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Full Length Research Paper

Phosphine resistance in *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) from different geographical populations in China

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This study was carried out to determine the level of phosphine resistance in 16 *Rhyzopertha dominica* (Fabricius) populations that were collected from ten provinces and one municipality in China following the Food and Agriculture Organization's (FAO) standard method. Results showed that the 50% lethal concentration (LC_{50}) of phosphine to these *R. dominica* populations ranged from 0.017 to 4.272 mg/L. Of the 16 populations, 5 were of low resistance, 6 were moderately resistant, and 5 were high resistant. The instantaneous rate of population increase (r_i) was correlated with phosphine resistance followed by the Exponential model ($y = 0.037e^{-0.005X}$, $R^2 = 0.937$). The relationship between the types of grain storage and the phosphine resistance of *R. dominica* population were also discussed.

Key words: Phosphine resistance, instantaneous rate of population increase, *Rhyzopertha dominica*, China.

INTRODUCTION

The lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae), is a widely studied stored-product insects originating from tropical areas, but actually distributed all over the world. Both larvae and adults are internal feeders, causing serious damages to grains, such as rice, maize, wheat and other stored commodities. The insect can develop and maintain a high rate of oviposition in dry grains, and can even survive in grains with as low as 8% moisture content (Birch, 1945a, b).

Existing projections indicate that future population and economic growth will require a doubling of current food production, including an increase from 2 billion to 4 billion tons of grains annually (Tubiello et al., 2007). However, the activities of insects and mites could frustrate these efforts. Arthropod pests are responsible for deterioration of stored food and can cause yearly losses estimated at about 30% of 1800 million tons of stored grain (Haubruge et al., 1997). In China, 60% grain yield is usually stored for further use, and as part of food security grain storage; during which about 6-12% grain loss as a result of pest damage is recorded in rural areas (Lan, 2006).

The main method for controlling stored-product insects in many countries, including China, is through fumigation with phosphine and methyl bromide. Use of methyl bromide is associated with depletion of the ozone layer and bromine residues in the soil (WMO, 1995; Ristaino and Thomas, 1998). Therefore, there is increased reliance on phosphine to control stored-product insects (Lorini et al., 2007). Phosphine gas has been used worldwide for more than half a century as a useful fumigant for the control of stored-product insects (Price and Mills, 1988; Chaudhry, 2000), and for the protection of stored grains and other commodities from pest attack.

However, widespread resistance to phosphine has emerged in several species of stored-product insects in many countries in which phosphine control failure has been detected (Champ and Dyte, 1977; Chaudhry, 2000; Collins et al., 2005; Lorini and Collins, 2006; Pimentel et al., 2007, 2010; Savoldelli and Süss, 2008; Collins, 2009). Several strong phosphine-resistant stored-product insects have been found in Morocco (Benhalima et al., 2004), Brazil (Ansell et al., 1990; Mills and Athie, 2001; Athie and Mills, 2005), Vietnam (Bui, 1999), China (Yan

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Table 1. Origin of Chinese populations of *R. dominica*.

Population	City	Province	Grain category	Storage facility	Time
ZC	Zhucheng	Shandong	Wheat	Wheat processing industry	September, 2007
XC	Xuchang	He'nan	Wheat	Local storage	August, 2007
HD	Handan	Hebei	Wheat	National storage	September, 2008
XN	Changsha	Hu'nan	Rice	Rice processing industry	August, 2008
ZG	Hefei	Anhui	Rice	Rice processing industry	August, 2008
GA	Gaoan	Jiangxi	Rice	Local storage	August, 2008
HP	Wuhan	Hubei	Rice	National storage	July, 2008
AL	Anlu	Hubei	Rice	National storage	September, 2007
SY	Shayang	Hubei	Rice	National storage	May, 2007
XF	Xiangfan	Hubei	Rice	National storage	May, 2007
BN	Chongqing	Chongqing	Rice	Rice processing industry	September, 2007
BB	Chongqing	Chongqing	Rice	National storage	September, 2007
LZ	Luzhou	Sichuan	Rice	National storage	October, 2008
GY	Guiyang	Guizhou	Rice	National storage	August, 2008
YC	Yangchun	Guangdong	Rice	Local storage	May, 2007
JM	jiangmen	Guangdong	Rice	Local storage	August, 2007

