Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 31–43

THE EFFECT OF TEMPERATURE ON SOME RESISTANT STORED-PRODUCT INSECTS EXPOSED TO PHOSPHINE

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ABSTRACT

Mixed-age cultures of susceptible strains of *Sitophilus oryzae, S. granarius, S. zeamais* and *Rhyzopertha dominica*, together with resistant strains of *S. oryzae* and *R. dominica*, were exposed to constant concentrations of 0.03, 0.05 and 0.1 mg L^{-1} phosphine (PH_3) at temperatures of 15, 25 and 35°C. All species and strains were generally more tolerant of PH_3 at 15°C than at higher temperatures. Higher temperatures reduced the required exposure time for complete mortality for susceptible strains of *Sitophilus* spp. and *R. dominica*, and for resistant strains of *S. oryzae*, at all concentrations. In contrast, the exposure time needed to achieve complete mortality of resistant *R. dominica* at concentrations of 0.03 and 0.05 mg L^{-1}, but not at 0.1 mg L^{-1}, was longer at 35°C than at 25°C. At 0.1 mg L^{-1}, complete mortality at 35°C was achieved in the same or slightly less time than at 25°C.

INTRODUCTION

Worldwide, phosphine (PH_3) fumigations are performed across a wide range of stored commodity temperatures. In Australia, and in many other parts of the world, grain is commonly stored at temperatures over 25°C. The effect of high temperatures is frequently not considered (Winks *et al.*, 1980) when disinfestation treatments are recommended since it is believed that the higher the temperature the greater the efficacy of PH_3 (Lindgren and Vincent, 1966; Hole *et al.*, 1976).

In laboratory studies, temperature has long been recognised as having complex effects on insect response to fumigants (e.g. Champ and Dyte, 1976). For this reason it is usually recommended that such studies be standardised on a single temperature that covers most species. In PH_3 toxicity studies, for example when using the FAO discriminating dosage test (Anon., 1975), the generally adopted exposure temperature has been 25°C.

Hole *et al.* (1976) found a positive correlation between temperature and dosage over the range of 10–35°C when they examined the response of immature stages of susceptible insects to PH_3. Price and Mills (1987) found a similar result when they exposed resistant

insects to PH_3 at 15 and 25°C. One of the ways temperature affects insect mortality is the rate of fumigant uptake. Price (1984) examined the effect of temperature on PH_3-uptake in susceptible and resistant adult laboratory insects exposed to PH_3 for 5-h periods. He found that increased temperature stimulates uptake in susceptible insects and enhances the active exclusion of PH_3 in resistant insects. Thus, resistant adult insects became more resistant with increased temperature.

This finding raised the question of whether resistant strains in the field would have an even greater likelihood of survival in grain at elevated temperatures (30°C or more). As part of the development of the SIROFLO® fumigation technique, studies were conducted to determine the response to PH_3 of a number of the major insect species infesting grain in Australia over a range of temperatures. SIROFLO® applies a continuous low concentration of PH_3 into a pressurised distribution system (Winks, 1992). This paper reports the results of studies in which mixed-age cultures of resistant insects were exposed to PH_3 at different temperatures and compares these results with those of Price (1984).

MATERIALS AND METHODS

The effects of temperature on PH_3 toxicity were determined by exposing mixed-age cultures to a continuous flow of PH_3 in air to determine the time to population extinction. The time to population extinction is an estimate of the minimum exposure time necessary to achieve complete mortality of all stages of the life cycle for a given concentration and temperature. In these experiments, concentration was held constant and time was the dosage variable. The methods used are generally those described in Winks and Hyne (1994). However, a more detailed description of the method using mixed-age cultures, including its rationale and repeatability, is given in Winks and Hyne (1997).

Insect material

A number of strains of four species of stored-product beetles, *Sitophilus oryzae, S. granarius, S. zeamais* and *Rhyzopertha dominica*, were used in these experiments. The strains of *S. oryzae* and *R. dominica* included PH_3-susceptible strains, PH_3-resistant field strains and laboratory-selected resistant strains (only susceptible strains of *S. granarius* and *S. zeamais* being used). Adults of all strains were diagnosed as susceptible (S) or resistant (R) by the FAO discriminating dosage test (Anon., 1975). Strain names and collection locations and dates of the insects are given in Table 1.

