*Donahaye, E.J., Navarro, S., Bell, C., Jayas, D., Noyes, R., Phillips, T.W. [Eds.] (2007) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, Gold-Coast Australia **8-13th August 2004**. FTIC Ltd. Publishing, Israel. pp. 267-273*

DEVELOPING FUMIGATION PROTOCOLS TO MANAGE STRONGLY PHOSPHINE-RESISTANT RICE WEEVILS, *SITOPHILUS ORYZAE* (L.).

MANOJ K. NAYAK, PATRICK J. COLLINS AND HERVOIKA PAVIC

Queensland Department of Primary Industries and Forestry, 80 Meiers Road, Indooroopilly, QLD 4068, Australia,
E-mail: manoj.nayak@dpi.qld.gov.au

ABSTRACT

FAO tests indicated that a strain of rice weevil, *Sitophilus oryzae*, from Santai, China has resistance to phosphine at levels stronger than previously documented in this species. These insects were genetically purified by mass selection with phosphine. Response of adults and mixed-age populations of this strongly resistant Chinese strain was compared with Australian susceptible and resistant strains. Resistance was characterised by exposing adults to phosphine in desiccators and cultures containing all life stages to constant concentrations of phosphine using a flow-through apparatus. Compared with the response of the susceptible strain at exposure periods of 48, 72 and 144 h, the resistance factors based on $LC_{99.9}$ values were calculated as x11, x10 and x11 for the Australian weak resistant strain; and x97, x122 and x169 for the Chinese resistant strain, respectively. Times to population extinction were significantly longer than known resistance already present in Australia. For example, exposure periods of 11 and 7 days are needed to completely control the Santai strain at 0.3 and 1 mg/L phosphine, respectively. This compares with 8 and 5 days at the same doses, required to control the most resistant strains of *S. oryzae* known in Australia. Times to population extinction of the Santai strain are also significantly longer than those for strongly resistant *Rhyzopertha dominica*. Control protocols developed from this research will enable Australian authorities to implement remedial action if strong resistance is detected in Australia.

INTRODUCTION

Although the rice weevil, *Sitophilus oryzae* (L) is a very serious pest of stored grain around the world, research on controlling this pest with fumigant phosphine is very limited (Hole *et al*., 1976; Price and Mills, 1988; Daglish *et al*., 2002; Daglish *et al*, 2004). Daglish *et al*., (2002) have evaluated the effects of exposure time and concentration of phosphine on mortality of a susceptible and a weak resistant strain of rice weevils from Australia. During this study, the authors have also confirmed strong resistance in a rice weevil strain from China, which was previously reported by collaborative researchers from China (Zeng, 1999). After this preliminary study, research on this strong resistant strain from China was discontinued due to quarantine restrictions. Only recently, establishment of a new quarantine facility have enabled us to resume further research on this strong resistant strain.

Phosphine fumigation is the major pest management option in Australia and it is used to disinfest up to 80% of the stored grain. There is a continuous effort to enhance the life span of phosphine in view of the unavailability of suitable alternative fumigants. Moreover, phosphine possesses several other advantages over other fumigants including its low cost; versatility in application and most importantly, its global acceptance as a residue-free treatment. Due to development of resistance to phosphine in several pest *spp*. in Australia, the industry relies on a national resistance management strategy (Collins, 1998). An important component of such a resistance management strategy is to characterise strong resistance in overseas pests. Characterising strong resistance and developing management strategies to control these overseas pests enables us to tackle strong resistance problems in future if they evolve in Australia. Therefore, it was considered important to characterise the strong resistance to phosphine in the Chinese rice weevils and to develop control protocols against them. In this paper, we compare resistance levels detected in the rice weevils from Australia and China, characterise the strong resistance in the Chinese strain and also suggest fumigation protocols to control them.

MATERIALS AND METHODS

Test insects: A susceptible strain and a weak resistant strain of the rice weevil, *Sitophilus oryzae*, from Australia were compared with a resistant strain from China. The susceptible strain (LS2) was collected from Brisbane in 1965, whereas the weak resistant strain (QSO335) was collected in 1990 from Millmerran, southeast Queensland and underwent selection to promote homozygosity (P.J. Collins, unpublished data). The Chinese resistant strain (Santai) was collected in 1998 from Sichuan province in China and has been selected with phosphine at the quarantine facility at the Food Protection Team laboratories in Brisbane. These weevils were cultured on whole wheat at 25ºC, 55% R.H. and 12:12 (L: D).

