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Phosphine Resistance in Thai Local Strains of *Tribolium castaneum* (Herbst) and Their Response to Synthetic Pheromone

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ABSTRACT

Tribolium castaneum (Herbst), one of the key pests of stored grains worldwide, has been reported to develop resistance to fumigants in several countries. This research was focused on assessing the toxicity of phosphine against two local strains of *T. castaneum* from Thailand by comparison with the standard susceptible strain (QTC-4) from Australia and evaluating the response of the selected strain to synthetic pheromone. By bioassay under laboratory conditions, it was found that *T. castaneum* collected from an animal feed mill in Samut Prakan province (SP strain) exhibited resistance to phosphine with resistance ratios of 3–25.8 while the strain from Khon Kaen province (KK strain) showed a much lower resistance level with resistance ratios of 1–1.45. The SP and KK strains were then observed for their responses to the pheromone in an arena of 1 m^3 in a glass cage. The results showed that synthetic pheromone could attract female beetles to the traps. Traps containing synthetic pheromone caught a significantly higher number of beetles than those of the untreated control. However, when comparing the response of the resistant strain (SP) with that of the susceptible strain (KK), it was found that both pheromone traps and untreated traps caught significantly lower numbers of resistant females than the susceptible ones. This may have been due to the resistant beetles being less active and wandering around less than the susceptible beetles. The lower response of resistant beetles to pheromone suggested that there is a need to find other alternatives for a monitoring system in areas where resistant populations occur in order to be able to make proper pest management decisions.

Keywords: phosphine resistance, *Tribolium castaneum* (Herbst), pheromone

INTRODUCTION

Thailand is an agricultural country which produces both crops and animals. Currently, most animal feeds are in the form of dried-ready-to-use feeds which use rice bran and broken rice seeds as main raw materials (Vaidya, 2013). It is common for raw materials used for animal feed production to be attacked by *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) as reported by Lyon (2000). Although passing through many steps during the production process, the odor of final feed products still has the ability to attract adult *T. castaneum* to the storage warehouse.

T. castaneum is a cosmopolitan pest associated with stored grain, flour and feed mills, food processing facilities, warehouses, and retail stores and the species can complete development between 20.0 and 37.5 ^oC, while under optimum conditions (35.0^oC and RH > 70%), development

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is completed within 19–20 d (Howe, 1956). Adults may live up to 3 yr or more, and adult females can lay eggs for more than 1 yr, although food resources and temperature strongly affect adult longevity (Good, 1936).

Various methods are widely used to prevent and control *T. castaneum* infestation such as warehouse sanitation, physical control by using high or low temperature, and the application of chemical insecticides. Among the insecticides used, fumigation by methyl bromide and phosphine is quite effective. However, both fumigants have some drawbacks due to their high toxicity to humans and other warm-blooded animals. Moreover, methyl bromide is known to affect the ozone layer and is one of the causes of global warming while phosphine at high concentration can burst into fire quite easily (Wisarathanonth *et al.*, 2008). Therefore, users or those who apply either fumigant must be extremely careful during the operation. However, the long-term use of a single fumigant increases the risk of resistance development in pest populations (Chaudhry and Price, 1990; Benhalima *et al.*, 2004). A global survey undertaken by the Food and Agriculture Organization in 1972/1973 showed that about 10% of the collected populations contained phosphine-resistant individuals, but phosphine resistance was not found in Brazil at that time (Champ and Dyte, 1976). However, 14 years later Pacheco *et al.* (1990) and Sartori *et al.* (1990) showed that populations of *Rhyzopertha dominica* (F.), *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.) and *Cryptolestes ferrugineus* (Stephens) in Brazil were highly resistant to phosphine. In Thailand, the levels of phosphine resistance in populations of *T.castaneum* are under investigation by testing against discriminating dosages. The knowledge of whether insects have resistance or not will facilitate the decision to select the appropriate control measure for each location.