Wheat (var. Corella or Rosella) used as a culture medium was conditioned to 12% m.c. prior to use and sterilised by heating to 60°C for a minimum of 1 h. Mixed-age cultures were established by placing 300 adults on approximately 1,000 g of whole wheat (*Sitophilus* spp.) or layers of whole wheat and flour (*R. dominica*) in 2-L glass jars. The adults were not removed from these cultures. After 6 weeks, samples of the cultures were taken and X-rayed to verify the presence and relative abundance of larval and pupal stages. Cultures of *Sitophilus* spp. were reared at 25°C, 57% r.h., and *R. dominica* at 30°C, 70% r.h.

TABLE 1
Strains, collection dates and origins of stored-product insects used in this study

Species (Strain)	Resistant/ Susceptible	Origin
Sitophilus oryzae		
LS2	S	Queensland 1962
CSO404P10	R	Laboratory selection with PH_3 for ten generations of CSO404 (formerly FAO strain SO476, from Karnal, India), 1976
CSO421	R	Goondiwindi, Queensland, 1991
Sitophilus granarius		
CSG4	S	Queensland, 1952
CSG46	S	Tutye, Victoria, 1974
Sitophilus zeamais		
CSZM9	S	Brisbane, Queensland, 1971
Rhyzopertha dominica		
CRD2	S	Pest Infestation Control Laboratory (UK), 1962
CRD316	R	Trangie, New South Wales, 1989
CRD235P10	R	Laboratory selection with PH_3 for ten generations of RD235 (formerly FAO strain CRD484, from Borivli, India), 1976

Fumigation chambers

Mixed-age cultures were placed in 3-L perspex or stainless steel fumigation chambers (Winks and Hyne, 1994) 72 h before fumigation. Clear acrylic (perspex) chambers were made from a perspex tube fitted with two perspex screw-top lids and made gastight with neoprene O-rings. Both top and bottom lids were fitted with an inlet and an outlet. The lids were lined with stainless steel mesh to prevent insects and dust from escaping from the inlet and outlet. The bases of the stainless steel chambers were secured with six hex screws and made gastight with a neoprene O-ring.

Fumigation procedures

The PH_3 concentrations used and the temperatures to which the insects were exposed are given in Table 2. A fixed concentration of PH_3 was continuously supplied to the bases of the fumigation chambers. PH_3 in nitrogen (BOC) was diluted with air using two Brooks mass flow controllers (5850E series). Atmospheric air was filtered through charcoal to a Charles Austin diaphragm pump supplying the mass flow controllers. The diluted PH_3

TABLE 2
Summary of the insects, PH_3 concentrations and temperatures used in experiments to determine times to population extinction

	Phosphine concentration (mg L^{-1})								
	0.03			0.05			0.1		
Strain	15°C	25°C	35°C	15°C	25°C	35°C	15°C	25°C	35°C
LS2		×	×	×	×	×	×	×	×
CSG4		×	×	×	×	×	×	×	×
CSZM9		×	×		×	×		×	×
CSO421		×			×	×			
CSO404P10		×			×	×		×	
CRD2		×		×	×	×	×	×	
CRD316	×	×	×	×	×	×	×	×	×
CRD235P10	×	×	×	×	×	×	×	×	×

was humidified to 57% r.h. for the experiments at 15 and 25°C and to 60% r.h. for the experiments at 35°C. Air was humidified to saturation by passage through distilled water maintained at 7, 15 and 26.5°C and then being reheated to laboratory temperatures of 15, 25 and 35°C, respectively.

Each chamber received a flow of the PH_3 air mixture at 30–40 ml min^{-1}, obtained by dividing the source flow equally using Brooks flowtubes (A125-5).

Throughout the experiment, PH_3 concentrations were monitored using a gas chromatograph fitted with a Tracor flame photometric detector in the phosphorus mode. The flame photometric detector was calibrated using accurate dilutions made from a concentrated source of PH_3. PH_3 was prepared from aluminium phosphide according to Anon. (1975), and its concentration was determined using a Gowmac gas density balance.

