Research Article

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High-dose strategies for managing phosphine-resistant populations of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)

Edwin Afful,[a] Tesfaye M Tadesse,[b] Manoj K Nayak[c,d] and Thomas W Phillips[a,d*]

Abstract

BACKGROUND: *Rhyzopertha dominica* is a serious pest of stored grains and many populations have resistance to the fumigant phosphine. Some populations contain beetles with a 'strong resistance' phenotype. Recent work found the LC_{50} values for two strong-resistant populations recently studied in North America, Belle Glade and Minneapolis were 100- and 595-fold higher, respectively, compared to LC_{50} of a lab-susceptible strain. Populations with 'weak-resistant' phenotypes had LC_{50} values 5- to 10-fold that of a susceptible strain. The work reported below aimed to determine the minimum phosphine concentrations and number of days of exposure needed to effectively control all life stages of representative weak- and strong-resistant strains, and then to recommend the treatment conditions needed to control strongly phosphine-resistant *R. dominica* in pest populations.

RESULTS: A dose-mortality assay estimated that phosphine fumigation over 48 h using 730–870 ppm at 25° C would control adults of both strongly resistant *R. dominica* populations. Fumigations with mixed life stage cultures found 200 ppm killed all susceptible and weak-resistant beetles in 2 days, but the strong-resistant Minneapolis and Belle Glade strains had substantial survivors at 200 ppm. Furthermore, the Belle Glade strain had beetles that survived 1000 ppm in 2-day fumigations. The strong-resistant Belle Glade strain needed nearly 10 days at over 400 ppm to have acceptable levels of control.

CONCLUSION: This study recommends protocols to manage strongly resistant *R. dominica* populations provided that a minimum phosphine concentration of 400 ppm be maintained at 25° C or higher for up to 10 days.

Keywords: lesser grain borer; stored products; insecticide resistance; fumigation, *Trtichum asetivum*

1 INTRODUCTION

A common method used to control insect infestations in stored grain and other durable stored products is fumigation with toxic gases. When properly applied, fumigants cause high levels of mortality and leave no or very little chemical residue on grain or food to pose a food safety concern.[1] Phosphine, hydrogen phosphide (PH_3), is the most commonly used fumigant to control many stored product insect pests worldwide. It is relatively inexpensive, easy to apply, allowed for use in many countries and compatible with a wide range of storage structures and commodities when compared to other fumigants.[2] Despite its cost-effectiveness and worldwide application, grain insect pests are evolving resistance to phosphine that requires alternative methods to enhance phosphine's effectiveness against them.

Phosphine resistance in pests of stored grain has been documented over the past 50 years. The Food and Agriculture Organization (FAO) of the United Nations undertook a global survey during 1972 and 1973 to assess the presence or absence of resistance to phosphine in major stored grain pests.[3] This survey reported that approximately 10% of the tested populations across several species had phosphine-resistant individuals.[3] Several researchers also reported higher frequencies of phosphine resistance in a range of stored product pests in several countries such as Morocco,[4] Brazil,[5] Vietnam,[6] China,[7] Australia[8–10] and Pakistan.[11] There are now at least 15 insect species known to have evolved resistance to phosphine[4,12,13] with a worldwide distribution.[4] Low levels of resistance to phosphine in stored-grain insects collected in the state of Oklahoma (USA) were first reported in the 1980s.[14] Thirty years later, high frequencies of resistance were found in the lesser grain borer, *Rhyzopertha dominica* (F.), and the red flour

* *Correspondence to: TW Phillips, Plant Biosecurity Cooperative Research Centre, GPO Box 5012, Bruce, ACT 2617, Australia. E-mail: twp1@ksu.edu*

a *Department of Entomology, Kansas State University, Manhattan, KS, USA*

b *Department of Plant Science, Bahir Dar University, Bahir Dar, Ethiopia*

c *Department of Agriculture and Fisheries, Queensland Ecosciences Precinct, Brisbane, Australia*

d *Plant Biosecurity Cooperative Research Centre, GPO Box 5012, Bruce, ACT 2617, Australia*

beetle, *Tribolium castaneum* (Herbst), in Oklahoma[15] and in several other locations in the USA and Canada.[16,17]

Phosphine resistance studies conducted in Australia over the past decade elaborated the genetic foundation for resistance and characterized two relatively distinct resistance phenotypes of *T. castaneum* and *R. dominica*, the so-called 'weak' and 'strong' resistance phenotypes.[18,19] These studies reported that beetles with the weak phenotype required phosphine concentrations of 10- to 50-fold greater than those needed to kill susceptible beetles, while beetles with the strong phenotype required 100-fold or greater concentrations relative to that required to achieve similar mortality levels of susceptible beetles exposed to the lower doses.[20] Genetic analysis of these insect species from Australia identified two unlinked genetic loci conferring strong resistance in both *R. dominica* and *T. castaneum*.[21–23] The first locus, *rph1*, is responsible for 'weak' resistance, whereas the second locus, *rph2*, acts synergistically with *rph1* to confer 'strong' resistance when resistance alleles are homozygous at both loci in an individual.[19] Pest populations with large numbers of strongly resistant insects cannot be adequately controlled using continuous fumigation with phosphine, and practical alternatives to phosphine are very limited.

