Developing effective fumigation protocols to manage strongly phosphine-resistant *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae)

Ramandeep Kaur\textsuperscript{a,b}\textsuperscript{*} and Manoj K Nayak\textsuperscript{a,b}

**Abstract**

BACKGROUND: The emergence of high levels of resistance in *Cryptolestes ferrugineus* (Stephens) in recent years threatens the sustainability of phosphine, a key fumigant used worldwide to disinfect stored grain. We aimed at developing robust fumigation protocols that could be used in a range of practical situations to control this resistant pest.

RESULTS: Values of the lethal time to kill 99.9% (LT\textsubscript{99.9}, in days) of mixed-age populations, containing all life stages, of a susceptible and a strongly resistant *C. ferrugineus* population were established at three phosphine concentrations (1.0, 1.5 and 2.0 mg L\textsuperscript{−1}) and three temperatures (25, 30 and 35 °C). Multiple linear regression analysis revealed that phosphine concentration and temperature both contributed significantly to the LT\textsubscript{99.9} of a population ($P < 0.003$, $R^2 = 0.92$), with concentration being the dominant variable, accounting for 75.9% of the variation. Across all concentrations, LT\textsubscript{99.9} of the strongly resistant *C. ferrugineus* population was longest at the lowest temperature and shortest at the highest temperature. For example, 1.0 mg L\textsuperscript{−1} of phosphine is required for 20, 15 and 15 days, 1.5 mg L\textsuperscript{−1} for 12, 11 and 9 days and 2.0 mg L\textsuperscript{−1} for 10, 7 and 6 days at 25, 30 and 35 °C, respectively, to achieve 99.9% mortality of the strongly resistant *C. ferrugineus* population. We also observed that phosphine concentration is inversely proportional to fumigation period in regard to the population extinction of this pest.

CONCLUSION: The fumigation protocols developed in this study will be used in recommending changes to the currently registered rates of phosphine in Australia towards management of strongly resistant *C. ferrugineus* populations, and can be repeated in any country where this type of resistance appears.


Keywords: *Cryptolestes ferrugineus*; strong resistance; phosphine; fumigation protocols

1 INTRODUCTION

The fumigant phosphine is used across the globe for disinestation of stored grains and other durable commodities. Its popularity as a fumigant over several decades can be attributed mainly to its ease of application, use on a wide range of commodities, low cost and, most importantly, its universal acceptance as a nearly residue-free treatment.\textsuperscript{1} Although several alternatives have been explored in recent years, they have failed to match the combined advantages offered by phosphine.\textsuperscript{2–5} Moreover, the recent phase-out of the ozone-depleter methyl bromide has left phosphine as the only economically and environmentally viable fumigant for the industry for routine disinestation of stored commodities. However, this overreliance on a single fumigant has resulted in the development of high levels of resistance to phosphine in key storage pest species across the globe.\textsuperscript{6–16}

In Australia, a high level of resistance to phosphine has been reported in a strongly resistant strain of *Rhyzopertha dominica* (F.) (600x) over a 48 h fumigation,\textsuperscript{6} *Tribolium castaneum* (Herbst) (431x) over a 20 h fumigation\textsuperscript{17} and *Cryptolestes ferrugineus* (Stephens) (1450x) over a 72 h fumigation, calculated on the basis of the LC\textsubscript{50} of each strain relative to their respective susceptible counterparts. The level of resistance in *C. ferrugineus* is the highest ever detected in any stored-product insect species\textsuperscript{10} and needs special attention. A proactive approach, however, has resulted in proposed successful management of the strong resistance in *R. dominica*,\textsuperscript{18} *Sitophilus oryzae*\textsuperscript{7} and *Liposcelis bostrychophila*\textsuperscript{11} in Australia through proper characterisation of each of these resistances and development of effective fumigation protocols by manipulation of phosphine concentration, exposure period and temperature. The Australian grain industry is currently facing a problem of highly resistant *C. ferrugineus* surviving currently registered rates of phosphine used in bulk grain storages, particularly in the northern and southern grain belts.\textsuperscript{19} Therefore, we aim to investigate the interaction of a range of phosphine concentrations.
and fumigation temperatures against strongly resistant *C. ferrugineus* and to use this information to establish practical fumigation protocols to manage resistance.

