Influence of concentration, temperature and humidity on the toxicity of phosphine to the strongly phosphine-resistant psocid *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae)

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Abstract

BACKGROUND: The psocid *Liposcelis bostrychophila* Badonnel, is a widespread, significant pest of stored commodities, has developed strong resistance to phosphine, the major grain disinfestant. The aim was to develop effective fumigation protocols to control this resistant pest.

RESULTS: Time to population extinction of all life stages (TPE) in days was evaluated at a series of phosphine concentrations and temperatures at two relative humidities. Regression analysis showed that temperature, concentration and relative humidity all contributed significantly to describing TPE ($P < 0.001$, $R^2 = 0.95$), with temperature being the dominant variable, accounting for 74.4% of the variation. Irrespective of phosphine concentration, TPE was longer at lower temperatures and high humidity (70% RH) and shorter at higher temperatures and low humidity (55% RH). At any concentration of phosphine, a combination of higher temperature and lower humidity provides the shortest fumigation period to control resistant *L. bostrychophila*. For example, 19 and 11 days of fumigation are required at 15 °C and 70% RH at 0.1 and 1.0 mg L^{-1} of phosphine respectively, whereas only 4 and 2 days are required at 35 °C and 55% RH for the same respective concentrations.

CONCLUSIONS: The developed fumigation protocols will provide industry with flexibility in application of phosphine.

Keywords: *Liposcelis bostrychophila*; strong resistance; phosphine; fumigation protocols

1 INTRODUCTION

Over the last decade, the importance of phosphine fumigation in the management of stored-product pests has risen significantly worldwide owing mainly to the gradual phasing out of methyl bromide, a lack of suitable alternatives and increasing consumer sensitivity towards insecticide residues. The foremost advantage of phosphine over other fumigants has been its global acceptance by most markets as a residue-free commodity treatment. Moreover, phosphine is cheap, easy to apply and can be used in a wide range of storage types and commodities. However, the development of resistance in a range of storage pests has emerged as a major threat to the sustainability of this unique material.[1–6]

Resistance to phosphine has been documented in a range of coleopterous and lepidopterous pests.[1–9] In addition, phosphine resistance has been reported in several species of wingless psocids belonging to the genus *Liposcelis*.[10–13] These insects have emerged as widespread, significant pests of stored commodities internationally in the past 15 years.[10,11,14–20] Resistance to phosphine has been reported in *L. entomophila* (Enderlein) from Indonesia[10] and China,[11] in *L. bostrychophila* Badonnel from India[12] and in three *Liposcelis* spp. from Australia.[13] In Australia, although resistance has developed in *L. entomophila*, *L. decolor* and *L. bostrychophila*, it is strongest in *L. bostrychophila*.[13] Effective control of these insects is problematic, as current fumigation recommendations are based on responses of the major coleopterous pests and are inadequate to control these highly resistant psocid strains.[13] There is a strong need to establish effective fumigation protocols to control these insects in grain in order to ensure continued market access, grain quality and food security.

Phosphine concentration, exposure period and grain temperature are major variables that determine the

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toxicity of phosphine. It had been demonstrated in several pest species that a change in any one of these factors independently or in combination would result in a change in efficacy against a target pest,[7,21–25] and these variables have been considered in this work. Psocids are highly dependent on environmental moisture and thrive at higher humidities.[26,27] Optimal environmental relative humidity for *L. bostrychophila* is 70% at 30 °C.[15] Therefore, the additional variable of humidity was also included in the study.

The aim of the present research was to quantify the influence of concentration, temperature and humidity on phosphine toxicity and to use these data to establish practical fumigation protocols for use by industry. The range of each variable examined in this work reflects the application of phosphine by industry and the range of storage conditions experienced.