Previous research showed that modifications to recommended phosphine concentration and exposure periods can be used to successfully control phosphine-resistant populations of several insect species.[18,24–27] For example, a recent study in Australia found that fumigation protocols in the laboratory with three phosphine concentrations (1.0, 1.5 and 2.0 mg L^{-1}; or 714.8, 1072.2 and 1429.6 ppm) and three temperatures (25, 30 and 35 °C) could control all life stages of a strongly resistant strain of the rusty grain beetle *Cryptolestes ferrugineus* Stevens. Findings of that study stipulated that successful control of resistant *C. ferrugineus* can be achieved using shorter fumigation periods at elevated grain temperatures, irrespective of the concentrations used.

R. dominica is one of the most destructive pests known to damage stored grain, and it has a record for phosphine resistance.[17,18,22,23,28,29] Both the larvae and adult stages cause damage to stored grain by boring irregularly shaped holes into undamaged kernels and larvae develop inside kernels. We recently reported that 32 out of 34 North American populations of *R. dominica* had frequencies of resistance ranging from 4 to 97% after testing adult beetles with a discriminating dose assay.[17] Populations in Kansas and Florida had beetles with the 'strong' resistance phenotype, which were characterized with resistant factors between 100 and 595-folds higher than those of susceptible strains and approximately 10-fold compared to their 'weak' resistant counterparts.[17] The concentration–mortality experiments that showed the 'strong' resistance phenotypes had the highest treatments at 1000–2000 ppm and were unable to kill more than 80 or 40% of tested beetles from the Minneapolis, Kansas and Belle Glade, Florida populations, respectively. Those results lead us to conclude that these *R. dominica* were among the strongest resistant insects known from recent studies. However, these bioassays were conducted only with adults and the phosphine exposure time was only 20 h. It is well known that the toxicity of a fumigant is a function of the gas concentration, the exposure time, the species and life stage when all other variables are equal.[1] Our data at that time suggested that beetles from these two strongly resistant populations would be very difficult to kill. However, this situation was an opportunity to assess methods for effective control using phosphine at higher concentrations and long exposure periods.

The aim of our study was therefore to determine the minimum gas concentrations and number of days of exposure needed to effectively control all life stages (adults, larvae, pupae and eggs) of strongly phosphine resistant populations of *R. dominica* from the USA, and to propose recommendations for phosphine resistance management of this grain pest. The specific objectives of our experiments were:

1. To re-characterize strong resistance in *R. dominica* adults from previously studied North American populations, Minneapolis and Belle Glade using dose-mortality experiments with 48 h exposure rather than the 20 h used in our earlier study.
2. To evaluate the efficacy of both high and low concentrations of phosphine applied over 48 and 96 h exposure periods to mixed life stages of laboratory colonies representing 'weak' and 'strong' resistant populations from a past study.
3. Determine the number of days of fumigation needed for complete control of mixed life stages of laboratory colonies from Minneapolis and Belle Glade populations when exposed to relatively high or low concentrations of phosphine.

