

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. 285-291

## **MORTALITY OF PHOSPHINE-RESISTANT AND SUSCEPTIBLE LESSER GRAIN BORER, *RHYZOPERTHA DOMINICA*, EXPOSED TO CONSTANT, FLUCTUATING AND FALLING CONCENTRATIONS OF PHOSPHINE**

DIANXUAN WANG<sup>1</sup> AND PATRICK J. COLLINS<sup>2</sup>

<sup>1</sup>Henan University of Technology, Zhengzhou, Henan, 450052, P.R.China.

E-mail: wangdian@public2.zz.ha.cn

<sup>2</sup>Department of Primary Industries, 80 Meiers Road, Indooroopilly QLD 4068, Australia.

E-mail: pat.collins@dpi.qld.gov.au

### **ABSTRACT**

Adults of a highly-resistant (YCRD, Rf=328) and a susceptible reference (QRD14) strain of the lesser grain borer, *Rhyzopertha dominica*, were fumigated with phosphine (in culture medium) in a sealed 12 litre chamber at three different gas concentration regimes for 18 days: constant concentration, rising and falling concentration and falling concentration. In the first regime, phosphine concentration was maintained at 100 ml/m<sup>3</sup>. In the second experiment, phosphine concentration was initially 140 ml/m<sup>3</sup> but then alternately increased for 3 days and decreased for 3 days to give the same cumulative concentration as regime 1. In the third set of experiments, phosphine concentration was initially 100 ml/m<sup>3</sup> and then gradually decreased every 3 days by 20~40 ml/m<sup>3</sup> to 0 ml/m<sup>3</sup>. Insect cages were removed every three days to count mortality.

Complete mortality was experienced in the susceptible strain by day 6. However, complete control of the resistant insects was not achieved by 18 days. Mortality of the resistant strain increased with exposure time and at about the same rate under each regime. Concentration regime appeared to have no influence on mortality at any time interval. The results indicate that under long fumigation times, fluctuations in phosphine concentration do not influence mortality as long as a threshold exposure period and concentration are reached. Adults of the highly-resistant strain of the lesser grain borer can be killed after several days' effective fumigation even when the concentration decreased subsequently.

### **INTRODUCTION**

Phosphine is a special fumigant. It produces different effects on different species and different populations or strains of insect pests (Bell, 1976; Bell *et al.*, 1977; Bond and Upitis, 1976; Nakakita and Winks, 1981, and others). Consequently its effective concentration is different in different cases and is affected by many factors (Price and

Mills, 1988; Proctor and Ashman, 1972; Rajendran and Muralidharan, 2001). Exposure time is a dominant factor for effective fumigation (Winks, 1984; Winks and Waterford, 1986). For many field fumigations, the phosphine concentration changes with an increase initially, followed by a decrease after the peak has been reached. In the case of fumigations carried out with insufficient gastight seal, a supplement of phosphine may be added causing concentration fluctuation. The phosphine level may also be maintained at a constant level using the SIROFLO method. The question arises therefore, as to whether there are differences in insect susceptibility between fumigations carried out under the above-mentioned regimes. This aspect has not been well covered. To provide further information on this subject, three different gas concentration regimes namely: constant concentration, rising and falling concentration and falling concentration, were carried out in the laboratory, for adults of a highly-resistant and a susceptible reference strains of the lesser grain borer, *Rhyzopertha dominica*.

#### MATERIALS AND METHODS

The laboratory tests were carried out using large jars of 12000 ml capacity and with a diameter of 210 mm (Figure 1.). Two glass tubes (6 mm in diameter) were installed for phosphine injection and for gas recirculation using the pump of the phosphine monitor. . Some fine wires used for suspending each group of removable cages were installed through a seal clamp (50 mm in diameter). A rubber stopper of 135 mm diameter was used to seal the jar and hold the fixed tubes and the seal clamp in place. 50 adults of *Rhyzopertha dominica* of each strain and some cracked wheat were put into one glass tube inside cages (70 mm in length and 10mm in diameter). Two replicates were done for each strain at each removal period which was set at three day intervals. The removed insects were cultured for fourteen days and then dead and live insects were counted. Phosphine was generated from zinc phosphide and monitored by an electronic monitor (Model: HL-210, made by Xinjialieng Co., Beijing, P.R.China) with a range of 0 to 1000 ml/m<sup>3</sup> after injection. Phosphine was also recirculated. When the cages were removed the phosphine concentration was checked and phosphine was added if required. Three large jars were used for fumigation and another one served as control. A 100 ml beaker containing saturated sodium hydroxide was placed in each jar to provide suitable humidity. Three phosphine concentration regimes (experiments) were applied in the experimental jars (Figure 2). In the first regime, phosphine concentration was maintained at 100 ml/m<sup>3</sup>. In the second regime, phosphine concentration was initially 140 ml/m<sup>3</sup> but then it was alternately increased for 3 days and decreased for 3 days to give the same cumulative concentration as in the first experiment. In the third regime, phosphine concentration was initially 100 ml/m<sup>3</sup> and was then gradually decreased every 3 days by 20~40 ml/m<sup>3</sup> to reach 0 ml/m<sup>3</sup>.