2 MATERIALS AND METHODS

2.1 Test insects

Experiments were conducted on a strain of *L. bostrychophila* (SLB3) collected from a central storage at Karkoo, South Australia, in 1997. With a 67-fold resistance compared with a reference susceptible strain, SLB3 has the strongest level of resistance to phosphine yet detected in Australia in this species.[13] This resistance factor was calculated by dividing 0.03 by 2.0 mg L^{-1}, the lowest concentrations that achieved complete mortality of all life stages of susceptible and resistant insects, respectively, in 6 days. SLB3 has maintained its original level of resistance in spite of not having been selected in the laboratory. The resistance genotype may have been already fixed in the original population sample of this parthenogenetic species. Cultures were maintained on a medium comprised of whole wheat, kibbled wheat, whole wheat flour and brewer's yeast (10 + 10 + 10 + 0.1 by weight) in constant conditions of 30 ± 1 °C, 70 ± 2% relative humidity (RH) and photoperiod of 12:12 h light:dark.[28]

2.2 Time to population extinction (TPE) assays

Response to phosphine was measured by exposing mixed-age cultures of psocids to fixed concentrations of phosphine in gas-tight desiccators (6 L capacity). Cultures of *L. bostrychophila* were specially generated over a 6 week period so that they contained all life stages living in the culture medium described in Section 2.1. About 25 g of culture medium containing a mixed-age sample of psocids (approximately 2000 individual nymphs and adults, with an unknown number of eggs) was placed in a plastic soufflé cup (30 mL capacity) and sealed with a perforated lid. Three cups were arranged in a desiccator for each treatment and each treatment was replicated. Controls were exactly the same as treatments except that they were not dosed with phosphine. Phosphine concentrations of 0.1, 0.17, 0.3, 0.5, 0.7 and 1.0 mg L^{-1} were tested at 15, 20, 25, 30 and 35 °C, and at 55 and 70% RH. All experiments were undertaken in constant conditions at specified temperatures ±1 °C and RH ±2%. The range of phosphine concentrations tested represents those likely to be used in practice, and, similarly, the environmental conditions chosen reflect those encountered by industry and in which phosphine may be applied. Experimental cups containing mixed-age psocid colonies were arranged in open desiccators and left in each test environmental regime for at least a week before fumigation to ensure equilibration to the specified conditions and to rule out any pre- or post-fumigation temperature effects on phosphine toxicity.[23]

The experimental fumigation procedure was the same as described previously.[16] Phosphine was generated from a commercial aluminium phosphide formulation and captured over acidified water. Its concentration was measured using a gas density balance chromatograph (Aerograph model 90-P; Varian, Mount Waverley, Victoria, Australia). Phosphine was collected from this source using a gas-tight syringe and was injected through a rubber septum in the lid of the experimental desiccator to give the required concentration. Insects were removed from the fumigation after a predetermined exposure period and left in the same temperature/humidity regime for up to 4 weeks. This period was intended to overcome the potential problem of any delay in hatching of psocid eggs that may have occurred owing to the fumigation.[29] At the end of the 4 week recovery period, each test cup was sieved and inspected for the presence of live insects. The criterion of response was population extinction (i.e. no live psocids in any of the three cups). In this way, the time taken, in days, to control all life stages completely was determined for each phosphine concentration under each temperature/humidity regime. Results were recorded simply as the least number of whole days taken to achieve population extinction. Phosphine concentration was monitored randomly throughout the experiments using a gas chromatograph (Varian Star 3600X®) fitted with a pulsed-flame photometric detector to confirm that there was no loss of the gas during exposure periods.

2.3 Statistical analysis

The relationship between time to population extinction and the variables concentration, temperature and relative humidity was examined using multiple linear regression analysis in GenStat 8.[30] A full model was fitted, including all possible two-way and the three-way interactions. Using backwards selection, terms that were not significant at the 5% level were excluded from the final model.

3 RESULTS

Throughout the experiments, even in the most extreme conditions (55% RH and 35 °C), psocid populations in control treatments survived as expected

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and continued to grow. Overall, there was a steady decrease in time to population extinction from the regimes of low concentration and low temperature towards the regimes of high concentration and high temperature (Figs 1a to e). At each temperature, except 35 °C, time to population extinction took longer at 70% than at 55% RH, although relative humidity had less effect on response to phosphine as temperature increased. At 35 °C, TPE was equal for the two humidities at 0.3 and 1 mg L^{-1}, and an additional day was required at concentrations of less than 0.3 mg L^{-1} to achieve population extinction at 55% RH compared with 70% RH, reversing the general trend (Fig. 1e). There was also an overall trend for concentration to have less effect on response to phosphine as temperature increased (Fig. 1), demonstrated by the flattening of response lines with each increase in temperature. Regression analysis revealed that concentration, temperature and relative humidity all contributed significantly to describing the time to population extinction ($P < 0.001$, $R^2 = 0.95$), with temperature being the dominant variable and accounting for 74.4% of the variation. The three-way interaction ($P = 0.6740$) and

