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Resistance of phosphine to eleven strains of *Tribolium castaneum* and stage-specific mortality of susceptible and resistant strains

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ABSTRACT

Tribolium castaneum (Herbst) is a common pest of stored grain around the world. Tolerance or resistance is one of the main factors for success with phosphine fumigation. There were few reports on the resistance over the last ten years in China. The resistance to phosphine for eleven strains of red flour beetle [*Tribolium castaneum* (Herbst)], that were collected from seven provinces of China, was assayed using the FAO method. The mortality of each life stage of four strains with different resistance factors (*Rf*) was determined at 100, 200, 300, 400 and 500 mL m^{-3} of phosphine. The strains were named: R_1, R_6, R_{131} and R_{274} with *Rf* ranging from 1 to 274. Five strains had *Rf* between 1 and 6 fold, three strains had *Rf* between 93 and 167 fold and three strains had *Rf* between 224 and 274-fold. Hundred per cent control of strain R_1 was achieved in 9 h with 300 mL m^{-3} or more of phosphine and in 7 h with 500 mL m^{-3} for eggs. It took 9 h with 400 mL $m^{-3}$and more for pupae. One hundred per cent control of strain R_6 was achieved in 27 h with 200 mL m^{-3} and more and in 21 h with above 400 mL m^{-3} for egg, and in 27 hours with 500 mL m^{-3} for pupae. The strain R_{131} and R_{274} can be completely controlled in 16 days with 200 mL m^{-3} and more for egg and in same number of days with 300 mL m^{-3} and more for pupae. For 100% control of egg in 13 days, the concentration was more than 300 mL m^{-3} for strain R_{131} and 400 mL m^{-3} for strain R_{274}. The results indicated that the pupae in all strains had the highest tolerance to phosphine for four strains examined. The exposure time should be more than 16 days with above 300 mL $m^{-3}$for the strains whose *Rf* was in 131–274 fold.

Key words: Full mortality, Life stages, Phosphine, Red flour beetle, Resistance

Red flour beetle [*Tribolium castaneum* (Herbst)] is one of the most important pests of stored cereals (Daglish et al., 2015). It is often found in Chinese grain storages and mills. In China, the most common method of control is fumigation with phosphine, using tablets of aluminum phosphide. There are always some failures of the fumigation due to poor sealing, low concentrations, short exposure time, resistance or tolerance. There are significant differences in tolerance between different life stages. For example, the highest resistances are present in early- and mid-pupae at levels which are much higher than adults (Nakakita and Winks, 1981). The order of tolerances for both the susceptible and the resistant strains are early-pupae > mid-pupae > late pupae > pre-pupae > 15-day larvae > 20-day larvae (Price and Mills, 1988). Resistance of this insect to phosphine is the main factor that affects the success of fumigation. Early-pupae of the susceptible strain were 32 times more tolerant of phosphine than 20-day larvae at the lethal dose of 50% of the population (LD_{50}) and 41 times more tolerant at the $LD_{99.9}$ (Price and Mills, 1988). Effective management of this pest with phosphine are threatened by widespread resistance to phosphine (Herron, 1990; Rajendran, 1994; Cao et al., 2003; Zhou et al., 2011; Opit et al., 2012; Daglish et al., 2015).

It is important to survey resistance to phosphine and to know the tolerance of the life stages of resistant strains to effectively manage insect pests in grain stores (Subramanyam and Hagstrum, 1995; Opit et al., 2012). Two factors i.e. concentration of phosphine and

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exposure time, are the main elements to a successful fumigation. The type of strain; resistant or susceptible and duration of exposure to phosphine were more critical than dosage. However, for control of the resistant strains, it is also necessary to achieve high dosages and maintained for long periods (Price and Mills,1988). There are few reports about the exposure time to obtain 100% control of the most tolerant life stage of the resistant strains under conditions of some phosphine concentrations or the effective concentration in some exposure time in practical or simulative fumigation. The resistance of eleven strains of *T. castaneum* from seven provinces in China was assayed using the FAO method. And the mortality of egg, larvae, pupae and adult of four strains with different levels of resistance was measured for different exposure times at five concentrations of phosphine from 100 to 500 mL $m^{-3.}$