Post-fumigation procedures

Samples of grain-and-insects, approximately 200 g each, were generally taken from the fumigation chambers at intervals of 2, 4, 7, 14, 21 and 28 d, although for some experiments daily samples were taken over a shorter period to obtain better estimates of time to population extinction. These samples were incubated at 25°C, 60% r.h., and assessed for the presence of live adults by sieving. Assessments were made 24 h after sampling and again after 8 weeks and 16 weeks incubation. The first sample from which no adults emerged (confirmed by subsequent samples) indicated the extinction of the population. The time of exposure of this sample was recorded as the estimate of the *time to population extinction* (Winks and Hyne, 1997). Data was presented graphically as split-column plots

according to the method described by Winks and Hyne (1997). The height of the split column shows the exposure time of the first sample from which no survivors were recorded. The split in the column shows the exposure time of the previous sample from which survival was recorded.

RESULTS

Susceptible strains

The times to population extinction for susceptible strains of all species exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C are given in Figs. 1, 2, 3 and 4. All susceptible strains were more tolerant of PH_3 at 15°C than at the higher temperatures for the concentrations examined. The *Sitophilus* species required longer exposure periods than *R. dominica* (CRD2) at all concentrations. The most tolerant strain at 15°C, LS2, required more than 22 d exposure for control at 0.05 mg L^{-1} PH_3. As the concentration increased to 0.1 mg L^{-1}, the time to population extinction decreased for the *Sitophilus* spp. but not for CRD2 (Figs. 1, 2 and 4).

At 25°C the time to population extinction for LS2 and CSG4 increased slightly while that of CSZM9 decreased slightly as concentration increased from 0.03 to 0.05 mg L^{-1}. Due to the differences in sampling frequency, for example a 7-d sample interval versus a daily sample, it was not possible to determine if these trends continued to 0.1 mg L^{-1}. The time to population extinction for CRD2 appeared to be similar

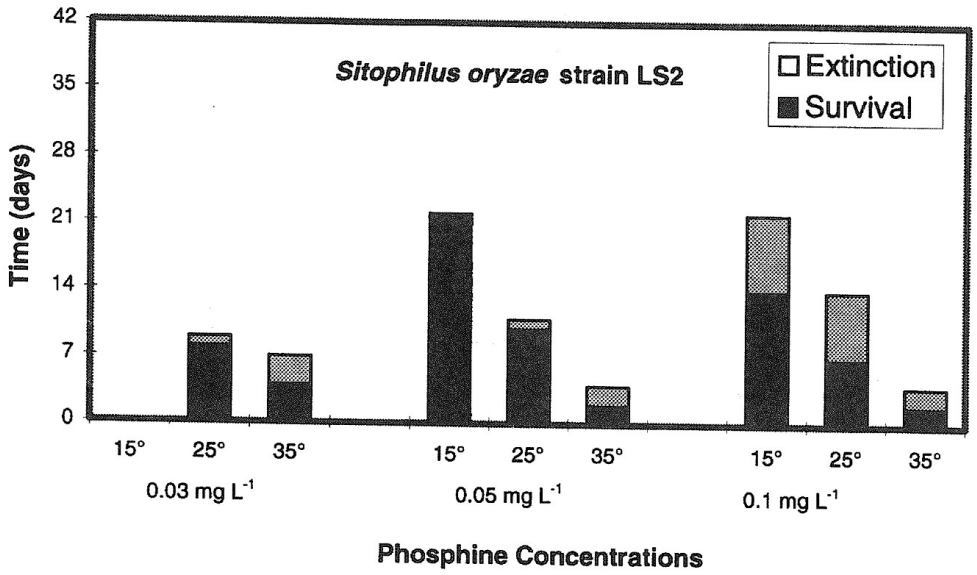

Fig. 1. Time to population extinction of mixed-age cultures of a PH_3-susceptible strain of *Sitophilus oryzae* (LS2) exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C.