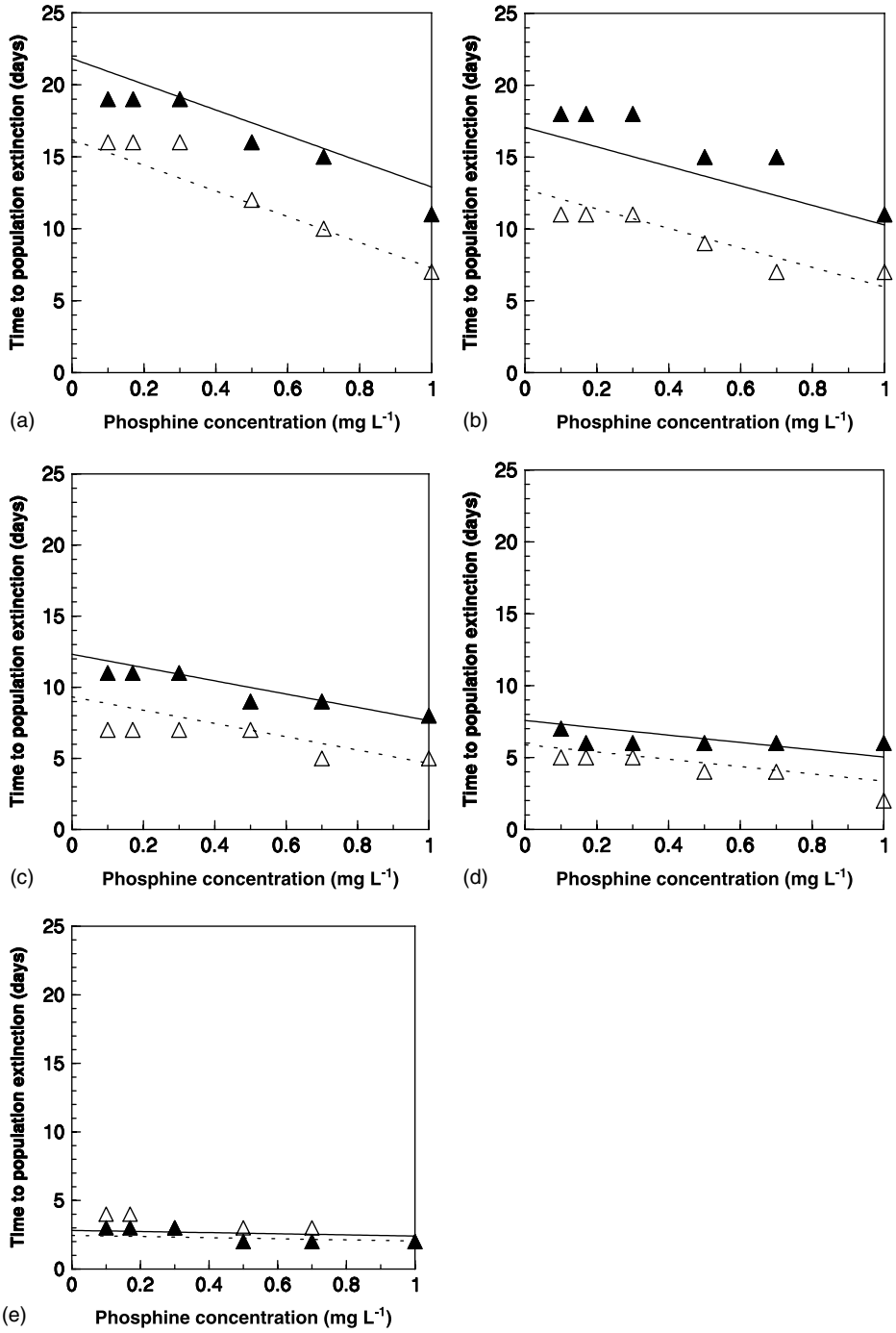

Figure 1. Time to population extinction (in days) of strongly resistant *Liposcelis bostrychophila* at a range of phosphine concentrations and temperatures [(a) 15 °C, (b) 20 °C, (c) 25 °C, (d) 30 °C and (e) 35 °C] and at two humidities (55 and 70% RH); observed data (△ 55%, ▲ 70%) and fitted regressions (dashed line 55%, solid line 70%).

