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CHARACTERISATION OF RESPONSE TO PHOSPHINE IN ADULTS OF REPRESENTATIVE STRAINS OF *SITOPHILUS ZEAMAIS* (MOTSCH.), *SITOPHILUS ORYZAE* (L.) AND *RHYZOPERTHA DOMINICA* (F.) FROM CHINA

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ABSTRACT

The effects of exposure period and phosphine concentration were quantified in adults of representative strains of *Sitophilus zeamais* (Motsch.), *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.) from China. All three species are major pests of stored grain in China. Although phosphine resistance is common in Chinese *S. oryzae* and *R. dominica*, with some very resistant populations, it is not present in Chinese *S. zeamais*. Therefore, the study was undertaken on a susceptible strain of *S. zeamais*, a strong resistant strain of *S. oryzae*, and a strong resistant strain of *R. dominica*. Adults were fumigated at a range of concentrations for periods of 20-144 h at 25ºC and 70% r.h. The data fitted the probit model well except for data from the Chinese strains of *S. oryzae* and *R. dominica* at 20 h, where the $LC_{99.9}$ values were much greater than the highest test concentrations. The LC_{50} and $LC_{99.9}$ tended to decrease with increasing exposure period, and the effects of concentration (C) and time (T) could be described for all strains by the equation $C^nT = K$. Time and concentration were equally important dosage factors for the susceptible strain of *S. zeamais* ($n \cong 1$), but time was a more important dosage factor than concentration for the resistant strains of *S. oryzae* and *R. dominica* ($n < 1$).

INTRODUCTION

Phosphine is one of the most important chemical control options for insect pests of stored grain, and has had been used for about half a century. However, phosphine resistance in the major pest species is becoming more common in many countries (Champ and Dyte, 1976; Zettler *et al.,* 1989; White and Lambkin, 1990; Sartori *et al.*, 1991; Zeng, 1999; Rajendran, 1999; Benhalima *et al.,* 2004). Among the most important species of grain insects in China are the maize weevil, *Sitophilus zeamais* (Motsch.), the rice weevil, *Sitophilus oryzae* (L.), and the lesser grain borer, *Rhyzopertha dominica* (F.). Phosphine resistance has not been detected in *S. zeamais* but is common in the other two species (Zeng, 1999). Many studies have investigated the effects of phosphine concentration (*C*) and exposure time (*T*) on phosphine toxicity against grain insects. Within certain ranges of time and concentration the equation, $C^nT = K$, has been shown to describe the effects of time and concentration on phosphine toxicity against adults of the beetles *Tribolium castaneum* (Herbst), *S. oryzae*, *R. dominica* and *Acanthoscelides obtectus* (Say) (Winks, 1984; Daglish *et al.,* 2002; Daglish, 2004; Hasan and Reichmuth; 2004). In addition, this equation has been shown to be useful for eggs of the psocid *Liposcelis bostrychophila* Badonnel, and mixed-age cultures of the beetles *S. oryzae* and *R. dominica* (Ho and Winks, 1995; Daglish *et al.*, 2002; Collins *et al.,* 2005). In this equation n is the toxicity index and K is a constant. Time is more important than concentration if $n < 1$ and vice versa. The study reported here was undertaken to characterise the response to phosphine of adults of representative strains of *S. zeamais*, *S. oryzae* and *R. dominica* from China, and to compare these results with published results on strains from other countries.

MATERIALS AND METHODS

Insects

Test strains of *Sitophilus zeamais* Motschulsky, *S. oryzae* (L.) and *Rhyzopertha dominica* F. were investigated. Each strain had been classified previously as susceptible or resistant using the FAO method (Anon., 1975). There was one susceptible strain of *S. zeamais* (ZZSz) originally collected from Henan in China. There was a resistant strain of *S. oryzae* (QSO335) originally collected from Queensland in Australia, and another (GDSo) was originally collected from Guangdong in China. There was one resistant strain of *R. dominica* (YCRd) originally collected from Guangdong, China. Except for *S. oryzae* strain QSO335, none of the strains underwent any laboratory selection before being tested. All strains were reared on wheat (14% moisture content) at 25 °, 75% r.h. in a controlled temperature and humidity cabinet. Adults were used for experiments approximately 7 wk after they were laid as eggs**.**

Fumigation

Phosphine was generated according to the general approach of the FAO method (Anon. 1975), except that phosphine was generated by placing a zinc phosphide in a sulphuric acid solution. The concentration of this source was determined by molybdenum-blue colorimetry. Glass desiccators (6 L) were used as fumigation chambers. Phosphine was injected into individual desiccators using airtight syringes, and a magnetic agitator was used for 15 min to ensure even distribution of the phosphine.