2 MATERIALS AND METHODS

2.1 Rearing of insects

Stock cultures of insects used for this study originated from field collections undertaken in our recent survey.[17] Insects were propagated in the laboratory for 2 years on a laboratory diet of 95% whole wheat kernels and 5% wheat flour under standard conditions at 28 °C and 65% relative humidity (RH) with a photoperiod of 16 h light and 8 h dark.[17] The phosphine-susceptible reference laboratory strain, referred to as 'USDA' in the current study, originated from a colony maintained at the United States Department of Agriculture (USDA), Center for Grain and Animal Health Research, Manhattan, Kansas, for over 40 years. In our previous work, we evaluated five populations for 'weak' vs 'strong' levels of resistance using LC_{50} values from dose-mortality experiments.[17] Laboratory colonies from these five field populations (see below) and the USDA strain were maintained following the same rearing method as described previously.[17] *R. dominica* adults were cultured on a mixture of 95% whole wheat kernels and 5% wheat flour in 473-mL Ball wide-mouth Mason jars (Hearthmark, LLC, Fishers, IN, USA). These jars have a ring-lid equipped with a metal screen and filter paper to prevent the adults from escaping but that allows air and moisture exchange. The culturing jars were held in a growth chamber at constant environmental regimes of 28 °C, 65% RH and a photoperiod of 16 h:8 h (light:dark) until used in a fumigation assay. Adults from these colonies were used for the initial concentration–mortality assays at 48 h exposures as described below. All other experiments reported here used mixed life stage cultures in 100-mL ventilated glass jars on a mixture of ~30 g of whole wheat kernels (*Tritichum aestivum*) and ~10 mg wheat flour. Fifty mixed sex adults from each population were added to each jar separately and kept in a growth chamber at the constant regimes described above for 6 weeks to have a mixture of all life stages for fumigation. *R. dominica* are long-lived adults[30] and cultures held for 6 weeks are expected to have the initial 50 adults, plus eggs laid daily by those initial females. All other immature life stages developing in the grain, and any new F1 adults that may emerge by the end of the 6 weeks, were expected to be present in the jar. Thus, each mixed life stage culture jar was intended to represent a small population that could possess a range of phosphine susceptibility.

2.2 Fumigation protocol

Fumigations followed our previous methods with some modifications.[17] Fumigations were conducted in a growth chamber kept at 25 °C and RH of 70%. Phosphine gas was from a pressurized cylinder of 10 000 ppm (1% in nitrogen), purchased from Linweld (Lincoln, NE, USA), that was diluted to a needed concentration at the time of an experiment. Fumigation chambers were 3.8-L glass jars, airtight and equipped with an injection port in the center of the metal screw-on lid. This port was fitted with a rubber injection septum that was used for the introduction and sampling of the fumigant.[15] The concentration of phosphine in each of the jars was measured at the beginning and end of each fumigation period using quantitative gas chromatography with a flame photometric detector (GC–FPD) as described below. The average between the starting concentration and the ending concentration during each fumigation period was the designated concentration for a given jar.

2.3 Quantitative analysis of phosphine concentrations with gas chromatography

In analyzing and quantifying the exact concentrations needed for each fumigation, we followed methods used recently, with slight modifications.[16] Phosphine concentrations were analyzed using a Shimadzu GC-17A (Shimadzu Scientific Instruments, Columbia, MO, USA) gas chromatograph and an integrator-strip chart recorder to report peak areas and retention times of analytes.[16] The GC was equipped with a GS-Q column (30 m long × 0.53 mm internal diameter, 0.25 μm film thickness; J and W Scientific, Folsom, CA, USA) and the FPD set for detecting only phosphorus. The carrier gas used was ultra-high purity helium (Linweld, Lincoln, NE, USA). To determine the phosphine concentration in each jar, an external standard curve was generated. The GC detector response measured as the area under the curve for an injected sample of phosphine was converted into the estimated ppm concentration of PH_3 in a jar. A 500-ppm phosphine standard was achieved by the dilution of a carefully measured volume of the 1% phosphine mixed into a carefully measured volume of air in a Tedlar PVF film bag (CEL Scientific Corp., CA, USA). Peak areas of FPD responses from injections of this standard gas at volumes of 25, 20, 15, 10 and 5 μL were analyzed, with the 15 μL injection set at 500 ppm. GC peak areas were used to make a standard curve from which the linear equation calculated the ppm of phosphine in a given fumigation jar from the area integrated under the GC peak for a given sample injection.

2.4 Concentration–mortality assay

Our previous study estimated the LC_{99} concentrations for strongly resistant *R. dominica* using 20 h fumigations, which are not typical for commercial fumigation in practice.[17] Dose–response experiments were carried out in the current study to predict the LC_{99} with Probit analysis as before, but using a 48 h exposure period that is typical for many commercial fumigations. Our intention was to determine a range of gas concentrations we could use on mixed life stage cultures of the Belle Glade and Minneapolis strains along with populations representing 'weak' resistance and susceptible phenotypes. Concentration–mortality data for Belle Glade and Minneapolis populations were generated by exposing adults to an incremental range of phosphine concentrations for 48 h. A similar protocol was followed for the USDA lab strain with the aim of estimating the lethal concentrations (LCs) required for achieving mortality of 50% and 99% of the tested individuals in each of the populations. A minimum of 50 adult insects in ventilated vials were placed in fumigation jars and three separate jars were set up at each target concentration and held at 25 °C for the 48 h exposure. After ventilation, the jars were maintained at 27.5 °C and 70% RH in clean air. The phosphine concentrations targeted against the USDA lab susceptible population were 0.5, 1, 1.5 and 2 ppm, while the two populations with the strong-resistant phenotype were tested with target concentrations of 50, 100, 200, 300, 400, 500, 650, 750, 850, 900 and 1000 ppm. Recorded average concentrations quantified for the fumigation jars used in these experiments ranged from 0.58 ppm tested against susceptible populations up to 994.07 ppm of phosphine for the strong-resistant populations. After fumigation, insects were removed from the jars and given a 14-day recovery period in the growth chamber. Using PROC PROBIT from SAS version 9.4,[31] the LCs were estimated from a Probit regression analysis. Comparison among populations for differences in their level of resistance were made after computing resistant ratios based on the LC_{50} value for the population of interest divided by the LC_{50} of the laboratory-susceptible strain, referred to as the RR_{50}.