Fumigation of adults

Adults were exposed to a range of concentrations of phosphine at a range of exposure periods, but only results from 48, 72 and 144 h are presented here. Fumigation was carried out following the FAO method (Anonymous, 1975) at laboratory conditions of 25ºC and 55% R.H. A phosphine source generated from aluminium phosphide tablets was used as the source, the concentration of which was determined through gas chromatography using a gas-density balance and a nitrogen standard. Adult rice weevils (1-3 weeks after eclosion) were confined in ventilated polystyrene cups inside gas-tight desiccators. Fifty adults were taken per cup containing 2 g of wheat as one replicate and a total of 3 cups were organised per desiccator. Phosphine was injected through a septum in the lid of each desiccator to give the required concentration. The cups were removed after fumigation and retained for 2 weeks at 25 ºC and 55% R.H. when mortality was assessed.

Fumigation of mixed-age cultures

Insect cultures were specially prepared for this set of experiments so that all life stages would be exposed to phosphine. The fumigation procedure was essentially the same as described by Daglish et al., (2002), where the mixed-age cultures of insects, held in plastic containers, were placed in stainless steel chambers. Phosphine and air were allowed to flow in and out in one direction through these chambers, controlled separately by mass flow controllers. Experiments were conducted at 25ºC and 55% R.H. for a range of phosphine concentrations (0.2, 0.3, 1, 1.5 and 2 mg/L), but results from a low dose (0.2 mg/L) and a high dose (1 mg/L) are presented here. Adult weevils were removed at the end of the fumigation and mortality was assessed after 2 weeks. A second assessment was done after 8 weeks from the end of fumigation to allow time for eggs, larvae and pupae to emerge to adults (Daglish et al, 2002). 'Time to population extinction' was defined as the exposure period (in days) at which 100% mortality of adults and no live progeny were achieved. Containers without adult progeny were re-checked after another 8 weeks to confirm the population extinction.

Data gathered from the above experiments were subjected to statistical analysis to obtain $LC_{99.9}$ (for adults) and LT_{50} and $LT_{99.9}$ values (for mixed-age populations) using GenStat 5 software (GenStat, 2000).

RESULTS AND DISCUSSION

Fumigation of adults

The $LC_{99.9}$ values recorded for all three strains of rice weevils indicate that at all exposure periods, much higher concentration of phosphine is required to achieve 99.9% mortality of adults of the Chinese strain (Santai), compared with the concentrations needed for the susceptible and weak resistant strains from Australia (Figure 1). Moreover, for all three strains, the $LC_{99.9}$ tended to decrease with increasing exposure periods (Figure 1). The current results confirm the preliminary observations made on rice weevils by Daglish et al (2002). Compared with the response of the susceptible strain LS-2 at exposure periods of 48, 72 and 144 h, the resistance factors based on the $LC_{99.9}$ values were calculated as x11, x10 and x11 for the Australian weak resistant strain (QSO335); and x97, x122 and x169 for the Chinese resistant strain (Santai), respectively. Similarly, compared with the response of QSO335, the resistant factors were x9, x12, and x16 for the Santai strain at exposure periods of 48, 72 and 144 h, respectively. The resistance factor for the Australian resistant strain (QSO335) remained constant regardless of exposure period. However, the resistant factor for the Santai strain tended to increase with increasing exposure period.