et al., 2004; Cao, 2006), Australia (Collins, 1998; Bengston et al, 1999; Valmas and Ebert, 2006) and Pakistan (Alam et al., 1999; Ahmedani et al., 2006, 2007).

Resistance to phosphine has been reported in several economical important insect species, including *R. dominica* (F.), *Sitophilus oryzae* (Linnaeus), *Sitophilus zeamais* (Motschulsky), *Tribolium castaneum* (Herbst) *Trogoderma granarium* Evert, *Oryzaephilus surinamensis* (L.), *Cryptolestes ferrugineus* (Stephens), *Liposcelis bostrychophila* (Badonnel), *L. entomophila* (Enderlein) and *L. decolor* (Pearman) (Champ and Dyte, 1977; Borah and Chahal, 1979; Benhalima, 1988; Leong and Ho, 1994; Lorini and Galley, 1999; Nayak et al., 2002, 2003; Cao et al., 2003; Athie and Mills, 2005; Pimentel et al., 2010; Nayak and Collins, 2008; Collins, 2009).

Resistance in insects is often linked (among other factors) to various fitness traits, such as intrinsic rates of increase (White and Bell, 1990), the change of rate of population growth (Fragoso et al., 2005; Haubruge and Arnaud, 2001), fecundity and male reproductive competition (Arnaud and Haubruge, 2002). Fragoso et al. (2005) detected that insect population that are resistant to pyrethroid showed a reduced rate of population growth compared with other populations. However, Haubruge and Arnaud (2001) suggested that resistant population did not always involve decreased fitness. Therefore, the growth rate of insect populations was fundamental to insecticide resistance management.

The aim of this study was to detect the phosphine-resistance status of *R. dominica* populations in China. Understanding the level of resistance to phosphine and assessing the instantaneous rate of population increase (r_i) would be very useful toward the development of an appropriate strategy for resistance management of this pest.

MATERIALS AND METHODS

16 populations of *R. dominica* were collected from ten provinces and one municipality in China between May, 2007 and October, 2008 (Table 1). Geographical distribution of *R. dominica* populations sampled in China includes: Shandong, He'nan, Hebei, Hubei, Guangdong, Sichuan, Jiangxi, Anhui, Guizhou, Hu'nan provinces and Chongqing municipality. The cities from which samples were obtained are as follows; Zhucheng (ZC), Xuchang (XC), Handan (HD), Changsha (XN), Hefei (ZG), Gaoan (GA), Wuhan (HP), Anlu (AL), Shayang (SY), Xiangfan (XF), Chongqing (BN), Chongqing (BB), Luzhou (LZ), Guiyang (GY), Yangchun (YC) and Jiangmen (JM). The sparseness of these locations with their latitude and longitude coordinates, and the type of grain storage facilities are shown in Figure 1 and Table 1, respectively. BB, LZ, HD, GY, HP, AL, XF and SY populations were collected from national storage facilities. ZC population came from wheat processing industry. BN, XN and ZG populations were collected from rice processing industry, while XC, YC, JM and GA populations were from local storage.

The 16 populations and standard susceptible population of *R. dominica* studied were then reared on cracked wheat grains (13 ± 1% moisture content) free of insecticide residues. The temperature in the rearing chamber was maintained at 30 ± 1°C and 75 ± 5% relative humidity. Non-sexed adults of *R. dominica* (14 to 21-day old) were used in phosphine resistance assays.