2.5 Effects of phosphine at 200 and 1000 ppm for control of *R. dominica* mixed life stage cultures

Based on the results from the 48 h concentration–mortality assay of adults, we decided to test the effect of phosphine concentrations on mixed life stages on all six populations studied for 'weak' vs 'strong' resistance in our past study.[17] The six beetle populations by name and resistance phenotype were as follows. The USDA strain and Starbuck were the phosphine susceptible phenotype, Junction City and Wamego represented the weak-resistant phenotype, and Minneapolis and Belle Glade represented the strong-resistant populations. Target concentrations of 200 and 1000 ppm were selected as they encompass the effective concentrations determined from the adult assays at 48 h. Each target concentration was replicated three times with an air control treatment for each of the populations. Average concentrations for each fumigation jar for the target concentration of 200 ppm were recorded as 269.25, 297.21 and 282.68 ppm while those targeting 1000 ppm were measured as 774.41, 931.88 and 857.90 ppm. Mixed life stage colonies of each population were obtained using the rearing protocol described above and fumigation was done for 48 h. After fumigation, all *R. dominica* adults were sieved and discarded. Each jar was then assessed weekly for emerged live adults over 6 weeks for both the treated and untreated jars. The total number of live adult progeny was determined for each jar and the mean values and standard errors calculated for each population.

2.6 Effects of phosphine concentrations and different exposure times on strong-resistant *R. dominica* mixed life stages

Mixed life stage cultures, small populations containing a mixture of all immature life stages and adults, of the strong-resistant populations from Belle Glade and Minneapolis were not completely killed with 1000 ppm and 200 ppm concentrations for 48 h fumigation, respectively. We hypothesized that 100% kill could be achieved with a treatment determined from experiments investigating a range of gas concentrations and exposure times.

Two experiments were conducted to meet this objective. First, the effect of varied phosphine concentrations on the progeny emergence of *R. dominica* after a 96 h fumigation was assessed. Target concentrations were 150, 300, 450, 600, 750, 900, 1050 and

Table 1. Probit analysis for mortality of adult *Rhyzopertha dominica* from two resistant field populations and one laboratory susceptible strain to varying concentrations of phosphine over 48 h exposure at 25° C

Population	LC_{50} (95% FL) ppm	LC_{99} (95% FL) ppm	Slope ± SE	Intercept±SE	RR_{50}	χ^2 (df)	*N*	*P* value
Minneapolis	495.16 (363.48–552.54)	732.84 (696.64–813.28)	5.93 ± 1.49	36.82 ± 9.74	761.78	35.3 (19)	50	0.96
Belle Glade	602.57 (570.72–625.72)	870.12 (836.55–921.18)	6.33 ± 0.68	−40.53 ± 4.49	927.03	16.02 (13)	50	0.25
USDA	0.65 (0.62–0.69)	1.32 (1.15–1.66)	7.61 ± 0.96	1.40 ± 0.18	1.00	7.04(4)	50	0.13

1200 ppm with two replications (i.e. two fumigation jars) each, and two jars with untreated control cultures exposed only to air under the same rearing conditions. Recorded average concentrations of phosphine quantified for fumigation jars ranged from 150.07 ppm up to 1423.70 ppm. Immediately after the 96 h fumigation all adults were sieved and discarded. The number of adult insects that emerged weekly for 6 weeks following fumigation were counted in both treated and untreated jars. Non-linear regression models $\ln y = a + bx^{1.5}$ and $\ln y = a + be^{-5}x^{1.5}$ were fitted to the total number of adult progeny (y) as a function of concentration (x) for Minneapolis and Balle Glade strains, respectively, using TableCurve 2D software version 5.01. Systat software, Inc. (San Jose, CA, USA) and graphs were plotted using SigmaPlot, version 12.5. (Systat Software, Inc.).