An insect survey using pheromone traps is one of the options for pest management of stored products. Pheromones have been isolated and lures are commercially available for many stored-product insects (Chambers 1990; Phillips *et al*. 2000). Several trap designs specific for stored-product pests have also been developed (Vick *et al*., 1990, Mullen, 1992, Mullen and Dowdy, 2001). Pheromone traps have been demonstrated to be effective to capture stored-product pests in anthropogenic ecosystems, primarily with moths in the family Pyralidae (Vick *et al*., 1986; Soderstrom *et al*., 1987; Pierce, 1994; Bowditch and Madden, 1996; Mankin *et al*., 1999). For *T. castaneum*, 4, 8-dimethyldecanal (DMD) has been identified as the aggregation pheromone by Suzuki (1980). However, Semeao *et al.* (2011) showed that *T. castaneum* adults were more likely to visit black pillars than white pillars while on a larger scale, *T. castaneum* was captured in traps in front of black panels more than in front of white panels. Traps placed in the corners were more effective in capturing *T. castaneum* than those placed midway on walls.

The efficacy of a pheromone trap depends on both environmental factors and the insect population. The objectives of this research were to evaluate phosphine resistance in two local strains by a comparison with the standard susceptible strain and to evaluate their response to a commercial pheromone. The findings will be helpful in decision making for the management of this insect.

MATERIALS AND METHODS

Insect mass rearing

About 300 adults of *T. castaneum* were confined in a glass jar (18 cm height and 8 cm diameter) with 100 g of rice bran as the diet. Each jar was sealed with filter paper and maintained at room temperature. The adults were allowed to mate and oviposit for 7 d and then they were removed by mean of sifting from the culture media. The following insect strains were used in the experiment:

1. KK strain, a laboratory population

collected from the Laem Thong mill in Khon Kaen province and reared under laboratory condition since 1 August 2003.

2. SP strain, a field population collected from the Betagro feed factory Phra Pradaeng, Samut Prakan province (SP strain) on 30 April 2011.

3. QTC-4, the standard susceptible strain from Queensland, Australia.

Preparation of phosphine

Four liters of approximately 5% sulfuric acid solution was prepared by mixing technical grade sulfuric acid with water in a 5 L beaker. A gas trap tube was submerged into the solution, covered with silicone septum, pulled up to the right position and then locked to a stand. One tablet of aluminum phosphide wrapped with filter paper and white cloth was placed into the beaker. An upside-down glass funnel (8 cm diameter) was immediately placed over the aluminum phosphide. The sunken funnel was moved to the center of the beaker under the gas trap tube using a glass rod. After the chemical reaction between sulfuric acid and aluminum phosphide had taken place, phosphine gas bubbles moved up and replaced the sulfuric acid solution in the gas trap tube.

Bioassay for phosphine resistance in *Tribolium castaneum* (Herbst)

The testing materials were prepared by releasing 50 adults of *T. castaneum* (aged 2–4 wk) in ventilated plastic containers (3 cm height and 6 cm diameter). Each insect strain was prepared separately. Two containers per strain (making a total of six containers) were placed into a desiccator (approximately 22.3 L) before subjecting them to fumigation. The total of 21 concentrations varied from 0.004 to 0.24 $mg.L^{-1}$ plus an untreated control were tested during the bioassay. Each treatment (concentration) was replicated three times.

Phosphine calculated for the specified concentration was injected with a gas-tight syringe through a septum on the lid of each desiccator. The required exposure period was 20 hr. After fumigation, the plastic containers were removed from the desiccators, added with 1 teaspoonful of sterile rice bran and kept for 14 d at room temperature before passing through detailed examination. Insects that did not respond to probing by a pencil tip were considered dead. Insect mortality and survival were recorded. The mortality data were corrected by Abbott's formula (Abbott, 1925) before subjecting them to a probit analysis ($p > 0.05$) using the Poloplus program (Version 2.0, Pacific Southwest Forest and Range Experiment Station, Berkeley, CA, USA).

Response of local strains of *Tribolium castaneum* to synthetic pheromone

The experiment was conducted in a glass cage arena ($1 \times 1 \times 1$ m^3). Each of the bottom corners of the cage was pasted with two pieces of vertical black cardboard (18×50 cm^2) on the inside of the cage (Figures 1 and 2). The black cardboard served as the background to where each trap was placed.

Two local insect strains were tested separately. The experiment consisted of two treatments: 1) dome traps containing synthetic aggregation pheromone (4,8-dimethyldecanal) (Storgard®, Trécé Inc., Adair, OK, USA) and 2) For preparation of the trap containing the pheromone, a pheromone septum was attached to the inner side of the dome lid.