Phosphine bioassays

Fumigation of adult *R. dominica* was based on the FAO standard method (FAO, 1975) and it took place in a controlled temperature and relative humidity chamber (25°C, 70% rh). Phosphine was obtained by a reaction of Zinc phosphide (Ji'ning City Yimin Chemical Plant, China) in acidified water (10% sulfuric acid). The concentration of the phosphine was always tested before the bioassays. Depending on the mortality at these concentrations, higher or lower concentration (at least 5 to 8 concentration) was

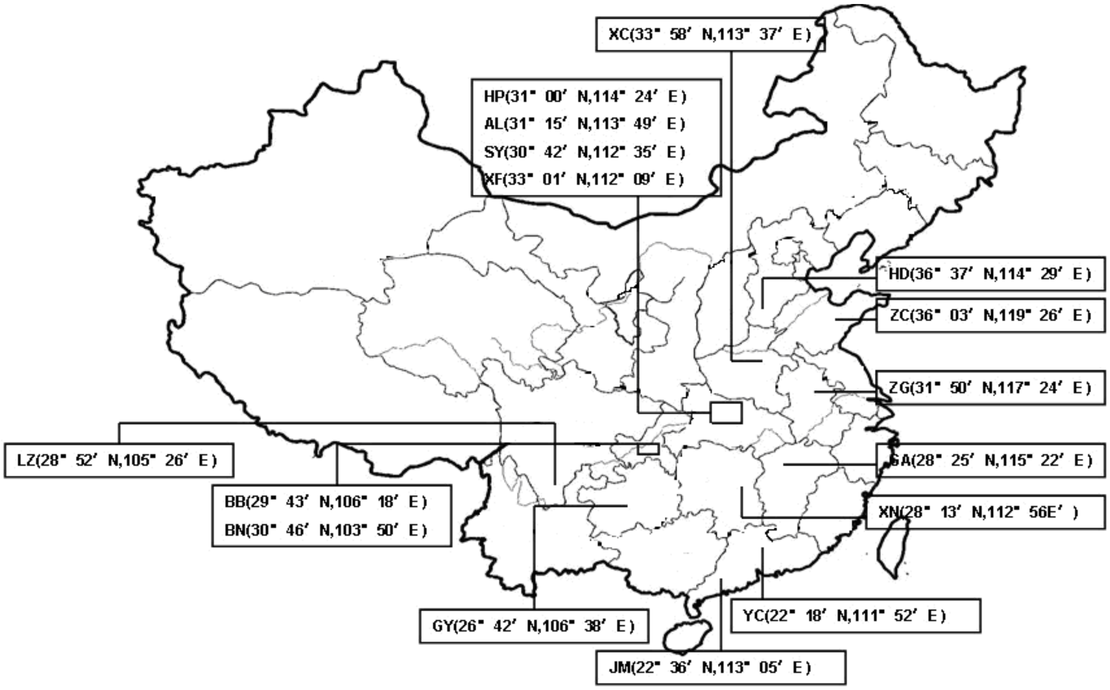

Figure 1. Distribution of *R. dominica* collection sites across China.

used to estimate the concentration-mortality curves after preliminary tests. Test adults were held in a perforated metallic tube closed with rubber stoppers and secured with adhesive tape inside modified gas desiccators. Gas was injected with micro-syringes through a septum in the lid of each desiccator. After 20 h of fumigation, test insects were transferred into glass tubes with cracked wheat, and were kept for 14 days (25°C, 70% rh). The mortality was then recorded. Each treatment was repeated three times.

Instantaneous rate of population growth (r_i)

The instantaneous rate of increase (r_i) test was carried out in 250 ml glass conical flask with 40 g cracked wheat. Each conical flask was infested with 20 non-sexed *R. dominica* adults (14 to 21- day old) and maintained at 30 ± 1°C, 75 ± 5% rh. Then, the conical flask was covered with a ventilated cloth. The number of surviving adults was counted after 60 days, and the instantaneous rate of increase in each population was calculated as follows:

$$r_i = \ln (N_f / N_0) / \Delta t$$

Where, N_f is the final number of observed live adults; N_0 is the initial number of *R. dominica* and Δt is the duration of the experiment (Walthall and Stark, 1997; Pimentel et al., 2007).