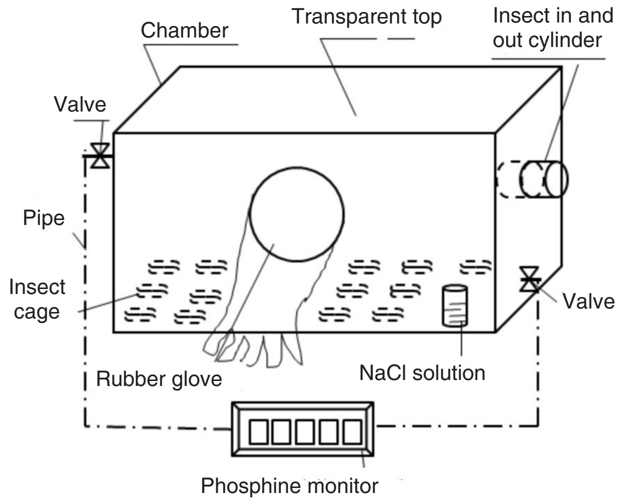

Fig. 1. Fumigation chamber and material arrangement

MATERIALS AND METHODS

Insects

Eleven strains of *T. castaneum* were collected from the grain depots, distributed in seven provinces located in south and central part of China in 2014 (Table 1). All strains after the collection were maintained without further exposure to phosphine in Stored Product Insect Research Laboratory of the Henan University of Technology, China. These populations were reared on wheat (*Triticum* sp.) flour (13% m.c.) plus 5% yeast in glass jars at 28±1ºC, 70 ± 5% r. h.

Resistance examination

Resistance factor was measured using the standard FAO test method for phosphine (FAO, 1975). Resistance factors were calculated using the 50% lethal dosage estimated from a dose-mortality experiment conducted using the standard FAO (1975) test method to phosphine.

Fumigation

The fumigation was carried out in the laboratory in several rectangle chambers (60 cm × 35 cm × 40 cm, Fig.1) that were made of metal armor plate (2 mm) except for transparent top which was made of Plexiglas. The air tightness of the chamber was guaranteed by airproof mat and bolt between rectangle bin and transparent top side. There was a hole on one vertical side that was sealed with rubber glove and fastening loop. Test insects taken out using a cylinder in the chamber sidewall of 80 mm diameter and 200 mm length. The cylinder could be sealed by two screwed caps on both ends separately. Insect cages could be taken out through this cylinder during fumigation, which reduced the loss of phosphine during sampling Insect cages were 10 mm in diameter and 70 mm in length. To check the mortality at each

Table 1 Probit analysis of phosphine mortality of eleven strains of *Tribolium castaneum* assayed by the FAO recommended method

Strain	City	Province	LC_{50} (fiducial limit in 95%)/(mg·L^{-1})	LC_{99} (fiducial limit in 95%)/(mg·L^{-1})	Slope	Intercept	X^2	Df	Rf
*R*1	Xiaoshan	Zhejiang	0.009(0.0707–0.012)	0.172(0.097–0.426)	1.83	8.73	1.183	3	1.0
R_{2a}	Baoshan	Yunnan	0.014(0.012–0.017)	0.162(0.097–0.354)	2.19	9.06	5.5267	3	1.6
R_{2b}	Jiaxing	Zhejiang	0.015(0.013–0.018)	0.097(0.066–0.176)	2.86	10.23	0.7255	2	1.7
R_{2c}	Huzhou	Zhejiang	0.021(0.017–0.028)	0.298(0.148–1.067)	2.02	8.39	0.3003	2	2.3
R_6	Fangshan	Beijing	0.053(0.039–0.071)	3.012(1.150–17.759)	1.33	3.69	1.4822	3	5.9
R_{93}	Shantou	Guandong	0.838(0.558–1.023)	8.848(5.188–30.323)	2.27	5.17	0.9335	5	93.2
R_{131}	Haikou	Hainan	1.178(0.940–1.364)	14.354(7.324–69.020)	2.14	4.85	0.6575	5	130.9
R_{167}	Sanya	Hainan	1.505(1.350–1.693)	9.321(5.981–21.162)	2.94	4.48	3.1319	5	167.2
R_{224}	Nanning	Guanxi	2.012(1.817–2.245)	11.047(7.416–22.449)	3.15	4.05	7.5539	5	223.5
R_{263}	Nanyang	Henan	2.363(2.137–2.704)	11.717(7.852–23.557)	3.35	3.75	3.4105	5	262.6
R_{274}	Guangzhou	Guangdong	2.462(2.206–2.876)	13.456(8.596–30.219)	3.15	3.77	1.8151	5	273.5