## 2 MATERIALS AND METHODS
The response of mixed-age cultures of *C. ferrugineus* to phosphine was evaluated at three temperatures (25, 30 and 35 °C) and three phosphine concentrations (1.0, 1.5 and 2.0 mg L⁻¹). In total, nine different combinations of temperature and phosphine concentration were tested for exposure periods of up to 19 days, and each combination was replicated 3 or 4 times.

### 2.1 Test insects and preparation of mixed-age cultures
A reference phosphine-susceptible strain (QCF31, population collected from Cecil Plains in Queensland in 1998) and a strongly phosphine-resistant strain (QNCF73, collected from a central stor-...
3 RESULTS

The mortality of mixed-age populations of susceptible *C. ferrugineus* that were fumigated for 1, 2 and 5 days at all temperature and concentration combinations was 100% for all exposure periods. No emergence of susceptible adults was observed after 8 weeks for any temperature and concentration combinations in any exposure periods. Therefore, data obtained from the susceptible strain were not suitable for probit analysis using Wadley's model to calculate LT$_{99.9}$ values.

Probit analysis using Wadley's method of the mixed-age populations of strongly resistant *C. ferrugineus* at each phosphine concentration and temperature provided LT$_{99.9}$ values that decreased concomitantly as phosphine concentration and temperature increased from 1.0 to 2.0 mg L$^{-1}$ and from 25 to 35°C (Table 1, Fig. 1). In general, experimentally observed TPEs for the strongly resistant *C. ferrugineus* were shorter than LT$_{99.9}$. Probit analysis also revealed that efficacies of phosphine concentrations (1.0, 1.5 and 2.0 mg L$^{-1}$) were significantly different, i.e. efficacy was highest at 35°C, as confirmed by 95% CI ratio test. However, mortalities for 2.0 mg L$^{-1}$ were significantly different across all temperatures. The shortest LT$_{99.9}$ (5.59 days) was recorded at the highest phosphine concentration (2.0 mg L$^{-1}$) and highest temperature (35°C). The longest time to kill 99.9% of the strongly resistant *C. ferrugineus* population was 20 days at 25°C for fumigation with 1.0 mg L$^{-1}$ of phosphine. No significant difference was observed in 99.9% mortalities of populations at 30 to 35°C with 1.0 mg L$^{-1}$ of phosphine, as confirmed by 95% CI ratio test (Table 1). The differences between the longest and shortest times to kill 99.9% of a population were 6, 3 and 5 days at 1.0, 1.5 and 2.0 mg L$^{-1}$, respectively, across all temperatures. However, within each temperature the difference between the longest and shortest times to population extinction were 10, 8 and 9 days at 25, 30 and 35°C, respectively, across all three phosphine concentrations.

Multiple linear regression analysis of the strongly resistant *C. ferrugineus* data revealed that phosphine concentration and temperature both contributed significantly to LT$_{99.9}$ ($P < 0.003$, $R^2 = 0.92$), with concentration being the dominant variable and accounting for 75.9% of the variation. The two-way interaction ($P = 0.523$) did not contribute significantly to the model and so was omitted.

The resulting regression equation is as follows:

$$LT_{99.9} = 27.51 (±3.35) − 0.38 (±0.11) \text{ (temperature)} − 27.41 (±3.61) \text{ (log concentration)}$$

Based on this equation, an increase in concentration and temperature lowers the LT$_{99.9}$ of strongly resistant *C. ferrugineus* (Table 1).

4 DISCUSSION

The aim of the present research was to develop robust fumigation protocols that could be used in a range of practical grain storage situations to control strongly resistant *C. ferrugineus* populations. We have established fumigation protocols in the laboratory for three phosphine concentrations (1.0, 1.5 and 2.0 mg L$^{-1}$) and three temperatures (25, 30 and 35°C) for the control of all life stages of strongly resistant *C. ferrugineus*. Our results follow an existing trend of either increasing phosphine concentration or increasing fumigation period for the control of strongly resistant populations. Moreover, it was also established that successful control of resistant *C. ferrugineus* can be achieved using shorter fumigation periods at elevated grain temperatures, irrespective of the concentration used. Based on our data, mixed-age populations of strongly resistant *C. ferrugineus* can be successfully controlled using a 20 day fumigation with 1.0 mg L$^{-1}$ at 25°C, which is similar to the results reported by Wang et al.$^{26}$ According to Wang et al.$^{25}$ an initial concentration of 1.0 mg L$^{-1}$ of phosphine with a further requirement to maintain a concentration above 0.4–0.7 mg L$^{-1}$ for 16–25 days was required to control strongly resistant *C. ferrugineus* in warehouses. In another study, Li and Yan$^{29}$ recommended a phosphine concentration of 0.3 mg L$^{-1}$ for more than 28 days to control strongly resistant *C. ferrugineus* in warehouses. Nayak et al.$^{10,30}$ reported that 1.0 mg L$^{-1}$ of phosphine maintained for 24 days at 20°C was required to attain population extinction of strongly resistant *C. ferrugineus*. The LT$_{99.9}$ times established in the present study for the various temperature