Three replicates were used for each population in this test.

Data analysis

The concentration-mortality data were analyzed using probit program software, where probit-transformed mortality was regressed against Log_{10}-transformed dose (SPSS16.0). The instantaneous rate of population increase (r_i) were subjected to analysis of variance (P<0.05) followed by Tukey's multiple range test (SPSS 16.0). The instantaneous rate of population increase (r_i) curve was subjected to regression analysis (SPSS 16.0).

RESULTS

Phosphine resistance of *R. dominica*

According to the FAO (1975) standard, the LC_{50} value of susceptible *R. dominica* strain was lower than 0.013 mg/L. All the 16 populations collected from ten provinces and one municipality were identified as resistant strains because their LC_{50} values exceeded 0.013 mg/L. The 16 populations of *R. dominica* however, had different sensitivities to the phosphine gas. The mortality expressed based on the LC_{50} values, ranged between 0.017 mg/L (ZC) and 4.272 mg/L (XC) (Table 2).

The authors tested logit and complementary log-log in addition to probits without transformation, and found that the logit model was most suitable. Mortalities predicted by the logit model did not differ significantly (P > 0.05) from the bioassay observed values (Table 2). The logit model was therefore appropriate to be used for concentration-mortality analysis. The concentration-mortality curves indicated that 5 populations of *R. dominica* (ZC, GY, XN, BN and ZG) exhibited low resistant to phosphine (RF value < 10-fold), while 6 populations (LZ, BB, HP, HD, JM and XF) were mo-derately resistant (10-fold < RF value < 100-fold). AL, SY, YC GA and XC populations showed high resistance (>100-fold) (Table 2).

Table 2. Relative toxicity to phosphine of sixteen populations of *R. dominica.*

Population	Slope ± SE[a]	LC_{50} (95% CL) (mg/L)	LC_{95} (95% CL) (mg/L)	X^2	P	RF[b]
SCS[c]	2.518±0.222	0.007(0.006-0.009)	0.033(0.022-0.064)	9.687	0.085	-----
ZC	1.893±0.165	0.017(0.012-0.023)	0.124(0.076-0.293)	9.837	0.080	2.429
GY	1.464±0.110	0.030(0.020-0.042)	0.395(0.229-0.935)	9.024	0.108	4.286
XN	1.090±0.129	0.033(0.021-0.048)	1.068(0.584-2.661)	0.965	0.810	4.714
BN	2.573±0.202	0.037(0.029-0.047)	0.162(0.109-0.321)	7.154	0.128	5.286
ZG	1.020±0.091	0.066(0.046-0.093)	2.723(1.487-6.203)	6.291	0.178	9.429
LZ	0.975±0.129	0.120(0.059-0.228)	5.870(1.671-131.673)	8.658	0.124	17.143
BB	1.044±0.085	0.155(0.107-0.215)	5.816(3.583-10.901)	5.701	0.223	22.143
HP	0.932±0.108	0.282(0.165-0.419)	16.414(8.944-40.849)	4.750	0.314	40.286
HD	1.653±0.182	0.302(0.137-0.518)	2.992(1.359-25.555)	6.344	0.096	43.143
JM	1.536±0.112	0.327(0.269-0.397)	3.850(2.703-6.071)	6.198	0.287	46.714
XF	1.551±0.239	0.338(0.208-0.467)	3.882(2.459-8.489)	5.091	0.165	48.286
AL	1.169±0.100	0.903(0.606-1.389)	23.040(10.06-94.899)	13.620	0.058	129.000
SY	1.397±0.149	0.913(0.757-1.125)	13.744(8.030-30.818)	3.530	0.473	130.429
YC	1.453±0.282	1.349(0.859-1.993)	18.293(8.697-87.505)	5.523	0.238	192.714
GA	2.276±0.208	1.826(1.565-2.114)	9.640(7.340-14.039)	2.894	0.408	260.857
XC	6.650±0.897	4.272 (3.876-4.595)	7.550(6.795-8.918)	3.382	0.336	610.286

[a]SEM = Standard error of mean, [b] RF = resistance factor (LC_{50} of resistant population/ LC_{50} of sensitive population, [c] = standard susceptible population.