the concentration × RH interaction ($P = 0.745$) did not contribute significantly to the model and so were omitted.

The regression equation is as follows:

$$\begin{aligned}\text{Time to Extinction} = &-8.65(\pm 4.69)\\ &+ 0.279\ (\pm 0.18)(\text{temperature})\\ &- 15.31\ (\pm 1.75)(\text{concentration})\\ &+ 0.6389\ (\pm 0.0734)(\text{relative humidity})\\ &+ 0.4257\ (\pm 0.0675)(\text{temperature}\\ &\times \text{concentration})\\ &- 0.01756(\pm 0.00282)(\text{temperature}\\ &\times \text{relative Humidity})\end{aligned}$$

This equation reflects the fact that an increase in temperature or concentration decreases the time to extinction, while an increase in relative humidity increases the time to extinction (Figs 1a to e).

In general, TPE decreased as temperature and relative humidity increased (Fig. 1). However, increases in phosphine concentration did not always result in concomitant decreases in TPE. At lower temperatures, 15, 20 and 25 °C, there was no change in TPE with increases in phosphine concentration from 0.1 to 0.3 mg L^{-1}, and there were only limited decreases in TPE with increasing phosphine concentration at 30 and 35 °C (Fig. 1). At the higher concentrations, there was a general trend of decreasing TPE with increasing concentration across all temperatures. Shortest TPEs were recorded against highest concentrations of phosphine at the highest temperature. In almost every case, insects fumigated at 70% RH were more tolerant than those fumigated at 55% RH. The maximum time recorded for population extinction of strongly resistant *L. bostrychophila* was 19 days at 15 °C and 70% RH fumigated with phosphine at 0.1, 0.17 or 0.3 mg L^{-1}, whereas the minimum TPE was 2 days for concentrations of 0.5, 0.7 and 1.0 mg L^{-1} at 35 °C and 70% RH (Fig. 1). This minimum time was also recorded for 1.0 mg L^{-1} at 30 and 35 °C at 55% RH. At 35 °C, the difference between the longest and shortest times to population extinction was only 2 days across all concentrations and two humidities.

4 DISCUSSION

The aim of the present work was twofold: to quantify the influence of concentration, temperature and humidity on phosphine toxicity to the psocid *L. bostrychophila*, and to employ these data to establish practical fumigation protocols for use by industry. Of the three variables tested in relation to time to population extinction, temperature exerted the most effect, accounting for almost three-quarters of the variation in response. Irrespective of phosphine concentration, time to population extinction in psocids was longer at lower temperatures and shorter at higher temperatures. Higher relative humidity increased survival of psocids at lower temperatures, but the influence of relative humidity on toxicity was reduced markedly as temperature increased. It is evident that, at any concentration of phosphine, a combination of higher temperature and lower humidity will provide the most effective control of strongly resistant *L. bostrychophila* populations. The observation that phosphine toxicity increases with increasing temperature in *L. bostrychophila* is consistent with data from studies of a range of beetle[21–23,25] and moth[7,24] species. Hole *et al*.[22] suggested that this phenomenon may be due to slowing down of several physiological processes such as metabolic rate and oxygen consumption, which in turn prolong the development of tolerant stages into more susceptible stages. More recently, Chaudhry *et al*.[25] demonstrated a progressive increase in uptake of phosphine in both susceptible and resistant strains of the cigarette beetle, *Lasioderma serricorne* (F.), with increase in temperature from 5 to 25 °C, eventuating in higher mortalities.