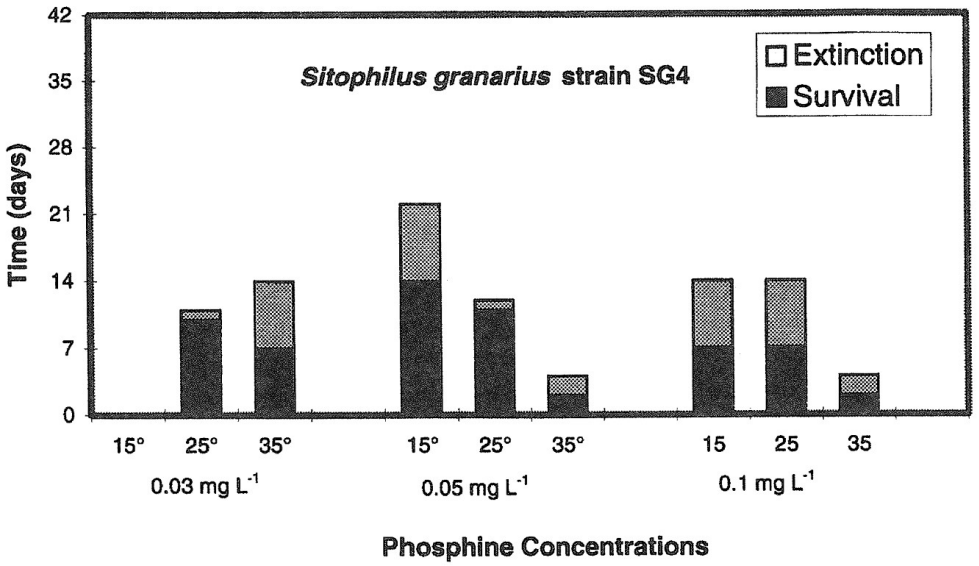

Fig. 2. Time to population extinction of mixed-age cultures of a PH_3-susceptible strain of *Sitophilus granarius* (CSG4), exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C.

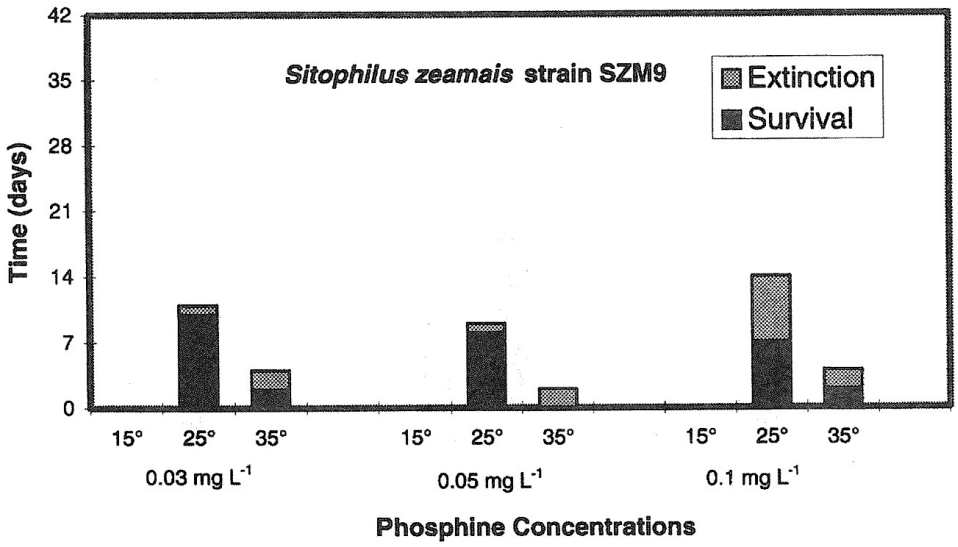

Fig. 3. Time to population extinction of mixed-age cultures of a PH_3-susceptible strain of *Sitophilus zeamais* (CSZM9), exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 25 and 35°C.