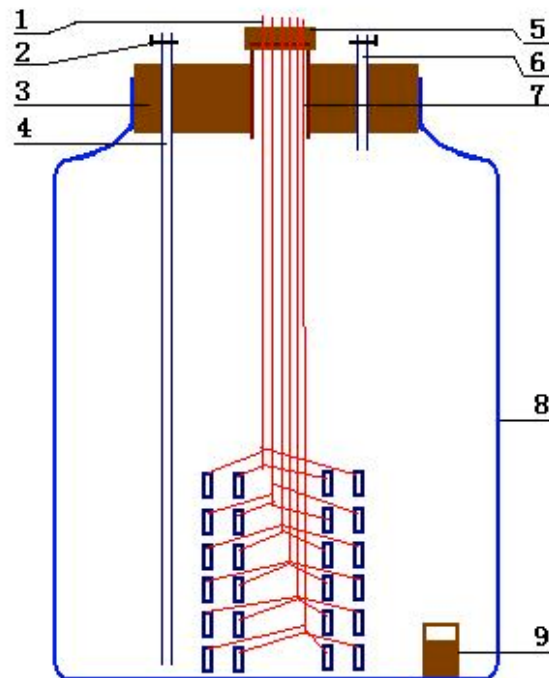


Figure1. Diagram of fumigation apparatus

1-fine wires for suspension of the cages 2-clamp seal 3-rubber stopper 4- tube for gas removal 5-cap seal 6-tube for gas injection 7- hole in rubber stopper to enable removal of the cages 8- Fumigation jar 9- saturated sodium hydroxide to provide suitable humidity

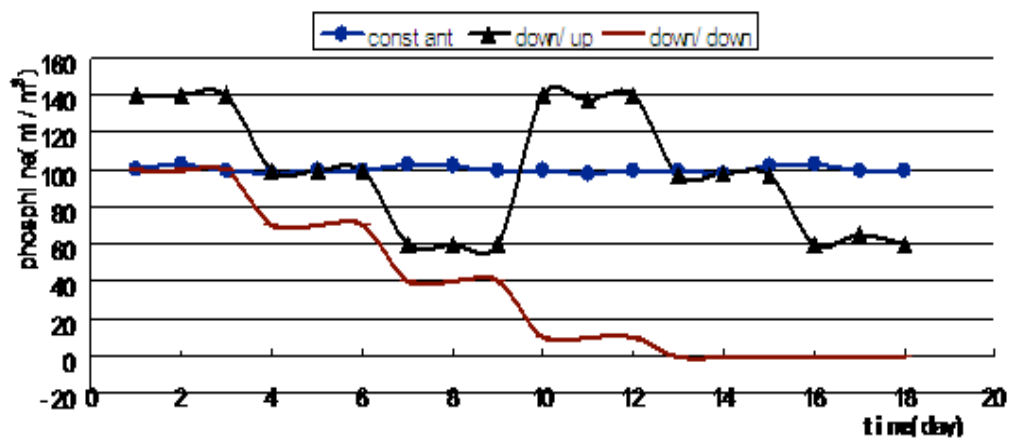


Figure 2. Changes in phosphine concentrations during experimental regimes

## RESULTS

The mortalities of the tested lesser grain borers are shown in table 1, table 2, figure 3 and figure 4. Complete mortality of the susceptible strain was experienced by day 6 even under the regime of falling concentration. However, complete control of the resistant insects was not achieved even after 18 days. Mortality of the resistant strain increased with exposure time and at about the same rate under each regime. The concentration regimes appeared to have no obvious influence on mortality at any time interval. A small difference may be observed where a slightly higher mortality was reached at the constant phosphine concentration regime.