Majority of the slopes of the concentration-mortality curves were similar among the populations of *R. dominica*, except for BN, GA and XC populations. Among these three populations, BN population showed low-resistance, while GA and XC populations had high-resistance. These results indicate that the resistance of *R. dominica* populations was not associated with the slopes of the concentration-mortality curves. Furthermore, response curves from all populations except XC had low slope values, indicating that there was high heterogeneity in these populations.

The populations were ranked in order of RF values; the populations from processing facilities cluster near the top, then the national storage populations and the populations from local storage. The average RF for processing facileties, national storages and local storage were 5.465, 61. 490 and 277.643, respectively. This result indicates that resistance is connected with the type of grain storage.

Instantaneous rate of population increase (r_i)

The instantaneous rate of population increase (r_i) was used to assess the fitness disadvantage associated with phosphine in the absence of the fumigant. There was significant variation in r_i among populations of *R. dominica* ($F_{15, 32}$ = 64.423, P < 0.001). The r_i curve was fitted using exponential regression curve ($y = 0.037e^{-0.005X}$, R^2 = 0.937; $F_{1, 14}$ = 209.488, P < 0.001).

R. dominica populations with high r_i showed low resistance factor (RF for LC_{50}), while low r_i presented high RF value (Figure 2). This indicated that the existence of fitness costs (r_i) was connected with the resistance of phosphine in *R. dominica* population.

DISCUSSION

A global survey undertaken by the FAO in 1972/1973 showed that about 10% of investigated populations contained phosphine resistant insects, including a *R. dominica* population collected from Keelung of Taipei, China (Champ and Dyte, 1976). The earliest phosphine resistance in Chinese populations of *R. dominica* was reported by the Guangdong Institute of Cereal Science Research in 1976 (Zeng, 1996). In this study, all 16 populations of *R. dominica* were identified as phosphine resistant strains. Of the 16 populations, 31.25% were low resistant, 37.5% were moderately resistant, while 31.25% were highly resistant. XC population showed the highest resistance to phosphine, and the RF value was 610.286. The results therefore reveal the seriousness of the problems of phosphine resistance in China.

Response curves with low slopes are correlated with high levels of heterogeneity, suggesting a high variation in response to phosphine in treated populations (Lorini et al., 2007). The low slope of the response curves for all but the XC population indicated that there was high heterogeneity in the tested populations and that there is high variation in response to phosphine in these samples. Some populations of *R. dominica* (HP, AL, SY and YC) showed high LC_{95} value (Table 2), revealing that these