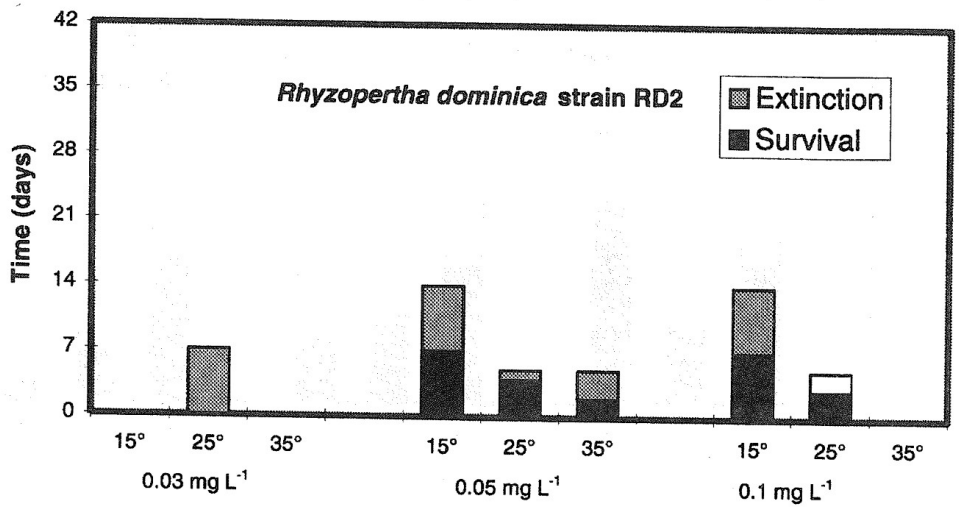

Fig. 4. Time to population extinction of mixed-age cultures of a PH_3-susceptible strain of *Rhyzopertha dominica* (CRD2), exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C.

for all concentrations (Note: as the first sample taken at 25°C for 0.03 mg L^{-1} was at 7 d, the time to population extinction could be earlier). By contrast, at 35°C, time to population extinction decreased for LS2 and CSG4 as concentration increased from 0.03 to 0.05 mg L^{-1}.

The time to population extinction for the *Sitophilus* spp. was longest at 15° and shortest at 35°C for all concentrations except CSG4 at 0.03 mg L^{-1}, where the time to population extinction at 25°C and 35°C appeared similar (Figs. 1, 2 and 3). There appeared to be a similar relationship with CRD2, although at 0.05 mg L^{-1} the observed times to population extinction were the same at 35 and 25°C (Fig. 4).

Resistant strains of *Rhyzopertha dominica*

The times to population extinction for resistant strains of *R. dominica* exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C are given in Figs. 5 and 6.

The relationship between temperature and time to population extinction for the resistant strains produced a complex picture. The time to population extinction of CRD316 was greatest at 35 and least at 25°C for 0.03 and 0.05 mg L^{-1}. The time to population extinction for CRD235P10 followed a similar trend at 0.05 mg L^{-1} but not at 0.03 mg L^{-1}, where the time to population extinction was greater at the higher temperatures. Although not apparent from Fig. 6, the laboratory selected strain CRD235P10, at 0.03 mg L^{-1}, appeared to be close to extinction after 35 d at 25°C when two insects were found

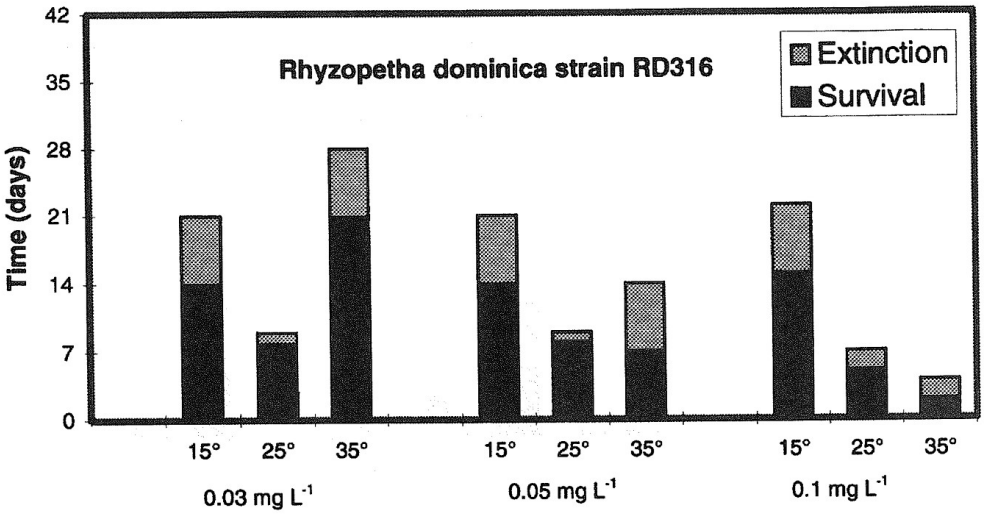

Fig. 5. Time to population extinction of mixed-age cultures of a field resistant strain of *Rhyzopertha dominica* (CRD316), exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C.