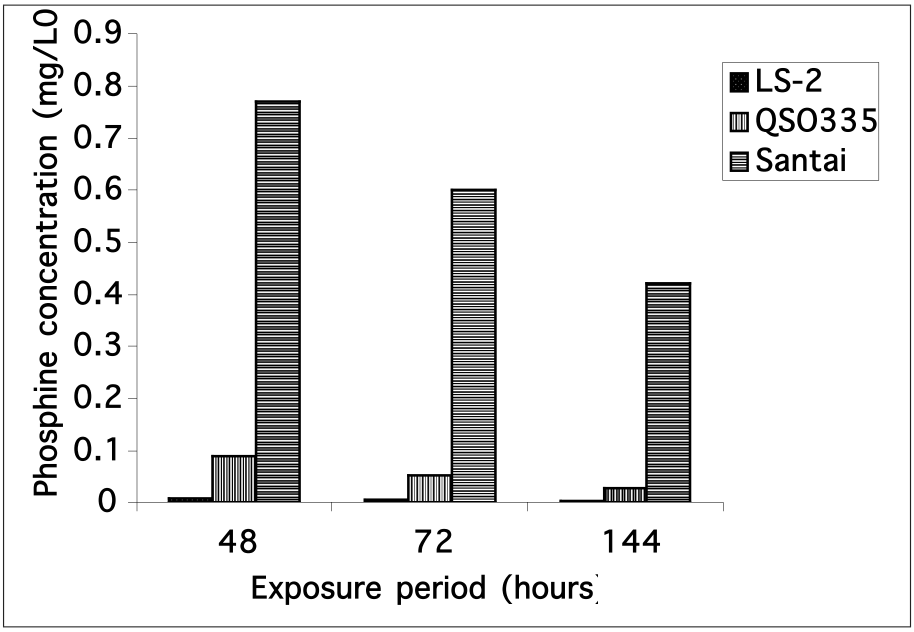

Fig. 1. $LC_{99.9}$ for adults of three strains of *S. oryzae*

Fumigation of mixed-age cultures

Both LT_{50} and $LT_{99.9}$ values obtained from this set of experiments indicate that at both lower (0.2 mg/L) and higher (1 mg/L) concentrations of phosphine, longer periods of exposure are needed to control the populations of Santai strain than that required for the susceptible and resistant populations of the Australian strains (Figures 2 and 3). Results recorded from 'time to population extinction' experiments also follow similar trend (Table 1). 'Time to population extinction' of the Santai strain was more than 6 and 3 days longer at phosphine concentrations of 0.2 mg/L and 2 mg/L, respectively, compared with the resistant Australian strain (QSO533) (Table1). Moreover, at concentrations of 0.2, 0.3, and 1 mg/L, the Santai strain had higher resistance and at 1.5 and 2 mg/L, it had equal resistance to that of the most resistant strain of lesser grain borer (*Rhyzopertha dominica*) reported in Australia (strain QRD569) (Collins et al., 2001) (Table 1). QRD569 is the most resistant strain of any grain storage insect pest in this country.