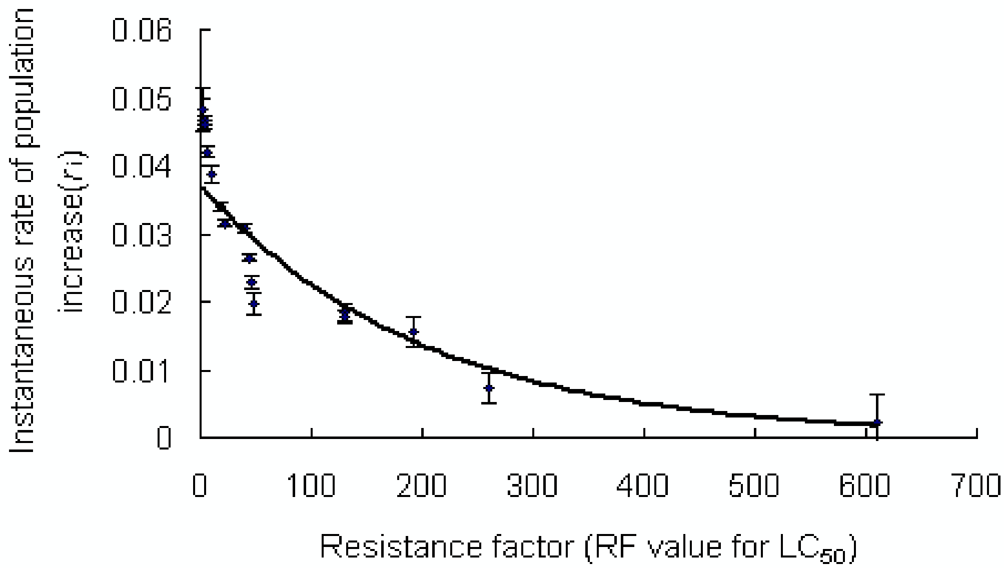

Figure 2. The instantaneous rate of population increase (r_i) for *R. domininca* as a function of resistance factor (RF value for LC_{50}). Curve was fitted using Exponential regression curve ($y = 0.037e^{-0.005X}$, $R^2 = 0.937$; $F_{1,14} = 209.488$, $P<0.001$).

populations have large numbers of highly resistant individuals, thus, suggesting that they have been under high selection pressure for many years (Benhalima et al., 2004).

Some factors have been known to contribute to the phosphine resistance in stored-product insects. Repeated ineffective fumigation in poorly sealed structures resulting in under-dosing could lead to the development of strong resistance in target pest (Benhalima, 1988; Pacheco et al., 1990; Zeng, 1999; Benhalima et al., 2004). The lack of phosphine concentration monitoring during fumigation may also cause under-dosing and consequent survival of insect pests (Mills, 2001). Finally, long-term use of a single fumigant would also result in selective resistance to phosphine in stored-product insects (Zeng, 1999; Benhalima et al., 2004). Meanwhile, another likely factor contributing to the resistance development is the exchange of insects via the trade in commodities (Champ and Dyte,1976; Benhalima et al., 2004; Ahmedani et al., 2007).

This study also detected that the phosphine resistance of *R. dominica* was associated with the types of grain storage. In china, national and local storage facilities are mainly used for the preservation of national grain and food surplus. The construction pattern in national storage facilities is better than those in local storages, thereby enabling more effective fumigation in the former. During fumigation, the concentration of the fumigant is an important factor. For example, if the minimum effective fumigation dosage is 0.28 g/m^3, then 2.0 to 3.5 g/m^3 dosage of aluminum phosphide would be required in the national storage, while in local storage, 6.0 to 8.0 g/m^3 dosage of aluminum phosphide was required (Song et al., unpublished). Due to the difference in gas tightness in the storage facilities, fumigation dosage and frequency were significantly different in national storage and local storage. The Chinese State Administration of Grain sets a standard of 100 ml/m^3 (about 0.14 g/m^3) as minimum concentration of phosphine for 18 days fumigations (Wang et al., 2002). However, the national storage facility used 200 ml/m^3 (about 0.28 g/m^3) of phosphine for 28 days once a year; and in local storage, 300 to 500 ml/m^3 (about 0.42 to 0.60 g/m^3) effective phosphine concentration for fumigation were applied for 28 days twice or thrice annually. In the processing facilities, phosphine is rarely used (Song et al., unpublished). Therefore, the difference in fumigation frequency and dosage of fumigant is a primary reason for the different phosphine resistance detected in the two types of storage facilities.

The instantaneous rate of growth represents the fraction by which a population would grow during a very short period of time. The results in this study showed that the high phosphine resistant population had lower rate of population increase than that of the low resistant populations, and this was consistent with the investigation of Fragoso et al. (2005), Longstaff (1991) and Pimentel et al. (2007). This finding could be useful in the management of phosphine resistance in *R. dominica*.

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