compared with the thousands found after 28 d at 35°C. This indicated a time to population extinction well beyond 28 d at this higher temperature. In contrast, at 0.1 mg L^{-1}, the time to population extinction was lowest at 35°C in strain CRD316 and was equally short at 25

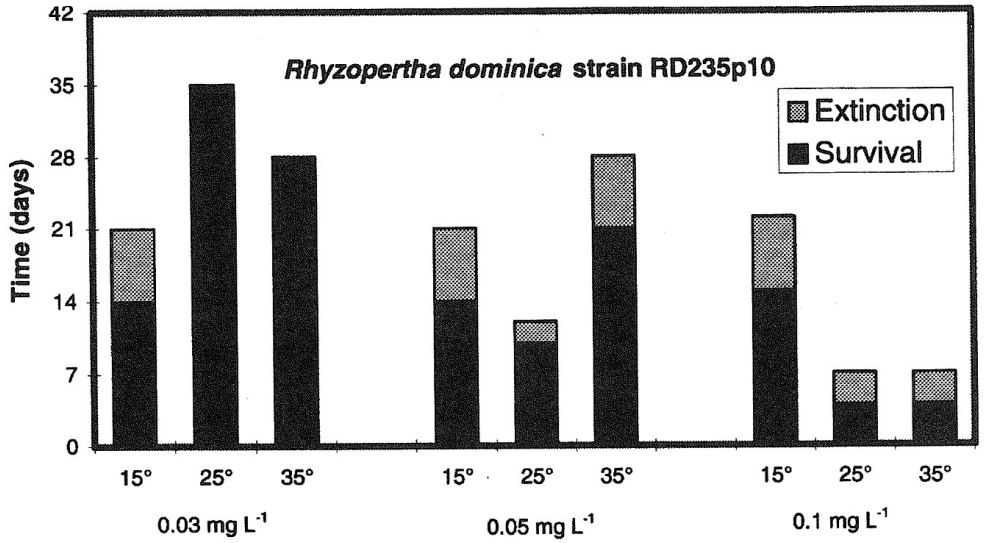

Fig. 6. Time to population extinction of mixed-age cultures of a laboratory selected resistant strain of *Rhyzopertha dominica* (CRD235P10), exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C.

and 35°C in CRD235P10. At both 25 and 35°C the time to population extinction became progressively shorter with each increase in concentration.

At 15°C the time to population extinction of both resistant strains, CRD235P10 and CRD316, and the susceptible strain, CRD2, appeared to remain the same at each of the three concentrations (Figs. 4, 5 and 6). As the concentration increased at 25°C, the time to population extinction of CRD235P10 and CRD316 decreased to a level similar to that of CRD2. For example, with CRD235P10, the time to population extinction decreased from more than 35 d at 0.03 mg L^{-1} to 7 d at 0.1 mg L^{-1}, similar to the susceptible CRD2 strain which, at the same concentration, took 5 d.

Resistant strains of *Sitophilus oryzae*

The time to population extinction for the resistant strains of *S. oryzae* are given in Figs. 7 and 8. This data set is incomplete as the resistant insects had not been exposed to PH_3 at 15°C and had been exposed at only one concentration at 35°C. From the available data, it seems that the time to population extinction for these strains became progressively shorter with each increase in concentration at both 25 and 35°C in a manner similar to that of the susceptible strain.

The resistant strains (CSO421 and CSO404P10) are most tolerant when exposed to 0.03 mg L^{-1} at 25°C, requiring more than 35 d for control, compared with the susceptible LS2 which required 14 d. At 0.1 mg L^{-1} the effect of resistance in the laboratory-selected CSO404P10 was lost, and the time to population extinction was the same as the susceptible LS2.