The second experiment assessed the role exposure time plays in the control of strongly resistant populations of *R. dominica*. Target concentrations of 150 ppm and 300 ppm were categorized as 'low dose' and 'high dose' and were selected based on results from the previous experiment on a range of concentrations at 96 h exposure. The exposure times to phosphine for this experiment were 0 (air control), 2, 4, 6, 8 and 10 days with two replications. The measured mean ± SE concentration in the jars for the 'low dose' was 232.71 ± 15.90 while for the 'high dose' it was 481.44 ± 36.15 over the course of the experiment. The numbers of adult insects that emerged from both treated and untreated jars weekly for 6 weeks were counted. Nonlinear regression models, $y^{0.5} = a + bx$ and $\ln y = a + bx$, were fit to number of progeny (y) as a function of days of exposure (x), for Minneapolis strains at low and high dose, respectively, and nonlinear regression models $y = a + bx^{0.5}$ and $\ln y = a + bx^2$ were fitted to the number of progeny (y) as a function of days of exposure (x) for Belle Glade strains at low and high dose, respectively, using TableCurve 2D software version 5.01. (Systat Software, Inc.). The parameters a and b were estimated by fitting equations to progeny production data as a function of days of exposure. SigmaPlot, version 12.5. (Systat Software, Inc.) was used to plot the regression graphs.

3 RESULTS

3.1 Concentration–mortality assay

Concentrations of phosphine estimated to kill 50% and 99% of the laboratory-susceptible *R. dominica* strain were compared with Belle Glade and Minneapolis populations. The RR_{50} values for Minneapolis and Belle Glade were 761 and 927-fold, respectively, compared to the susceptible laboratory strain (USDA). The three Probit regressions reported in Table 1 had a good fit of model to the data collected, with P values > 0.05 for the X^2 values in each case. The LC_{50} and LC_{99} values and their associated fiducial limits were within the range of phosphine concentrations, approximately 363–921 ppm, used commercially.[1] In contrast to our original concentration–response studies using 20 h exposure,[17] this 48 h exposure resulted in 100% kill of adult beetles at the highest concentrations tested for these populations, 781 ppm for Minneapolis and 824 ppm for Belle Glade.

Table 2. Mean number of adults emerged from mixed life stage cultures of *R. dominica* representing different phosphine-resistant phenotypes 6 weeks after exposure to 200 ppm of PH_3 for 48 h compared to untreated control

	Living adults emerged	Living adults emerged
Population, phenotype	Mean from treated jars ± SE[a]	Mean from control jars ± SE
USDA, susceptible	0	715.67 ± 6.88
Starbuck, susceptible	0	506.67 ± 8.67
Junction city, weak resistance	0	492.67 ± 12.99
Wamego, weak resistance	0	480.33 ± 0.33
Minneapolis, strong resistance	263.67 ± 8.08	430.67 ± 4.48
Belle glade, strong resistance	296.67 ± 11.01	489.33 ± 4.70

[a] Pairwise comparisons of adults emerged from treated jars compared to untreated control jars were all significantly different (*t*-test; $P < 0.05$).

3.2 Effects of phosphine on mixed life stages of six *R. dominica* populations at 200 and 1000 ppm at 48 h exposure

The mean number of adult progenies emerged was lower at both phosphine concentrations compared to the number of adults emerged in untreated control cultures. Phosphine at 200 ppm for 48 h completely suppressed progeny production in the USDA susceptible strain and the susceptible field population from Starbuck, and in the two weakly resistant populations, Junction City and Wamego (Table 2). High numbers of adult progenies were produced in cultures of strongly resistant populations of Belle Glade and Minneapolis that were exposed to 200 ppm phosphine. However, the mean adult progeny emergence was significantly lower compared to that in the untreated controls (Table 2). Belle Glade was the only population tested that did not have complete control when fumigated with a target of 1000 ppm for 48 h, which suggests immature stages of this strong-resistant population were more difficult to kill than adults (Table 3).

3.3 Effects of phosphine on emergence of progeny of two strongly resistant *R. dominica* populations exposed for 96 h

Emergence of progeny from both strongly resistant *R. dominica* populations, Belle Glade and Minneapolis, fumigated for 96 h over a range of phosphine concentrations from 150 to 1200 ppm is shown in Fig. 1. The regression plots show a strong relationship between concentration and progeny emergence in both populations. With the increase in target concentration, there was a significant reduction in the number of progenies produced.

