.7 – 12.9)	40.7 (33.6 – 52.5)	69.1 (53.4 – 98.3)	42.1 (19) [2.2]

Discriminating dose for IMM larvae - 98.3 ppm for 20 hours

Summary

- **The discriminating dose for RFB eggs based on 3-day fumigation is <u>73.6 ppm</u>**
- **The discriminating dose for IMM eggs based on 3-day fumigation is <u>109.8 ppm</u>**
- **The discriminating dose for IMM larvae based on 20-h fumigation is <u>98.3 ppm</u>**

Resistance Frequencies in Indianmeal Moth Larvae

- 2 field populations
- A discriminating dose of 98.3 ppm was tested for 20 hours at 25°C.

Populations	% Survival
	Rep 1
Sus.	0
Box E	90
Box F	78

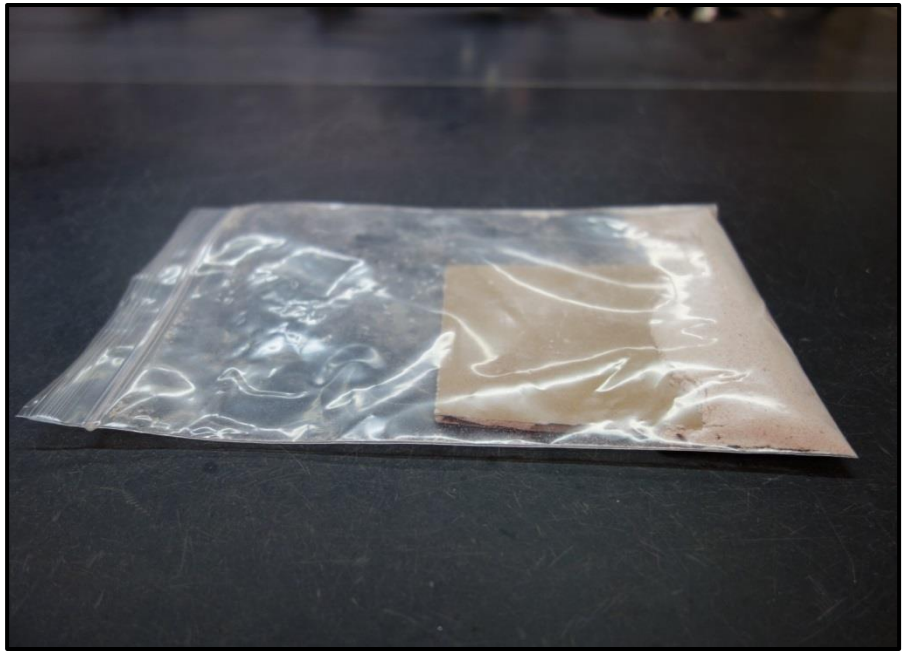
Saw Toothed Grain Beetle

- 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)

Populations	% Survival		
	Rep 1	Rep 2	Rep 3
Sus.	0	0	0
Box A	4	0	2
Box BR	98	98	100
Box BF	100	94	80

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 <u>many</u> 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 in future surveys of PH_3 resistance in stored-product insect pests in almond storage facilities.**