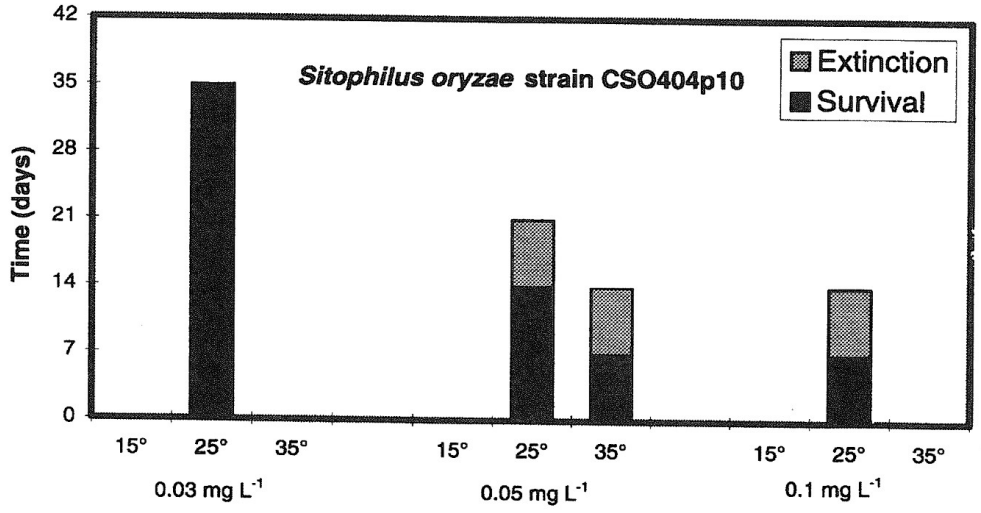

Fig. 7. Time to population extinction of mixed-age cultures of a field resistant strain of *Sitophilus oryzae* (CSO421), exposed to 0.03 and 0.05 mg L^{-1} PH_3 at 15, 25 and 35°C.

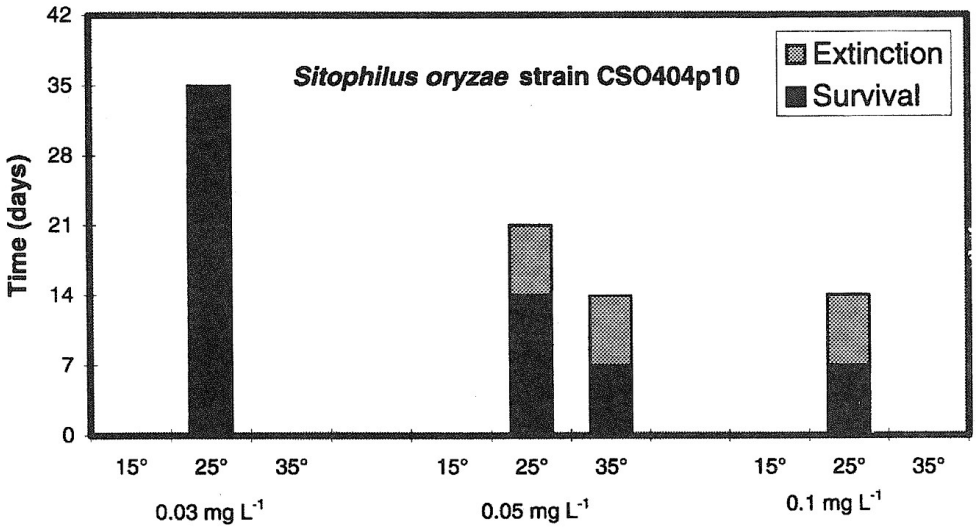

Fig. 8. Time to population extinction of mixed-age cultures of a laboratory selected strain of *Sitophilus oryzae* (CSO404P10), exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C.

DISCUSSION

Susceptible strains

The susceptible strains of all species tolerated PH_3 for longer periods at 15°C than at the higher temperatures. This finding is supported by the work of Hole *et al.* (1976) and Price and Mills (1987). Hole *et al.* (1976) suggested that the long exposure periods needed to kill insects at 15°C were due, in part, to the time taken for tolerant stages to develop into more susceptible stages. For many stored-product insect pests, 15°C is close to the temperature threshold at which development of immature stages can occur. For example, the developmental thresholds are 16.6°C for *R. dominica* and 11.4°C for *S. oryzae* (Beckett *et al.*, 1994). Development of the immature stages of *R. dominica* would be substantially protracted at 15°C, and this raises doubts as to whether the mortality observed in the present study was due entirely to tolerant stages developing into more susceptible stages.