Each treatment was tested separately by setting one trap in each of the bottom corners of the cage. A Petri dish with 100 female beetles was put at the center of the cage. Four pieces of paper strip were placed perpendicularly to each cage wall from inside the dish on the cage floor. The paper strips served as ramps for beetles to walk out of the dish. Each treatment was replicated four times.

After a period of 24 hr after release, the number of beetles in each of the corner traps was recorded. All traps and the cage were cleaned before a new set of experiments was conducted.

Treatments and strains were compared using a *t*-test.

RESULTS AND DISCUSSION

Bioassay for phosphine resistance in *Tribolium castaneum* (Herbst)

The lethal concentration values for 50%, 90% and 99% (LC_{50}, LC_{90}, and LC_{99}) and the parameter estimates for the probit analysis are shown in Table 1. The results indicated that the beetles from the KK strain were quite similar to those from QTC-4, the standard susceptible strain. By comparing the LC_{50} value of the KK strain (0.012 $mg.L^{-1}$) with that of the QTC-4 strain (0.012 $mg.L^{-1}$), the resistance ratio (RR_{50}) was found to equal 1. The comparison of the LC_{90} and LC_{99} values indicated that the RR_{90} and RR_{99} values were slightly increased (1.25 and 1.4, respectively). However, all tested insects from both strains died at the discriminating dosage set by Food and Agriculture Organization (1975) of

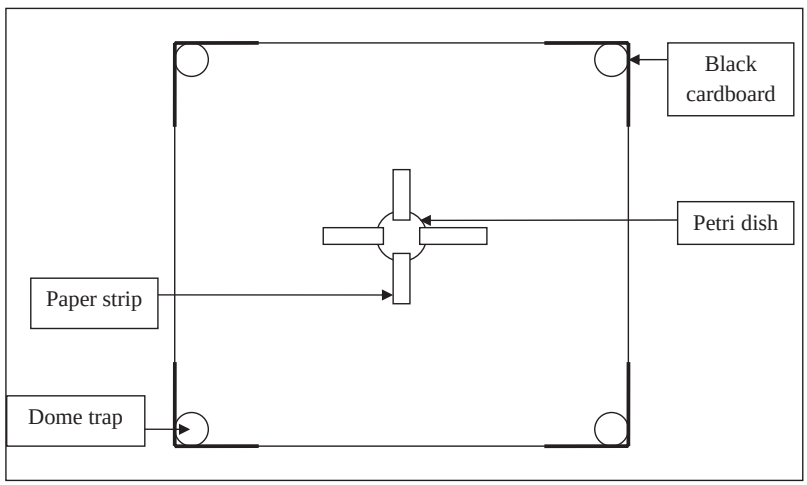

Figure 1 Top sketch view of the pheromone testing arena of 1 × 1 × 1 m^3.

Figure 2 Glass cage used as pheromone testing arena.

0.04 mg.L^{-1} indicating that both strains can still be considered as susceptible according to this standard.

At the discriminating dosage, some tested insects from the SP strain still survived and the LC_{50} value from the probit analysis was 0.036 mg.L^{-1} which was three times higher than for both the QTC-4 and KK strains (RR_{50} = 3). In comparison with the standard susceptible strain (QTC-4), the values of RR_{90} and RR_{99}, however, were much higher (RR_{90} = 9.75 and RR_{99} = 25.80). Although the resistance ratio was not as high as those reported from other countries (Pimentel *et al*., 2006; Opit *et al*., 2012), the SP population should be considered as one of the resistant strains in Thailand.

This was not a surprising result considering the habit of phosphine use in the collecting area of this strain. The animal feed factory conducted phosphine fumigation quite often and the duration of fumigation was too short (3 d instead of 7 d). Such practices clearly increased the selection pressure in favor of a phosphine-resistant population. The rate at which insecticide resistance develops depends on several factors, including how rapidly the insects reproduce, the insecticide's persistence and specificity, and the rate, timing and number of applications of insecticide made (Insecticide Resistance Action Committee, 2013).

Response of resistant and susceptible *T. castaneum* to synthetic pheromone

Synthetic pheromone was effective in attracting female beetles. Traps with pheromone were able to catch significantly ($P < 0.01$) higher numbers of female *T. castaneum* for both susceptible and resistant strains (Table 2). Approximately 50% of susceptible female beetles were caught by the pheromone trap while about 15% got caught in the empty traps. For the resistant strain, pheromone traps could catch only less than 30% while empty traps could collect about 6% of the released females.