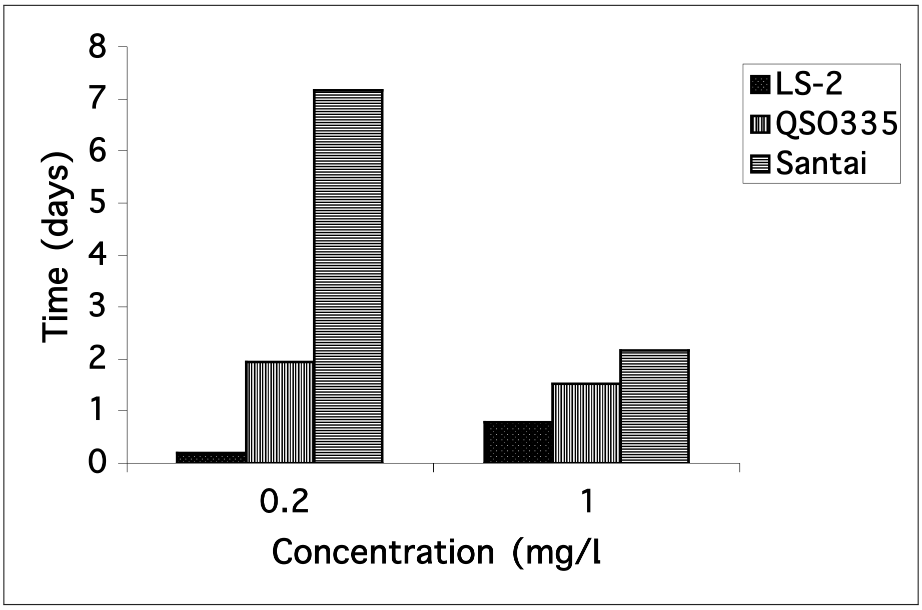

Fig. 2. LT_{50} for populations of three strains of *S. oryzae*

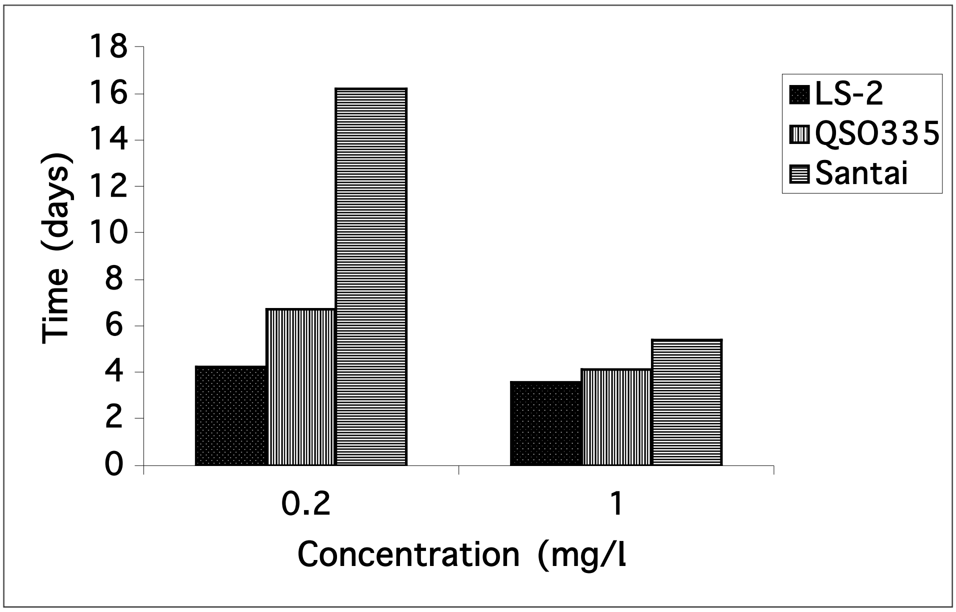

Fig. 3. $LT_{99.9}$ for populations of three strains of *S. oryzae*

TABLE 1

Time to population extinction (in days) of resistant strains of the rice weevil (*Sitophilus oryzae*) from Australia and China compared with the Australian resistant Lesser Grain Borer (*Rhyzopertha dominica*) at fixed concentrations of phosphine, 25ºC and 55% R.H.

Phosphine ppm (mg/l)	Chinese rice weevil (Santai)	Australian rice Weevil (QSO 335)	Australian lesser grain borer (QRD569)
140 (0.2)	>14	8	10
215 (0.3)	11	8	8
700 (1)	7	5	5
1000 (1.5)	6	4	6
1400 (2)	6	3	6

The current research has shown that the Australian resistant rice weevils are far easier to control compared with the strongly resistant Chinese strain. Due to the lack of suitable alternatives, phosphine will continue to be extensively used in Australia. Therefore, it is possible, that resistance in Australian rice weevils may be selected to the levels now found in Chinese strains. If this resistance develops here, it will pose a serious threat to the industry. Recent research on resistance management suggests, however, that irrespective of the development of resistance in several pests, the viability of phosphine can be sustained because it is possible to develop suitable protocols to control strong resistant insects (Collins et al., 2001). It is therefore necessary that monitoring of resistance in rice weevils should continue to alert industry in future when strong resistance develops. A revision of the phosphine label will be required at that point to combat this strong resistance and our current findings on ‘time to population extinction’ will be valuable at that time.

ACKNOWLEDGEMENTS

This research was supported by Grains Research and Development Corporation.

REFERENCES

Anonymous, (1975) Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative method for adults of some major species of stored cereals with methyl bromide and phosphine. FAO Method No 16. FAO Plant Protection Bulletin 23, 12-25.

Collins, P. J. (1998) Resistance to grain protectants and fumigants in insect pests of stored products in Australia. In: *Proc. 1st Australian Postharvest Technical Conference, 1998* (Edited by Banks, H.J., Wright, E.J. and Damcevski, K.A.,) pp. 55-57, Canberra, Australia.

Collins, P.J., Daglish, G. J., Nayak, M.K., Ebert, P., Schlipalius, D., Chen, W., Pavic, H., Lambkin, T.M., Kopittke, R.A. and Bridgeman, B.W. (2001) Combating Resistance to Phosphine in Australia. In: *Proc. International Conference on Controlled Atmosphere and Fumigation in Stored Products 2000*. (Edited by Donahaye, E.J., Navarro, S. and Leesch, J.,) pp. 593-607, Fresno, CA, USA.

Daglish, G.J. Collins, P.J., Pavic, H. and Kopittke, R.A. (2002) Effects of time and concentration on mortality of phosphine-resistant *Sitophilus ory*zae (L) fumigated with phosphine. *Pest Management Science*, **58**, 1015-1021.

Daglish, G.J., Kopittke, R.A., Cameron, M.C. and Pavic, H. (2004) Predicting mortality of phosphine-resistant adults of *Sitophilus oryzae* (Coleoptera: Curculionidae) in relation to changing phosphine concentration. *Pest Management Science,* **60**, 655-659.

GenStat 5 Committee, (2000) Genstat for Windows Fifth Edition, Numerical Algorithms Group, Oxford, UK.

Hole, B.D., Bell, C.H., Mills, K.A. and Goodship, G. (1976) The toxicity of phosphine to all developmental stages of thirteen species of stored product beetles. *Journal of Stored Products Research*, **12,** 235-244.

Price, L.A. and Mills, K.A. (1988) The toxicity of phosphine to the immature stages of resistant and susceptible strains of some common stored product beetles, and implications for their control. *Journal of Stored Products Research*, **24**, 51-59.

Zeng, L. (1999). Development and countermeasures of resistance in stored grain insects in Guangdong of China. *Proc. 7th International Working Conference on Stored-Product Protection, 1998* (Edited by Jin, Z., Liang, Q., Liang, Y., Tan, X. and Guan, L.) pp. 642-647, Beijing, China.