TABLE 1  
Mortality of susceptible lesser grain borer fumigated by phosphine under three different regimes

Days	Constant concentration	Decreasing concentration	Fluctuating concentration	Control
3	99.3	99.3	96.8	0.7
6	100	100	100	1.3
9	100	100	100	0.7
12	100	100	100	3.3
15	100	100	100	6
18	100	100	100	1.7

TABLE 2  
Mortality of highly-resistance lesser grain borer fumigated by phosphine under three different regimes

Days	Constant concentration	Decreasing concentration	Fluctuating concentration	Control
3	43.8	42.8	26.3	0.0
6	62.6	48.7	43.3	2.7
9	68.2	54.7	54.6	9.5
12	74.7	71.2	65.4	8.0
15	76.8	78.0	76.1	6.7
18	86.0	82.2	84.8	10.0

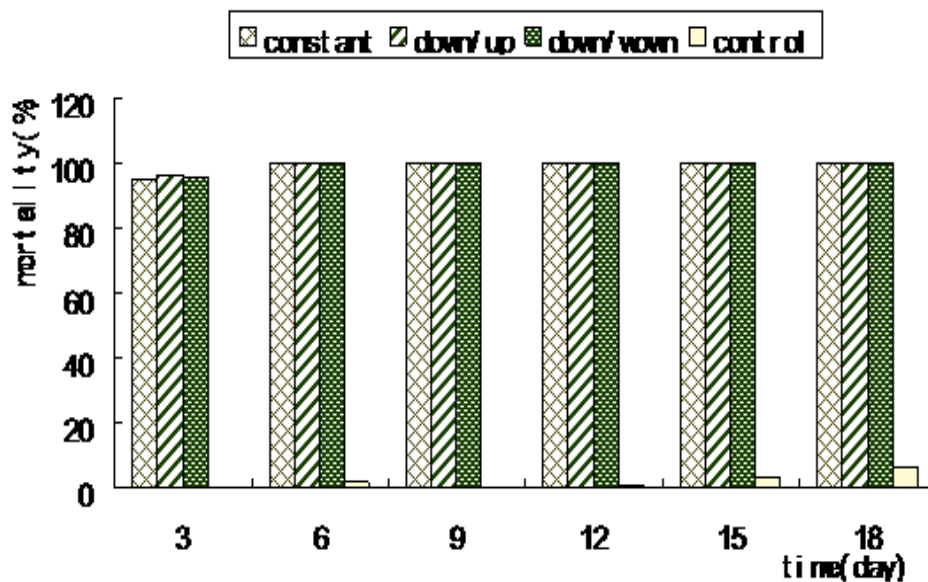


Figure 3. mortality of susceptible strain fumigated with phosphine in 18 days

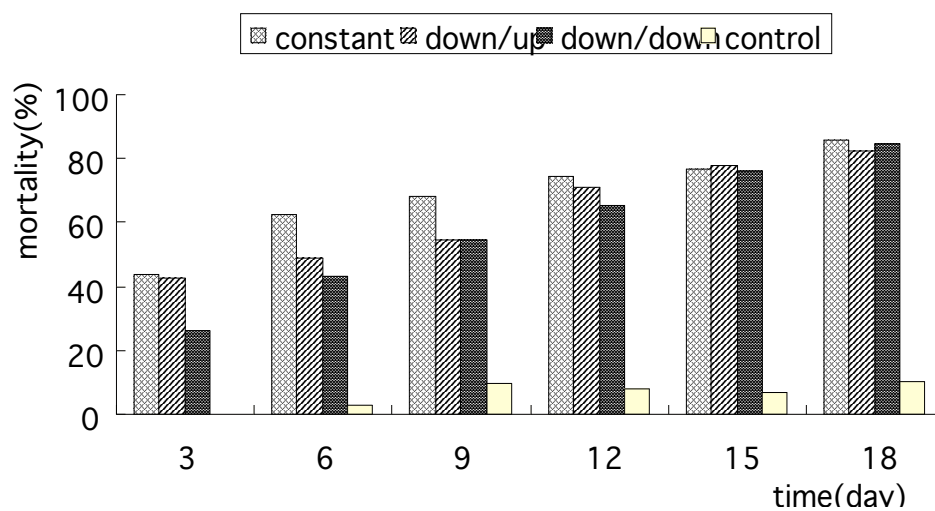


Figure 4. Mortality of resistant strain fumigated with phosphine over 18 day exposure period