Table 3. Mean number of adults emerged from mixed life stage cultures of *R. dominica* representing different phosphine-resistant phenotypes, 6 weeks after exposure to 1000 ppm of PH_3 for 48 h compared to untreated control

	Living adults emerged	Living adults emerged
Population, phenotype	Mean from treated jars ± SE[a]	Mean from control jars ± SE
USDA, susceptible	0.00	628.67 ± 15.59
Starbuck, susceptible	0.00	409.67 ± 5.36
Junction city, weak resistance	0.00	433.33 ± 15.62
Wamego, weak resistance	0.00	377.00 ± 9.71
Minneapolis, strong resistance	0.00	352.33 ± 6.06
Belle glade, strong resistance	143.00 ± 15.58	388.67 ± 9.24

[a] Pairwise comparisons of adults emerged from treated jars compared to untreated control jars were all significantly different (*t*-test; $P < 0.05$).

Target concentrations above approximately 770 ppm resulted in no emergence of progeny in the Belle Glade population, whereas any concentration greater than 400 ppm was sufficient to completely suppress adult progeny production from the Minneapolis population.

The effects of a 'low dose' exposure with an average of 232 ppm and a 'high dose' exposure to about 481 ppm phosphine concentrations on adult progeny emergence from treated mixed life stage colonies of the strong resistant Belle Glade and Minneapolis populations after fumigation for 2, 4, 6, 8 or 10 days at those concentrations are summarized in Fig. 2. As the length of exposure increased, the number of progenies emerged decreased in the colonies from both populations. However, we were unable to achieve 100% suppression of adult progeny emergence from the colonies of either population in a 10-day period when using the low dose that averaged 232 ppm concentration across all the jars. The Belle Glade colonies were reduced by 78.5% after 10 days of exposure at the low dose, while the Minneapolis colonies had a larger reduction, 95.6% at the same concentration. However, phosphine applied at high concentration of 481 ppm for 6 days and longer resulted in total elimination of the Minneapolis colonies. The Belle Glade population had a 100% reduction after exposure to high concentration for both 6 and 10 days, but there was 2.5% beetle survival after 8 days of exposure to high gas concentration. Of the two jars held for 8 days, one jar had no survivors and was recorded with an average phosphine concentration of 549.6 ppm, while the second jar averaged 392.0 ppm and had 29 surviving adults.

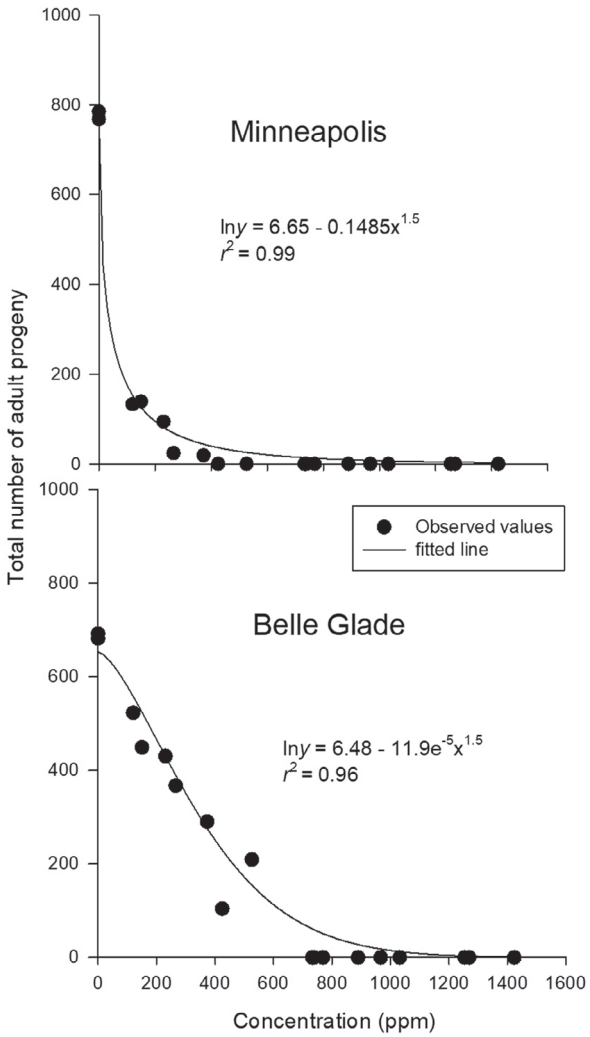

Figure 1. Effects of phosphine concentrations on progeny emergence of Belle Glade and Minneapolis populations over 96 h exposure periods at 25° C.