interval the screw cap in chamber was opened, the insect cage of three replications for each strain put in cylinder, the screw cap in chamber was closed using the rubber glove. Then the cage was taken out of the cylinder through the screwed cap that was outside of the chamber. The fumigant in the chamber was re-circulated and monitored by an electronic phosphine monitor with a pump and two rubber pipes that controlled the valves. The detecting range of phosphine monitor was in 0–1,000 mL m^{-3} and 0.01 mL m^{-3} in precision (model HL-210, Xinjialiang Co., Beijing, P. R. China). Here the unit of phosphine was shown in mL m^{-3} that meant phosphine volume in the volume of fumigating chamber. The relative humidity in the chamber was controlled by supersaturated solution of sodium chloride held in Petri dish on the bottom of the chamber. The half-loss-time of pressure decay at 500 Pa was over 2 minutes for the chambers.

Phosphine was generated from zinc phosphide in acidified water based on FAO method (FAO, 1975). The fumigant was injected using agastight syringe through the recirculation rubber pipes. Relatively stable concentrations of 0, 100, 200, 300, 400 and 500 mL m^{-3} of phosphine were maintained in six fumigation chambers. If a loss of phosphine was detected after the daily check, phosphine was added.

According to the results of resistance assay by the FAO recommended method the adults, eggs, larvae and pupae of strain R_1, R_6, R_{131} and R_{274} were fumigated with 0, 100, 200, 300, 400 and 500 mL m^{-3} of phosphine. The insect samples were withdrawn after different exposure times. Mortality of adults was checked after 14 d. Eggs were held for 20 d and emergence to adult checked every three days until there was no new emergence. Larvae and pupae were held and checked in same interval until no new emergence.

Experimental units or replicates of beetles for mortality studies were generated as follows. Fifty adults of 14 days age were placed with 2 g of whole wheat flour were put into one insect cage. Fifty eggs laid by 2,000 adults in one day accompanied with 0.5 g feed were in an insect cage. Fifty larvae of 21 days age were combined with 0.5 g feed and were taken as a batch in one cage. Fifty pupae of one day old were placed with 2 g of the feed were filled in same cage. There were three replications for each strain of the insect stage and phosphine concentration. The cages were sealed by 120 mesh of nylon sieve. The duration of exposure was modified according to the level of resistance and tolerance of the insect stage.

Statistical analysis

The statistical analysis was performed using DPS 3.11 software and Microsoft Excel 2007.

Table 2 Mortality (%) of different insect stages for strain R_1 with different exposure times and different phosphine doses

Stage	Exposure time (h)	100 mL m^{-3}	200 mL m^{-3}	300 mL m^{-3}	400 mL m^{-3}	500 mL m^{-3}
Eggs	1	12.00±2.08*	16.67±2.96	21.67±2.96	20.67±3.71	25.67±5.04
	3	27.00±4.16	36.00±4.04	41.33±2.73	54.00±3.06	59.67±2.60
	5	40.33±2.96	60.00±3.79	69.33±3.48	83.67±2.96	82.00±3.06
	7	54.33±2.33	66.67±4.41	88.67±2.96	94.33±3.48	100.00±0.00
	9	76.67±3.48	94.33±3.48	100.00±0.00	100.00±0.00	100.00±0.00
	24	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Larvae	1	30.00±3.06	40.00±2.00	40.00±2.31	55.33±3.53	70.67±4.06
	3	82.00±3.06	84.67±3.71	90.67±3.71	100.00±0.00	99.33±0.67
	5	90.00±3.06	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	7	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Pupae	1	8.67±1.76	12.67±1.76	17.33±1.33	20.00±1.15	23.33±1.76
	3	18.00±2.31	27.33±1.76	32.67±1.76	32.67±0.67	34.67±1.76
	5	26.67±2.40	42.67±1.76	55.33±2.40	61.33±2.91	68.67±1.76
	7	48.00±2.00	63.33±2.40	71.33±2.40	88.00±1.15	91.33±2.40
	9	67.33±1.76	82.00±1.15	93.33±4.06	100.00±0.00	100.00±0.00
	24	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Adults	1	39.33±2.91	58.00±3.06	69.33±1.76	77.33±1.76	84.67±1.76
	3	70.00±2.31	88.67±2.40	90.67±2.91	100.00±0.00	100.00±0.00
	5	89.33±2.40	96.67±1.76	100.00±0.00	100.00±0.00	100.00±0.00
	7	96.00±2.31	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	9	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