---

Table 1. Effective phosphine protocols to achieve 99.9% mortality in strongly phosphine-resistant *Cryptolestes ferrugineus* in a range of temperatures and concentrations

<table>
<thead>
<tr>
<th>Insect</th>
<th>Temperature (°C)</th>
<th>Phosphine concentration (mg L$^{-1}$)</th>
<th>Deviation</th>
<th>LT$_{99.9}$ (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly resistant <em>C. ferrugineus</em></td>
<td>25</td>
<td>1.0</td>
<td>16.0</td>
<td>19.26 (17.70–23.06)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>1077</td>
<td>11.66 (10.98–15.06)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>1436</td>
<td>9.98 (9.22–13.08)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.0</td>
<td>718</td>
<td>14.13 (13.54–16.36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>1077</td>
<td>10.64 (9.54–13.16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>1436</td>
<td>6.72 (6.05–9.17)</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>1.0</td>
<td>718</td>
<td>6.80 (6.23–12.32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>1077</td>
<td>4.05 (3.76–11.14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>1436</td>
<td>5.14 (3.82–8.79)</td>
</tr>
</tbody>
</table>

* LT$_{99.9}$ values followed by the same lower-case letter across three concentrations for an individual temperature are not significantly different, and LT$_{99.9}$ values followed by the same upper-case letter for an individual concentration across three temperatures are not significantly different, based on the lethal time ratio test (95% confidence intervals [lower and upper levels] for LT$_{99.9}$ are omitted for clarity) (see Section 2.3).

*Replicated observed times to population extinction.

*Replicated 4 times (the other treatments were replicated 3 times).
and concentration combinations for strongly resistant *C. ferrugineus* are much longer than previously established times for other strongly phosphine-resistant stored-product pests. For example, fumigation periods of 5 and 7 days were required for 1.0 mg L\(^{-1}\) of phosphine at 25 \(^\circ\)C to attain population extinction of mixed-age populations of strongly resistant *R. dominica* from Australia\(^{18}\) and India.\(^{31}\) In the Philippines, Sayaboc *et al.*\(^{32}\) reported 98.3 and 99.1% mortality of a strongly resistant population of *R. dominica* in 3 and 7 days at 1.0 and 0.71 mg L\(^{-1}\) respectively, whereas Liang *et al.*\(^{33}\) reported that 99.9% mortality of a strongly resistant Chinese strain was achieved at 0.4 mg L\(^{-1}\) in 9.8 days. Similarly, it was reported that, to achieve population extinction of strongly resistant *S. oryzae* at 25 \(^\circ\)C, protocols of 4 days at 1.0 mg L\(^{-1}\) and 7 days at 0.25 mg L\(^{-1}\) were required.\(^{31}\) According to Nayak and Collins,\(^{11}\) at 15 \(^\circ\)C and 70% RH, 19 and 11 days are required to control the phosphine-resistant *psocids* *L. bostrychophila* using 0.1 and 1.0 mg L\(^{-1}\) respectively. At a higher temperature of 35 \(^\circ\)C and 55% RH, however, only 4 and 2 days of fumigation were required at the respective phosphine concentrations to achieve population extinction of this strongly resistant pest.