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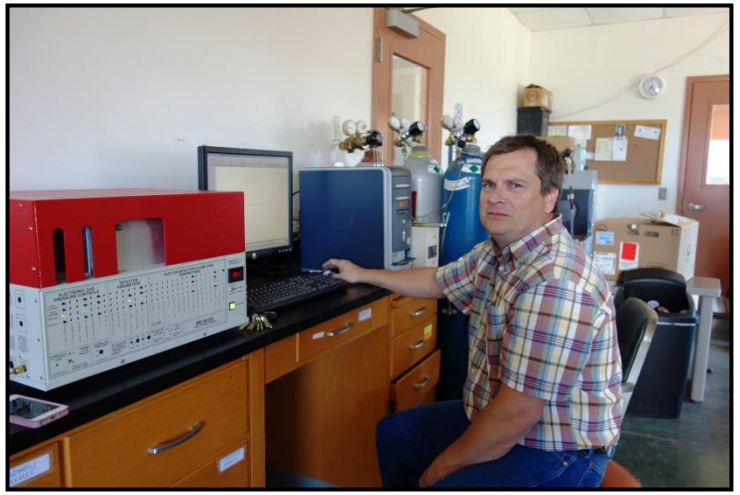
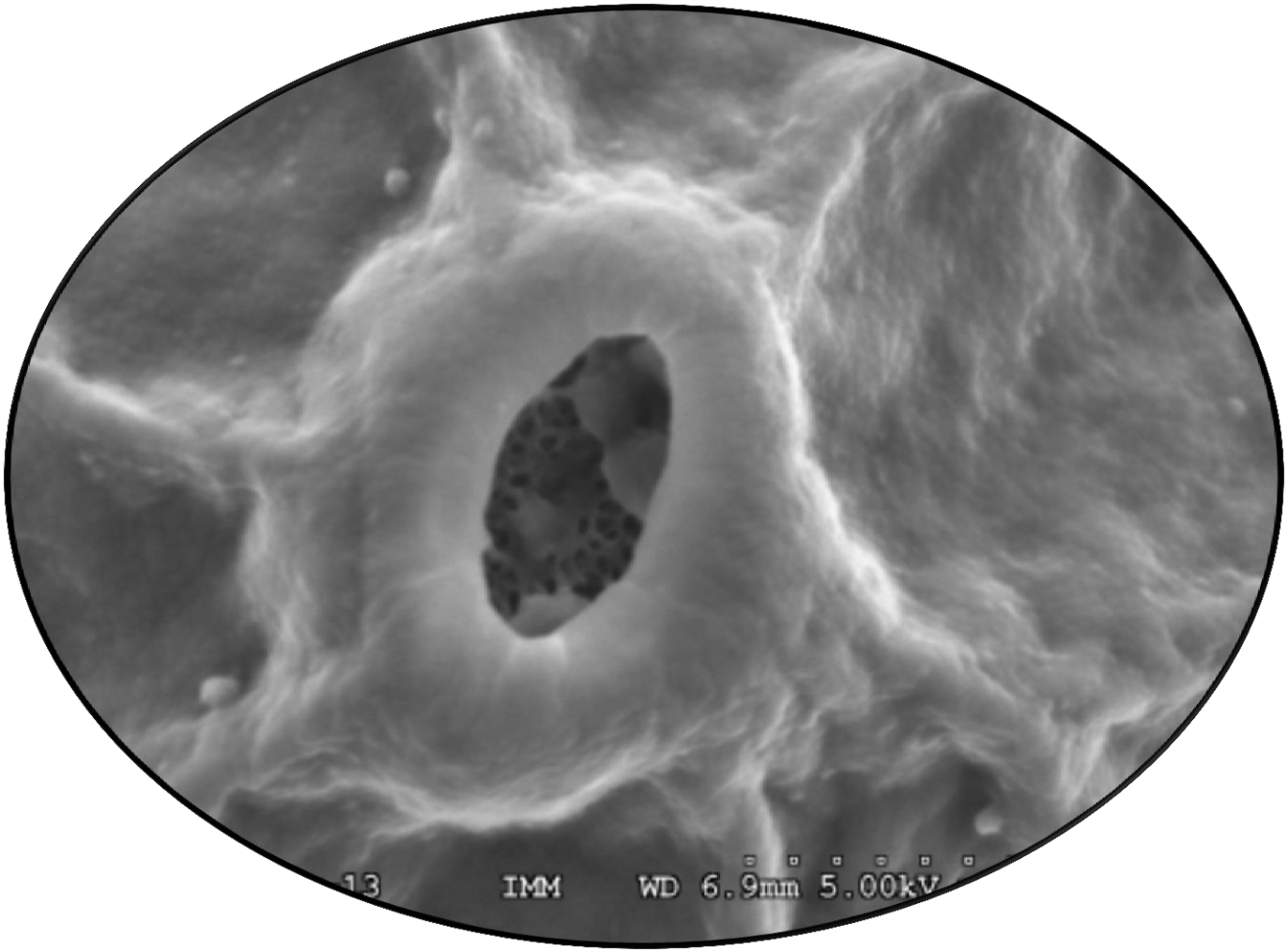


Almond Board of California

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.**

Thank You!!