*Mean±SE, the same below

Table 3 Mortality (%) of different insect stages for strain R_6 with different exposure times and different phosphine doses

Stage	Exposure time (h)	100 mL m^{-3}	200 mL m^{-3}	300 mL m^{-3}	400 mL m^{-3}	500 mL m^{-3}
Eggs	3	9.33±3.28	18.00±2.08	20.33±2.33	20.33±2.33	25.33±2.33
	9	31.33±3.93	42.67±4.41	56.67±3.48	62.33±2.96	66.33±4.91
	15	58.33±0.67	67.00±4.04	74.00±1.00	83.67±3.33	84.67±1.45
	21	73.67±3.48	85.33±1.45	88.67±3.48	100.00±0.00	100.00±0.00
	27	86.33±3.48	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	33	99.00±1.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	39	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Larvae	3	39.33±2.91	56.67±2.40	64.00±3.06	75.33±2.40	76.67±1.76
	9	89.33±2.40	95.33±2.91	100.00±0.00	100.00±0.00	100.00±0.00
	15	98.00±1.15	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	21	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Pupae	3	10.67±0.67	15.33±0.67	22.00±1.15	23.33±1.76	22.67±1.33
	9	30.67±1.76	34.67±0.67	37.33±1.76	42.00±2.00	41.33±1.33
	15	39.33±2.40	46.00±2.31	52.00±1.15	56.67±2.40	66.00±1.15
	21	48.67±2.40	63.33±7.69	73.33±2.40	78.67±1.76	84.67±2.40
	27	62.00±1.15	80.67±1.76	90.00±1.15	97.33±1.76	100.00±0.00
	33	88.67±1.33	98.00±1.15	100.00±0.00	100.00±0.00	100.00±0.00
	39	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Adults	3	33.33±1.76	56.00±1.15	68.00±2.31	73.33±2.40	78.00±1.15
	9	77.33±0.67	85.33±0.67	92.67±2.40	96.00±2.31	99.33±0.67
	15	90.00±2.31	97.33±1.76	100.00±0.00	100.00±0.00	100.00±0.00
	21	98.67±1.33	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	27	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

Table 4 Mortality (%) of different insect stages for strain R_{131} with different exposure times and different phosphine doses

Stages	Exposure time (d)	100 mL m^{-3}	200 mL m^{-3}	300 mL m^{-3}	400 mL m^{-3}	500 mL m^{-3}
Eggs	4	24.33±4.33	29.33±3.93	33.33±2.96	42.00±4.04	50.67±3.48
	7	36.00±3.79	46.67±5.21	58.00±3.79	66.33±4.33	70.00±4.73
	10	52.67±4.26	66.67±4.70	79.67±4.70	93.00±4.73	93.67±4.91
	13	81.33±3.93	87.67±2.33	100.00±0.00	100.00±0.00	100.00±0.00
	16	96.67±1.67	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	19	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Larvae	4	62.67±1.76	71.33±1.76	90.67±1.76	96.67±2.40	100.00±0.00
	7	76.00±2.31	80.00±2.31	94.67±2.91	99.33±0.67	100.00±0.00
	10	86.00±1.15	91.33±2.40	100.00±0.00	100.00±0.00	100.00±0.00
	13	95.33±2.40	99.33±0.67	100.00±0.00	100.00±0.00	100.00±0.00
	16	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Pupae	4	17.33±1.76	25.33±1.76	32.67±1.76	38.67±2.91	41.33±2.40
	7	28.67±2.40	34.67±2.40	40.00±2.31	47.33±2.40	48.67±2.40
	10	42.67±1.76	49.33±1.76	56.67±2.40	62.67±1.76	65.33±1.76
	13	59.33±1.76	68.00±3.06	84.00±2.31	86.00±2.31	88.67±2.91
	16	80.67±1.76	92.00±2.31	100.00±0.00	100.00±0.00	100.00±0.00
	19	98.00±1.15	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	21	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Adults	4	60.67±2.91	76.67±2.40	90.00±1.15	98.67±0.67	98.00±1.15
	7	76.00±3.06	92.67±2.40	100.00±0.00	100.00±0.00	100.00±0.00
	10	90.67±0.67	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	13	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