4 DISCUSSION

The results reported here provide the following information regarding use of phosphine for controlling and managing phosphine resistance of *R. dominica*. (i) Concentration–mortality experiments and calculations of resistance ratio values are important to characterize resistance phenotypes of *R. dominica*, but alone are not adequate to develop a useful concentration and exposure time to achieve high mortality of a given life stage. (ii) Insects with the weak resistance phenotype for phosphine resistance can be controlled at an application rate of 200 ppm or higher exposed for 48 h or longer, which is a treatment plan that could be effective for all grain pests, whether susceptible or resistant to phosphine, as per common application guidelines.[32] (iii) Our concentration–mortality experiments indicated that strongly resistant *R. dominica* adults can be controlled at 48 h of exposure with approximately 1000 ppm of phosphine, but mixed life stage experiments showed that one of the two strong-resistant populations could survive at the same treatment. (iv) Mixed life stage experiments in which phosphine concentrations were kept relatively constant at different exposure times, or with a fixed exposure time tested with different gas concentrations, showed that a strong-resistant *R. domininca* population like Minneapolis could be controlled with a minimum 4-day exposure time at a concentration of 500 ppm or higher. (v) Our results could support a recommendation to control strong-resistant *R. dominica* using a high phosphine concentration and a longer exposure time. However, populations like Belle Glade pointed to the risk that such a treatment may not result in complete control, and that a small number of strongly resistant beetles could survive and develop into the next generation.

Stored grain integrated pest management (IPM) suggests fumigators collect as much information as possible about the phosphine resistance status of pests at a given location prior to fumigation, whether from historical records of efficacy at the

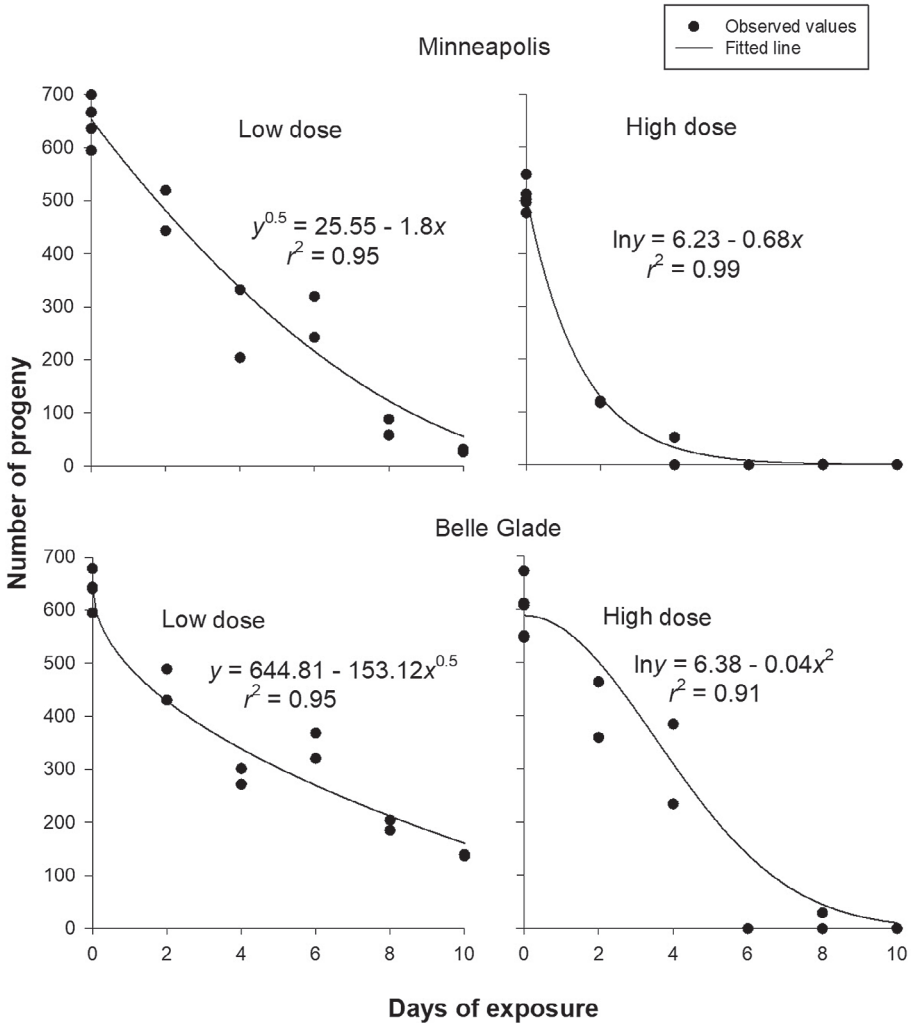

Figure 2. Effects of exposure time on survival and emergence of adult progeny of *R. dominica* emerged 6 weeks after a given exposure time to target phosphine concentrations of 150 ppm (low dose) or 300 ppm (high dose), which in fact averaged 232 ppm and 481 ppm, respectively.