With each progressive increase in temperature, the time to population extinction generally became successively shorter for all strains. There were a few exceptions however. For example, at 0.03 mg L^{-1} the time to population extinction of susceptible CSG4 at 35°C appeared greater than at 25°C, but this may have been due to the 7 d interval in the data at 35°C. These data support the finding that susceptible species become less tolerant of PH_3 with increased temperature (Price, 1984).

Resistant strains

Resistant *S. oryzae* do not demonstrate increased tolerance to PH_3 with increased temperature, as suggested by Price (1984) for *R. dominica*. *S. oryzae* was easier to control at 35°C than at 25°C for the single concentration examined. This was not the case for resistant *R. dominica* which was able to tolerate PH_3 longer at some concentrations at higher temperatures. For example, at 0.05 mg L^{-1} the laboratory-selected CRD235P10 required 28 d at 35°C to be killed, compared with 21 and 12 d at 15 and 25°C. At 0.1 mg L^{-1}, however, the insects were killed in 7 d at the higher temperatures compared with 21 d at 15°C.

The ability of resistant *R. dominica* and *S. oryzae* to tolerate PH_3 was affected by both temperature and concentration. At 15°C the time to population extinction for both strains of resistant *R. dominica* did not appear to change with each increase in concentration. At the higher temperatures the tolerance to PH_3 of the resistant strains of *R. dominica* and *S. oryzae* became shorter with each increase in concentration. For example, at 25°C resistant *S. oryzae* (CSO404P10, Fig. 8) required over 35 d to be killed at 0.03 mg L^{-1}, and 14 d at 0.1 mg L^{-1}, compared with 9 and 14 d to control LS2 at the same concentrations (Fig. 1). Similarly, resistant CRD235P10 required more than 35 d exposure at 0.03 mg L^{-1}, and only 7 d exposure at 0.1 mg L^{-1}, compared with CRD2 which required 5 to 7 d exposure at all concentrations. At 35°C, the time to population extinction of CRD235P10 was more than 28 d at 0.03 mg L^{-1} and 7 d at 0.1 mg L^{-1}.

It is possible that the differences in mortality response among the species at 35°C are due to differences in the individual species' ideal metabolic conditions. Resistance mechanisms operate more effectively in *R. dominica* at 35°C, 0.03 and 0.05 mg L^{-1}, which is close to the insects' optimum development temperature of 34°C (Birch, 1945; Beckett *et al.*, 1994). Further work, exposing resistant *S. oryzae* to PH_3 at 30°C, their optimum development temperature, would verify whether resistance in this species was or was not enhanced by temperature.

What does seem clear from this study is that, over the range of concentrations and temperatures observed for both the resistant strains, time is the critical component of dosage.

CONCLUSIONS

The temperature-dependent model proposed by Price (1984), in which susceptible insects become more susceptible to PH_3 with successive increases in temperature, appears to apply to all the susceptible insects in this study. However, the mortality response of resistant strains of *R. dominica* is not described by this model. Resistant *S. oryzae* appear, from the limited data, to follow the model for susceptible insects, while *R. dominica* is more tolerant at higher temperatures (but only at some concentrations).

The data of this report have important implications in the management of resistant *R. dominica* in countries with stored-product temperatures above 30°C. The exposure period is clearly more important than the level of concentration in determining effective dosages of PH_3 for control of insect pests of stored products. Hence, increasing exposure time rather than concentration would appear to be a better approach to the control of resistant *Sitophilus* spp. However, a concentration of at least 0.1 mg L^{-1} would seem to be essential for practical control of strains of *R. dominica* which are as resistant as the laboratory-selected strain CRD235P10.

ACKNOWLEDGEMENTS

Elizabeth Hyne would like to thank the Ian McLennan Achievement for Industry Award which enabled her to attend the 1996 CAF Conference in Cyprus.

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