Table 5 Mortality (%) of different insect stages for strain R_{274} with different exposure times and different phosphine doses

Stage	Exposure time (d)	100 mL m^{-3}	200 mL m^{-3}	300 mL m^{-3}	400 mL m^{-3}	500 mL m^{-3}
Eggs	4	7.33±2.73	9.00±2.89	14.67±3.48	26.33±1.45	26.33±4.33
	7	13.67±2.96	26.00±3.79	39.33±3.48	38.67±5.81	47.00±3.79
	10	31.67±5.21	50.33±2.96	68.33±3.48	69.33±2.33	81.00±3.79
	13	50.67±2.33	87.67±2.33	86.67±4.26	100.00±0.00	100.00±0.00
	16	66.00±2.89	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	19	96.67±1.67	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Larvae	4	11.33±1.76	22.67±2.40	42.00±1.15	61.33±2.40	74.00±2.31
	7	24.00±1.15	46.67±2.40	60.00±3.46	75.33±2.40	85.33±1.76
	10	47.33±2.40	58.67±2.40	72.00±1.15	84.00±3.06	96.67±2.40
	13	72.67±2.40	80.67±1.76	91.33±2.40	96.67±1.76	100.00±0.00
	16	92.00±3.06	98.67±1.33	99.33±0.67	100.00±0.00	100.00±0.00
	19	96.67±1.76	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	21	98.67±0.67	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	28	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Pupae	4	4.00±1.15	13.33±1.76	22.00±2.31	34.67±1.76	36.67±2.40
	7	18.00±1.15	26.67±1.76	32.67±1.76	40.67±1.76	44.00±2.31
	10	34.67±2.91	37.33±1.76	40.00±1.15	50.67±0.67	52.67±2.40
	13	49.33±2.40	77.33±1.76	79.33±1.76	84.67±1.76	90.67±1.76
	16	66.67±1.76	89.33±2.40	100.00±0.00	100.00±0.00	100.00±0.00
	19	88.00±1.15	96.67±1.76	100.00±0.00	100.00±0.00	100.00±0.00
	21	94.67±2.40	98.67±0.67	100.00±0.00	100.00±0.00	100.00±0.00
	28	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Adults	4	6.67±1.76	20.00±2.31	49.33±1.76	64.67±2.91	79.33±2.91
	7	25.33±2.40	64.00±2.31	91.33±1.33	97.33±1.33	100.00±0.00
	10	59.33±1.76	87.33±1.76	100.00±0.00	100.00±0.00	100.00±0.00
	13	80.67±1.76	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	16	93.33±1.76	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
	19	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

Table 6 CT product (gh/m^3) to achieve 100% mortality of different insect stages for different strains with different exposure times and different phosphine doses*

Stage	Strains	CT Product (gh m^{-3})				
		100 mL m^{-3}	200 mL m^{-3}	300 mL m^{-3}	400 mL m^{-3}	500 mL m^{-3}
Egg	R_1	3	7	4	5	5
	R_6	5	7	11	12	14
	R_{131}	63	106	129	172	215
	R_{274}	--	106	159	172	215
Larvae	R_1	1	1	2	2	2
	R_6	3	4	4	5	6
	R_{131}	53	106	99	132	66
	R_{274}	93	126	189	212	215
Pupae	R_1	3	7	10	5	6
	R_6	5	11	14	18	19
	R_{131}	70	126	159	212	265
	R_{274}	93	185	159	212	265
Adult	R_1	1	2	2	2	2
	R_6	4	6	6	8	10
	R_{131}	43	66	70	93	116
	R_{274}	63	86	99	132	116

*Assume a density of phosphine of 1.379 g/L, gas (25 °C); There was no full mortality in any life stage of strain R274

RESULTS AND DISCUSSION

Resistance levels

Five strains had *Rf* 1–6 fold, three strains had *Rf* 93–167 fold and three strains had *Rf* 224–274 fold (Table 1). There were significant differences among the different strains. Significant differences were also observed between the strains collected from same province such as Zhejiang and Guangdong province. High or low resistance did not appear to be related to regional climate, high or low temperature in insects collected, e.g. R_{263} from Henan where temperature is low compared to R_1 from Zhejiang, where temperature is higher. More warmer southerly provinces (Guangdong) had strains with both high and low resistance, as did the northerly provinces.