The 600 adults were picked out at random from each strain and divided into 12 groups of 50 adults. Each group was enclosed in a cage made from a length of glass pipe (120 mm long and 12 mm in diameter) which was sealed on both ends with porous cloth to allow gaseous exchange but prevent the escape of insects. Broken wheat (10-20 g) was added to each cage. Two cages were placed into each desiccator and there was one desiccator for each of five test concentrations and one control desiccator.

Insects were fumigated for 20, 48, 72, 96,120 and 144 h, and there were five concentrations per exposure period, increasing in geometric proportion. The control insects were kept under same conditions in desiccators without phosphine. After exposure the cages were removed and the insects were transferred to fresh wheat for 2 wk at 25^{o} and 75% r.h., after which the numbers of dead and living insects were recorded.

Data analysis

The mortality data were analysed using probit analysis (Finney, 1971). The equation $C^nT = K$, was then derived from the resulting equations, where C is concentration (mg/L), T is time (h), n is the toxicity index, and K is a constant. Resistance factors were calculated by dividing the LC_{50} of the relevant resistant strain by the LC_{50} of a susceptible strain. The FAO method (Anon., 1975) lists LC_{50} values for susceptible *S. zeamais* (0.007 mg/L), *S. oryzae* (0.011 mg/L) and *R. dominica* (0.008 mg/L) exposed for 20 h. Results of probit analyses are available also for susceptible strains of *S. oryzae* and *R. dominica* exposed for 20, 48, 72 and 144 h (Daglish *et al.*, 2002; Daglish, 2004). The LC_{50} values used for these exposure times were 0.0088, 0.0031, 0.0020 and 0.0011 mg/L for *S. oryzae*, and 0.0052, 0.0017, 0.0011 and 0.00064 mg/L for *R. dominica*.

RESULTS

Sitophilus zeamais

The LC_{50} and $LC_{99.9}$ values for the *S. zeamais* strain ZZSz tended to decrease with increasing exposure period (Table 1). The resistance factor for 20 h exposure was estimated to be 1.1 based on the LC_{50} given in the FAO method (Anon., 1975) confirming its susceptible status. The effects of time and concentration were quantified for exposure periods of 20-144 h (Table 2). The toxicity index, *n*, was approximately equal to 1.0 showing that time and concentration made approximately equal contributions to toxicity in this range of exposure times.

Sitophilus oryzae

The LC_{50} and $LC_{99.9}$ values for the *S. oryzae* strains QSO335 and GDSo tended to decrease with increasing exposure period (Table 1). The results for the Chinese resistant strain GDSo exposed for 20 h must be interpreted with care, because the $LC_{99.9}$ value (250 mg/L) greatly exceeded the highest concentration tested (8.3 mg/L). The resistance factor for 20 h exposure was estimated to be 3.7 for the Australian resistant strain (QSO335) and 574.0 for the Chinese resistant strain (GDSo) based on the LC_{50} given in the FAO method (Anon., 1975). The Chinese strain was more resistant than the Australian strain at all exposures. At the LC_{50} level, for example, the Chinese strain was 10-154 times more resistant, depending on the exposure period. The effects of time and concentration were quantified for strain QSO335 for exposure periods of 20-144 h, and for strain GDSo for exposure periods of 48-144 h (Table 2). In both strains the toxicity index, *n*, was <1.0 showing that time made a greater contribution to toxicity in these ranges of exposure times.

Rhyzopertha dominica

The LC_{50} and $LC_{99.9}$ values for the *R. dominica* strain YCRd tended to decrease with increasing exposure period (Table 1), but the results for 20 h exposure must be interpreted with care, because the $LC_{99.9}$ value (180 mg/L) greatly exceeded the highest concentration tested (11.1 mg/L). The resistance factor for 20 h exposure was estimated to be 416.3. The effects of time and concentration were quantified for exposure periods of 48-144 h (Table 2). The toxicity index, *n*, was <1.0 showing that time made a greater contribution to toxicity than concentration in this range of exposure times.

TABLE 1
Probit analysis of results of exposure of adults of *Sitophilus zeamais*, *S. oryzae* and *Rhyzopertha dominica* to phosphine for fixed periods of time at 25ºC.