Psocids are soft-bodied animals dependent on atmospheric moisture,[26,27] so it is not surprising that relative humidity should influence their survival under the stress of fumigation. In this case, *L. bostrychophila* were more tolerant to phosphine at their optimum relative humidity, 70%,[15] than at a suboptimal 55% RH. This effect was severe at low temperatures but became less so as temperature increased. A likely explanation for this observation is that air at higher temperatures contains more water vapour than air at lower temperatures. For example, at 35 °C, air at 70% RH contains about 25 g kg^{-1} of water vapour, while air at 55% RH contains about 20 g kg^{-1} of water vapour. In contrast, at 20 °C, air at 70% and 55% RH contains only about 12 and 9 g kg^{-1} water vapour respectively.[31]

An unexpected result was the plateau of survival at lower phosphine concentrations (0.1, 0.17 and 0.3 mg L^{-1}). Based on previous responses of beetle species, progressively longer times to population extinction as concentration decreased would have been expected.[5,9] This phenomenon was independent of temperature or relative humidity, as it occurred in all treatments and was not due to a delayed egg hatching effect,[29] as this was accounted for in the experimental design. The authors believe that this result is due to the significantly higher tolerance to phosphine shown by eggs compared with adults and nymphal stages.[13,32] It appears that existing adults and nymphs and any nymphs emerging from eggs are quickly killed at even the lowest concentration of phosphine. However, at these concentrations, eggs are apparently not affected, and time to population extinction is only reached once all eggs have hatched and nymphs are exposed to the toxin. At somewhere between 0.3 and 0.5 mg L^{-1}, a threshold is reached where phosphine begins to have a toxic effect on eggs, and, as concentration increases, times to population extinction progressively shorten.

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Industry experience in Australia is that, although always present in low numbers, outbreaks of *L. bostrichophila* generally occur in rainy periods during summer, when the relative humidity reaches 70–80%, or in situations where grain moisture content is greater than 13%, which is equivalent to about 70% equilibrium relative humidity in wheat.[31] In addition, infestations are more prevalent at maritime export terminals, where atmospheric relative humidity remains high throughout the year. The authors suspect that the cases where current label rates of phosphine have been failing to control the resistant psocid populations may be due to the survival of populations longer than the recommended exposure periods in areas within the storages with equilibrium relative humidity higher than 55%.

The present experiments used the range of minimum concentrations registered in Australia for use with cylinderised phosphine.[33] There are several practical implications from the present study. Firstly, these recommendations, based essentially on the response of strongly resistant *Rhyzopertha dominica* (F.),[5] will be sufficient to control the strongly resistant *L. bostrychophila*, provided that the relative humidity of the storage environment remains at 55%. However, if fumigations are to be carried out at 70% RH at temperatures of 30 °C or less, then longer exposure periods will be needed at several concentrations. For example, at 0.3 mg L^{-1}, exposure periods would have to be increased by 5, 6 and 4 days at temperatures of 15, 20 and 25 °C respectively. Similarly, at the highest registered minimum concentration of 1 mg L^{-1}, fumigation periods would have to be increased by 1, 2, 3 and 3 days at temperatures of 15, 20 and 25 and 30 °C respectively. At 35 °C, however, the effect of high humidity on phosphine was found to be overshadowed by the temperature effect, and the current recommendations for 30 °C at 55% RH should be able to control the strongly resistant psocids at both humidities.

In these experiments, the toxicity of a range of phosphine concentrations under various environmental conditions was explored. The aim was to recommend robust protocols that could be used in a range of practical situations. The concentrations and exposure periods shown in Fig. 1 are the minimum requirements to achieve complete control under the conditions of relative humidity and temperature specified. These protocols provide industry with some flexibility in application of phosphine, depending on conditions. For example, the temperature of the freshly harvested grain in storages sometimes reaches 35 °C in Australia, and a fumigation period of only 2 days will be required at 1.0 mg L^{-1} to control resistant psocid infestations, irrespective of relative humidity. This type of flexibility allows pest managers to operate more effectively within the logistical constraints of a dynamic grain marketing environment. To take advantage of this potential flexibility, however, grain managers must be equipped to sample and measure grain temperature and moisture content and to monitor phosphine concentrations during fumigations to ensure that the minimum requirements for effective fumigation are met.

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