Mortality of different life stages

The mortality of different stages of four strains of *T. castaneum* on having levels of resistance in different exposure time fumigated by five concentrations of phosphine can be seen in Tables 2 to 5. There was always some mortality even at the shortest duration and the lowest dose for all life stages in all the strains. This shows that there will always be some mortality with fumigation. The concentration × time, or CT product, shows that pupae and egg stages are more tolerant than larvae and adults (Table 6). Resistant strains needed higher CT products than susceptible strains, there was always a combination of duration and dose that could control all life stages, regardless of the strain.

The exposure time required to get 100% mortality was approximately 24 h for the adult and larvae of strain R_1 and R_6 with of 100 mL m^{-3} of phosphine. And the time required was 24 to 39 h for the eggs and pupae of the two strains with same dose. The eggs and pupae were more tolerant than adults and larvae to phosphine. There were a few hours of tolerance different between strain R_1 and R_6 for same stage with fumigation of 100 mL m^{-3} that the different was in hours. Strain R_1 and R_6 showed similar results for the same stage in the fumigation with higher concentrations which caused full mortality.

The exposure time to completely control all life stages was more than 13, 16, 19 and 21 d for adults, larvae, eggs and pupae, respectively, of strain R_{131}, and 19, 28, >19 and 28 d for the same stages of strain R_{274} fumigated with 100 mL m^{-3}. The eggs and pupae of strain R_{131} and strain R_{274} were found more tolerant to phosphine than adults and larvae. Resistant strains, even the most tolerant stages of egg and pupae could be controlled with long durations at high doses. For example, the exposure time needed to kill 100% of pupae and eggs was more than three times longer than that of larvae of strain R_1 at all doses. The exposure time needed to kill 100% of pupae was less than three times longer than that of larvae of the resistant strains R_{274} and strain R_{131}.This indicates that differences between the stages are smaller for the resistant strains than the susceptible strain.

Implications for pest management

Effective management of *T. castaneum* is threatened by phosphine resistance which has been reported from many parts of the world (Daglish et al., 2015). There were a few reports about it in China during the last decade (Zhang, 1999; Cao et al., 2003; Zhou et al., 2011). The results of this research indicate that there was a great difference among of the strains collected from different locations in China. The development of resistance phosphine may be still on the increase in some places, whereas it may not change too much in other places.

The highest resistances were present in early- and mid-pupae, much higher than adults. Early-pupae of the susceptible strain were 32 times more tolerant of phosphine than 20-day larvae at the LD_{50} and 41 times more tolerant at the $LD_{99.9}$ (Nakakita and Winks,1981). We found that differences in tolerance to phosphine for life stages were greater for susceptible strains than in resistant strains. Dyte and Halliday (1985) described the problems and implications of resistance to phosphine found in the adult stage of insect pests. The present tests show that the most tolerant stages were eggs and pupae even for susceptible strain. The stages that are difficult to control are the pupae, eggs and larvae for resistance strain. The complete control of immature stages (eggs, larvae and pupae) of resistant strains needs to be given longer durations and higher doses, and these stages are difficult to detect in the grain stores.

The previous work on susceptible strains showed that the duration of exposure is of more consequence than the concentration in achieving mortality at practical concentrations (Bell, 1976). The greater importance of high phosphine concentrations was determined in our research due to that the most tolerant pupae can be killed by high concentration of phosphine in shorter exposure time. To control the resistant insects it was also found necessary to achieve high dosage levels, which must be maintained for long durations (Price and Mills, 1988).

As a replacement fumigant for phosphine is unlikely to be found, the need to amend current fumigation practices to prevent further development of resistance is urgent. Great improvements in the gas-tight sealing of storages, lengthening of exposures to the gas and high applications rates are needed to insure control and to prevent the development of resistance strains (Price and Mills, 1988; Emery et al., 2011).

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