In relation to LT\(_{99.9}\) of the strongly phosphine-resistant *C. ferrugineus* and the interactions of the two variables investigated in this study (temperature and concentration), phosphine concentration exerted the maximum effect, accounting for 75.9% of the variation in response. Irrespective of the phosphine concentrations used, LT\(_{99.9}\) and TPE in strongly resistant *C. ferrugineus* were longer at lower temperature and shorter at higher temperature. This observation of increased phosphine toxicity with increasing temperature is in accordance with previous studies undertaken on a range of stored-grain beetles, moths and psocids.\(^{11,16,34–36}\) Based on our data, for example, 1.0 mg L\(^{-1}\) of phosphine is required for 20, 15 and 15 days, 1.5 mg L\(^{-1}\) for 12, 11 and 9 days and 2.0 mg L\(^{-1}\) for 10, 7 and 6 days at 25, 30 and 35 \(^\circ\)C, respectively, to attain 99.9% mortality of the strongly resistant *C. ferrugineus* population. This phenomenon of increased phosphine toxicity with increasing temperature has been correlated with increase in insect respiratory rate, metabolic rate and oxygen consumption in response to increasing temperature, which increases the uptake of phosphine and leads to higher mortalities.\(^{34,36}\) Bond *et al.*\(^{37}\) also demonstrated that environmental factors that lower the rate of metabolism, such as reduced oxygen atmosphere or decreased temperature during fumigation, would lead to increased tolerance to phosphine in insects. Further evidence of this phenomenon was observed in studies of resistant *Caenorhabditis elegans* (Maupas), where it was found that an increase in metabolic rate conferred increased susceptibility to phosphine,\(^{38}\) while a constitutively lowered metabolic rate conferred resistance.\(^{39}\)

The protocols developed in this study aimed at recommending practical minimum fumigation periods and phosphine concentrations that would control all life stages (eggs, larvae, pupae and adults) of strongly resistant *C. ferrugineus* in Australia. The LT\(_{99.9}\) data give us guidelines for achieving a successful fumigation. They can be used to determine the phosphine concentrations required to attain complete control of resistant populations within a certain time period needed for a specified temperature. Failure of fumigation normally occurs when phosphine concentrations are not maintained at the required levels, and generally this is the case for large bunker (pad) storages or old leaky silos, where it is difficult to achieve airtightness. There have been changes to the cylinder phosphine label in Australia over the last decade to address the development of strong phosphine resistance in *R. dominica*. For example, the current label of phosphine (ECO\(_2\)FUME\(^{38}\)) in Australia recommends that, to control a strongly resistant population of *R. dominica*, fumigation with a concentration of 1 mg L\(^{-1}\) of phosphine should be undertaken within a gas-tight storage structure for 10, 9, 5 and 3 days at temperatures of 20, 25, 30 and 35 \(^\circ\)C respectively. Given that they are much higher than the current registered protocols, we suggest that the protocols developed in the present study to manage strongly resistant populations of *C. ferrugineus* need to be incorporated in the label through changes to the current registration. To achieve this, it is imperative that industry-scale trials be undertaken.

In conclusion, protocols developed in the present study provide industry with some flexibility in application of phosphine at a range of temperatures of the stored commodity for management of infestations of strongly resistant *C. ferrugineus*. This type of flexibility allows grain storage managers to operate more economically, provided that the storage structures are properly sealed and gas is monitored during the course of fumigation. In large commercial bulk storage structures, high concentrations of phosphine such as the ones we are suggesting from this study (e.g. 2 mg L\(^{-1}\) for 10 days) can be practically maintained by using a cylinderised formulation (e.g. ECO\(_2\)FUME\(^{38}\) and VAPORPH\(_3\)OS\(^{38}\)). Moreover, connecting a recirculation system enables rapid and

**Figure 1.** Response of mixed-age populations of strongly resistant *Cryptolestes ferrugineus* to phosphine at three concentrations and temperatures. Curves are presented as mortality calculated by probit analysis using Wadley’s model.
even distribution of the gas throughout the storage structure. This approach also has the advantage of topping up the gas during the fumigation period if the monitoring system indicates the loss of gas. Phosphine is generally considered to be a cheap fumigant, and managing strongly resistant C. ferrugineus far outweighs the additional costs involved in the higher dosages such as 2 mg L\(^{-1}\) for 10 days. Moreover, additional costs in the form of higher phosphine dosages are sufficiently justified in the case of fumigating oilseeds and pulses, where use of an alternative fumigant such as sulfuryl fluoride is not applicable. We conclude that, given these advantages, the research output from the present study will help to sustain the usefulness of phosphine into the foreseeable future.

ACKNOWLEDGEMENTS

The authors thank CYTEC, Australia, and the Plant Biosecurity Cooperative Research Centre (Project No. PBCRC3036) established and supported under the Australian Government’s Cooperative Research Centres Programme (http://www.crcplantbiosecurity.com.au) for supporting this research. Thanks also to Dr Gregory J Daglish for his valuable comments on the manuscript, to Hervolka Pavic for technical support and to Kerri Chandra (née Dawson) for statistical advice.

REFERENCES