**DISCUSSION**

The results indicate that over prolonged fumigation times, fluctuations in phosphine concentration do not influence mortality so long as a sufficient threshold exposure period and concentration are reached. After the insects are injured by phosphine, the

mortality will rise continuously whether the concentration in the regime changes or not.. The concept of end-point mortality where mortality of the insects continues to increase after phosphine fumigation has ended, may provide some explanation of the results (Winks, 1984). Furthermore, for fumigated insects that are cultured and observed after 14 days, as in accordance with FAO Method No.16 there is also evidence of increasing mortality. Actually, in some commercial fumigations, where the effective level of phosphine is maintained over 3-5 days, most of the adult insects will be killed after a 7-10days fumigation. For example Nayak *et al.*, (2000) reported succesful results when 1.5 tablets per cubic metre are applied in an enclosure sealed for 7 days, when the temperature of the produce was above 25°C, or for 10 days at lower temperatures. The treated produce should be aerated before it is handled. In the third set of experiments an effective fumigation (above 60~70ml/m<sup>3</sup>) should be obtained after 6 days, according to the report on the Siroflo technology of phosphine fumigation (Pratt, 1998). Under this regime, some adults were killed and some were seriously injured indicating that the mortality can continue to increase.

#### REFERENCES

- Bell C. H. (1976) The tolerance of developmental stages of four stored product moths to phosphine. *Journal of Stored Products Research*. **12**, 77-86.
- Bell C. H., Hole B. D. and Evans P. H. (1977) The occurrence of resistance to phosphine in adult and egg stages of strains of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) *Journal of Stored Products Research*. **13**, 91-94.
- Bond E. J. and Uptis E. (1976) Response of three insects to sublethal doses of phosphine *Journal of Stored Products Research* **8**, 307-312.
- FAO. (1975) Recommended methods for detection and measurement of agriculture pests to pesticide. Tentative method for adults of some major pest species of stored cereals with methyl bromide and phosphine. FAO Method No, 16, FAO Plant Protection Bulletin, 1975, **23**:12-26.
- Hole B. D., Bell, C. H., Mills K. A. and Goodship Gwen (1976) The toxicity of phosphine to all developmental stages of thirteen species of stored product beetles. *Journal of Stored Products Research*. **12**, 235-244.
- Howe R. W. (1973) The susceptibility of the immature and adult stages of *Sitophilus granarius* to phosphine. *Journal of Stored Products Research* **8**, 241-262.
- Nakakita H. and Winks R. G. (1981) Phosphine resistance in immature stages of a laboratory selected strain of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) *Journal of Stored Products Research*. **17**, 43-52.
- Nayak M. and White, G. (2000) Grain storage Psocid and mite pests, DPI note, <http://www.dpi.qld.gov.au> File No: FS0439 • Date created: October 2000
- Pratt, S. (1998) Workspace and environmental phosphine monitoring during Siroflo® fumigation of horizontal sheds at Coolamon and Illabo, NSW. *CSIRO Entomology Technical Report No. 79*, 23pp.

- 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.
- Proctor D. L. and Ashman F. (1972) The control of insects in exported Zambian groundnuts using phosphine and polyethylene lined sacks. *Journal of Stored Products Research* **8**,127-137.
- Rajendran S. and Muralidharan N. (2001) Performance of phosphine in fumigation of bagged paddy rice in indoor and outdoor stores. *Journal of Stored Products Research*. **37**, 351-358.
- Reynolds E. M., Robinson J. M. and Howells Carol (1967) The effect on *Sitophilus granarius* (L.) (Coleoptera, Curculionidae) of exposure to low concentrations of phosphine. *Journal of Stored Products Research* **2**, 177-186.
- Winks R. G. (1984) The toxicity of phosphine to adults of *Tribolium castaneum* (Herbst): Time as a dosage factor. *Journal of Stored Products Research*. **20**, 45-56.
- Winks R. G. and Waterford C. J. (1986) The relationship between concentration and time in the toxicity of phosphine to adults of a resistant strain of *Tribolium castaneum* (Herbst). *Journal of Stored Products Research*. **22**, 85-92.