Time (h)	_^{2}	Slope (± SE)	LC_{50} (mg/L) (95% fiducial limits)	$LC_{99.9}$ (mg/L) (95% fiducial limits)
S. zeamais (strain ZZSz)				
20	1.7	4.66 (± 0.61)	0.0077 (0.0072 - 0.0085)	0.036 (0.025 - 0.065)
48	11.2	4.82 (± 0.34)	0.0043 (0.0041 - 0.0046)	0.019 (0.016 - 0.024)
72	7.1	4.99 (± 0.08)	0.0026 (0.0022 - 0.0030)	0.011 (0.0082 - 0.0178)
96	10.9	5.52 (± 0.47)	0.0020 (0.0019 - 0.0021)	0.0073 (0.0061 - 0.0093)
120	45.2	8.12 (± 1.59)	0.0010 (0.0009 - 0.0012)	0.0025 (0.0019 - 0.0049)
144	23.6	5.80 (± 1.26)	0.0016 (0.0014-0.0020)	0.0053 (0.0032 - 0.0268)
S. oryzae (strain QSO335)				
20	39.0	5.87 (± 0.84)	0.041 (0.037 - 0.046)	0.14 (0.10 - 0.25)
48	28.7	5.70 (± 1.07)	0.023 (0.018-0.027)	0.081 (0.057 – 0.20)
72	13.6	6.35 (± 0.45)	0.0096 (0.0091 - 0.010)	0.029 (0.025 - 0.036)
96	0.3	5.09 (± 0.52)	0.010 (0.0095 - 0.011)	0.041 (0.032 - 0.059)
120	25.3	5.69 (± 1.69)	0.0033 (0.0028 - 0.0056)	0.0116 (0.0063-1.1)
144	4.5	6.40 (± 0.71)	0.0035 (0.0033-0.0038)	0.011 (0.0061 – 0.89)
S. oryzae (strain GDSo)				
20	17.7	1.92 (± 0.22)	6.3 (5.4-7.9)	250 (120-870)
48	26.1	2.64 (± 0.20)	0.28 (0.26 - 0.31)	4.2 (2.8 - 7.1)
72	19.3	4.45 (± 0.34)	0.13 (0.13-0.14)	0.67 (0.54-0.88)
96	15.2	5.39 (± 0.68)	0.097 (0.092 - 0.10)	0.36 (0.31 - 0.45)
120	18.6	4.79 (± 0.59)	0.081 (0.072 - 0.090)	0.36 (0.27 - 0.61)
144	16.3	4.20 (± 0.37)	0.063 (0.059 - 0.067)	0.34 (0.27 - 0.49)
R. dominica (strain YDRd)				
20	6.0	1.77 (± 0.32)	3.3 (2.6 - 4.0)	180 (69 – 1400)
48	0.5	4.40 (± 0.72)	0.55 (0.42 - 0.64)	2.8 (2.1 - 4.7)
72	3.7	2.31 (± 0.29)	0.091 (0.072 - 0.11)	2.0 (1.2 - 4.6)
96	8.8	2.81 (± 0.29)	0.081 (0.070 - 0.092)	0.85 (0.56 - 1.6)
120	16.4	3.56 (± 0.40)	0.067 (0.059 - 0.075)	0.60 (0.41 - 1.13)
144	13.8	2.74 (± 0.26)	0.051 (0.045 - 0.056)	0.68 (0.48 - 1.1)

Resistance factor from LC_{50}

The resistance factor for the Chinese strains of *S. oryzae* (GDSo) and *R. dominica* (YCRd) decreased as exposure period increased (Table 3). In the present study, the resistance factor for *S. oryzae* strain QSO335 was not affected by exposure period of 20-144 h.

TABLE 2
Equations describing relationship between phosphine concentration (C) and exposure period (t) on mortality of adults of *Sitophilus zeamais*, *S. oryzae* and *Rhyzopertha dominica* to phosphine at 25ºC.

Species	Strain	Equation	r^2
S. zeamais	ZZSz[1]	$LC_{50} = 0.1351T^{-0.9325}$ (or $C^{1.0724}T = 0.1169$)	0.883, P < 0.01
		$LC_{99.9} = 1.0117T^{-1.0836}$ (or $C^{0.9228}T = 1.0108$)	0.747, P < 0.05
S. oryzae	QSO335[1]	$LC_{50} = 2.6237T^{-1.3106}$ (or $C^{0.7630}T = 2.0875$)	0.898, P < 0.01
		$LC_{99.9} = 8.8192T^{-1.3041}$ (or $C^{0.7668}T = 5.3083$)	0.863, P < 0.01
S. oryzae	GDSo[2]	$LC_{50} = 39.612T^{-1.3049}$ (or $C^{0.7663}T= 16.765$)	0.972, P < 0.01
		$LC_{99.9} = 16083T^{-2.2482}$ (or $C^{0.4448}T = 74.301$)	0.827, P < 0.05
R. dominica	YDRd[2]	$LC_{50} = 798.84T^{-1.9850}$ (or $C^{0.5038}T= 28.991$)	0.835, P < 0.05
		$LC_{99.9} = 1012.4T^{-1.5104}$ (or $C^{0.6621}T= 97.689$)	0.909, P < 0.01

[1]20-144 h exposure, [2]48-144 h exposure.