Table 1 Toxicity of phosphine against the standard susceptible strain (QTC-4 from Queensland, Australia) and two local strains (KK = Khon Kaen strain, SP = Samut Prakan strain) of *Tribolium castaneum*. RR-values indicate the resistance ratio computed by comparing the lethal concentration values of the tested strains with the respective values from the standard strain.

Parameter/Strain	QTC-4	KK	SP
LC_{50} (mg.L^{-1})	0.012	0.012	0.036
Range LC_{50} (mg.L^{-1})	0.011–0.013	0.012–0.013	0.031–0.041
RR_{50}	1.0	1.0	3.0
LC_{90} (mg.L^{-1})	0.016	0.020	0.156
Range LC_{90} (mg.L^{-1})	0.014–0.019	0.018–0.022	0.125–0.207
RR_{90}	1.0	1.25	9.75
LC_{99} (mg.L^{-1})	0.020	0.029	0.516
Range LC_{99} (mg.L^{-1})	0.017–0.027	0.025–0.035	0.363–0.814
RR_{99}	1.0	1.45	25.8
Parameter estimates*			
a	20.135	12.032	2.905
b	10.491	6.311	2.012

LC_{50}, LC_{90}, LC_{99} = lethal concentration values for 50%, 90% and 99%.

* Parameter estimates for regression line: Y = a + b X where a = the Y intercept and b = the slope of the line.

The number of resistant females caught by pheromone traps was significantly ($P < 0.05$) lower than that of the susceptible females (Table 2). The untreated control with empty traps was also able to catch significantly ($P < 0.01$) more susceptible females than resistant females. The result indicated that resistant beetles may be less active and wander around less than the susceptible beetles. Pimentel *et al*. (2006) indicated that phosphine resistance in *T. castaneum* was associated with a lowered respiration rate. Such a mechanism should then affect insect behavior and activity. Foster *et al.* (2005) reported the reduced response of insecticide-resistant aphid (*Myzus persicae*) to its alarm pheromone and suggested a potential fitness trade-off. Resistance to insecticides is often accompanied by fitness costs, such as a decreased rate of development, fecundity, survival or mating competitiveness relative to susceptible insects (Roush and McKenzie, 1987).

The results from this experiment suggested that cautious consideration must be taken when applying phosphine in storage or in an animal feed factory or both in order to delay the development of resistance in insect pests. Once the resistant population is established, the efficacy of the pheromone traps in attracting female beetles will be reduced and hence other alternatives for insect monitoring must be sought in order to obtain more accurate information for making pest management decisions.

CONCLUSION

The field population of *Tribolium castaneum* (SP strain) collected from an animal feed mill in Samut Prakan province exhibited some degree of phosphine resistance as the value of RR_{50} was 3.00 while the RR_{90} and RR_{99} values were 9.75 and 25.80, respectively. However, a laboratory population (KK strain) maintained under laboratory conditions for several years did not show any notable increase in the resistance level when compared to the standard susceptible strain ($RR_{50} = 1$, $RR_{90} = 1.25$ and $RR_{99} = 1.4$). The habit of phosphine use in the field is one of the key factors which determine resistance development. When testing the response of both strains to the synthetic pheromone in a testing arena, it was observed that less resistant females were caught by both pheromone traps and untreated control traps when compared to the susceptible females. The result suggested that resistant females could be less active and wander around less than the susceptible females and hence showed the lowered response to pheromone trapping. Therefore, in areas where phosphine resistance occurs in the population of *T. castaneum*, other monitoring techniques must be sought.

Table 2 Numbers of susceptible (KK) and resistant (SP) female *T. castaneum* caught by pheromone traps in a testing arena of $1 \times 1 \times 1$ m^3 glass cage.

Design/strain	Susceptible (KK)	Resistant (SP)	P[a]	CV%
Pheromone	47.75±4.589	28.75±3.092	0.013*	16.1
Untreated control	14.25±1.750	5.75±1.315	0.010**	26.6
P[b]	0.000**	0.000**		
CV (%)	31.8	40.8		

* = Significantly different ($P < 0.05$); ** = Highly significantly different ($P < 0.01$).

[a] = *P*-values from independent *t*-test comparing susceptible and resistant strains.

[b] = *P*-values from independent *t*-test comparing pheromone and untreated control traps.

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