DISCUSSION

This study investigated the effects of time and concentration on toxicity of phosphine against adults of representative strains of *S. zeamais*, *S. oryzae* and *R. dominica* from China. The *S. zeamais* strain was susceptible, reflecting the fact that phosphine resistance has never been detected in this species in China. In China, resistance is common in *S. oryzae* and *R. dominica* populations, and the strains investigated are believed to among the most resistant found. A resistant strain of *S. oryzae* from Australia was used as a reference strain. In general, the data from each exposure time fitted the probit model. However, for the Chinese strains of *S. oryzae* and *R. dominica* exposed for 20 h, the $LC_{99.9}$ was much higher than the highest test concentration. Although other similar instances have been reported, they have also been limited to short exposure times, and are unlikely to be of any practical significance (Winks, 1984; Winks and Waterford, 1986; Daglish et al., 2002; Hasan and Reichmuth, 2004).

In all strains, the LC_{50} and $LC_{99.9}$ values decreased as exposure period increased from 20 h through to 144 h. In contrast, Daglish (2004) found that the resistance factors for less resistant strains of *S. oryzae* (QSO335) and *R. dominica* (QRD14) were not affected by exposure periods of 20-144 h.

The relationships between LC_{50} and $LC_{99.9}$ with exposure time fitted the $C^nt = K$ model well. Time and concentration were approximately equally important dosage factors for the susceptible strain of *S. zeamais* ($n \cong 1$), but time was a more important dosage factor than concentration for the resistant strains of *S. oryzae* and *R. dominica* ($n < 1$). Comparing K values of the C^nt models on a strong resistant-strains with those reported by Daglish (2004) on susceptible-strains in *R. dominica* and *S. oryzae*, the K of YCRd is 138 (28.991/0.21) times more than that of QRD14, and K of GDSo is 86 (16.765/0.1949) times more than that of QSO335. These results indicated that to control the strong resistant-strains needed a concentration of phosphine, which was higher than to control the susceptible one except for prolonging the exposure period.

Eggs and pupae are often more tolerant to phosphine than adults (Hole *et al.,* 1976), so further research is needed to determine the practical significance of the resistance in the Chinese strains of *S. oryzae* and *R. dominica*. It is possible, however, to draw some tentative conclusions by comparing the results with published data on other strong resistant strains. Adults of an Indian strain of *S. oryzae* had an LC_{50} of 1.7 mg/L for 20 h exposure at 27ºC (Rajendran, 1999), and this strain required 500 ppm (0.7 mg/L) for complete control of mixed-age cultures exposed for 168 h at 27ºC. The Chinese strain tested in the current study had an LC_{50} of 6.3 mg/L for 20 h at 25ºC. This suggests that mixed-age cultures of the Chinese strain will be harder to control than the Indian strain. Rajendran *et al.* (2001) reported an Indian strain of *R. dominica* with an LC_{50} of 1.8 mg/L for adults exposed for 48 h at 27ºC, and Collins *et al.* (2002) reported an Australian strain with an LC_{50} of 1.0 mg/L for adults exposed for 48 h at 25ºC. Rajendran *et al.* (2002) found that the Indian strain required 1000 ppm (1.4 mg/L) for complete control of mixed-age cultures exposed for 168 h at 27ºC, and Collins *et al.* (2005) found that the Australian strain had an $LT_{99.9}$ of 170 h for mixed-age cultures fumigated at 0.3 mg/L at 25ºC. The Chinese strain was broadly similar to these strains with an LC_{50} of 0.55 mg/L for adults exposed for 48 h at 25ºC. It is likely, therefore, that relatively high concentrations will be needed to control of mixed-age cultures of the Chinese strain.

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TABLE 3
Estimated resistance factors for *Sitophilus zeamais*, *S. oryzae* and *Rhyzopertha dominica* exposed to phosphine for fixed periods of time at 25ºC.

Species	Strain	Source	Estimate	Exposure time (h)			
				20	48	72	144
S. zeamais	ZZSz	China	A[1]	1.1	-	-	-
S. oryzae	QSO335	Australia	A[1]	3.7	-	-	-
			B[2]	4.7	7.4	4.8	3.2
	GDSo	China	A[1]	572.7	-	-	-
			B[2]	715.9	90.3	65.0	57.3
R. dominica	YDRd	China	A[1]	412.5	-	-	-
			B[3]	634.6	323.5	82.7	79.7

[1]Susceptible LC_{50} value from Anon. (1975), [2]Susceptible LC_{50} values from Daglish *et al.* (2002), [3]Susceptible LC_{50} values from Daglish (2004).

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