site or from more direct measurements for resistance prior to the treatment.[1,12,20] If a facility was fumigated in the past with records of acceptable control each time, then the fumigator could proceed with a good fumigation using application methods from the past, especially regarding gas concentration and hold time. Any information on failed fumigations or suboptimum success in past fumigations should direct the fumigator to making the next phosphine fumigation one that could eliminate the current pest populations. If past failures are due to resistant insects, rather than to excessive leaks or incorrect application rates, then the next treatment could be one recommended for weak-resistant insects, such as the *R. dominica* characterized here and in past work.[15,17,18]

Our results suggest that a treatment of 200 ppm or greater held for 48 h or longer at a temperature of 25 °C or higher should control all life stages of weak-resistant *R. dominica*. Successful fumigation of commercial structures requires that any targeted treatment for weak-resistant beetles not be compromised during its course to have either less gas, a shorter hold time or at a cooler temperature. We recommend that gas concentration and hold time be increased as much as possible to ensure successful control. A study in Western Australia demonstrated consistent success over several years in controlling weak-resistant populations of different insect pest species in wheat.[33] A key IPM component in that work was monitoring resistance levels in commercial storage bins and then applying an adequate treatment protocol for weak phosphine resistant insects.[33] Same-day 'quick test' bioassays to assess resistance status on samples of insects prior to fumigation have been developed for *Cryptolestes ferrugineus*,[34] *Triboloium* castaneum[35] and *R. dominica*.[36] Such techniques are critical IPM tools to determine if resistant insects are present and to make decisions for application of higher concentration and longer exposure time treatments if needed. Prior information or a same-day quick test of sampled beetles could likely help control populations of *R. dominica* like those studied here from Junction City and Wamego.

Our data show that high phosphine concentrations with long hold times can achieve control in mixed life stage colonies of strong-resistant beetles. Afful *et al.*[17] characterized phosphine resistance phenotypes for the strongly resistant Minneapolis and Belle Glade populations of *R. dominica* by using a concentration–mortality assay with a 20 h exposure. Probit analysis on those data estimated extremely and unrealistically high LC_{99} values, such as 59 681 ppm for the Minneapolis population and 8086 ppm for Belle Glade. We had surviving adults in both populations at concentrations over 1000 ppm, but we can now attribute those survivors to the short exposure time of that 20 h assay. Similar experiments on adults reported here

used a 48 h exposure with a range of phosphine concentrations that resulted in estimated concentrations less than 1000 ppm for LC_{99}s. The resistance ratio factors computed here from the new LC_{50} values for Minneapolis and Belle Glade populations indicated these were 761- and 927-fold more difficult to kill compared to the USDA-susceptible strain, compared to RR_{50}s of 100 and 596, respectively, reported in our 2017 paper based on 20 h exposure times[17] Immobile life stages of grain insects, the eggs and pupae, are known to be much less susceptible to phosphine compared to adults[18] so any high-dose treatment for strong-resistant pests would require that gas concentrations and hold times should be greater than suggested by our RR_{50} values derived from adult testing.[1,18,20]

It is commonly accepted that the control of any pest population with a fumigant at a given temperature will require a gas concentration maintained for a given time to completely kill the most tolerant life stages of the target pest. Experiments with our two most strongly resistant populations of *R. dominica* investigated mortality when exposed for 96 h (4 days) to several different gas concentrations (Fig. 1) and when insects in chambers with relatively constant gas concentrations were held for different exposure times, from 2 to 10 days (Fig. 2). Results with the Belle Glade beetles had complete control of mixed life stages at about 1000 ppm held for 4 days, or 500 ppm held for 6–10 days. Phosphine 'doses' representing a combination of an effective concentration held for a minimum effective time, sometimes referred to as a 'concentration–time product', are known for strongly resistant populations of other storage pest species. All our work was done at 25 °C, but it is likely we could achieve good kill at shorter exposure times and lower gas concentrations if the exposure temperature was increased. Kaur and Nayak[24] controlled a strongly resistant strain of *C. ferrugineus* with 1.0 mg L^{-1} (719 ppm) of phosphine held for 20, 15 and 15 days, 1.5 mg L^{-1} (1078 ppm) for 12, 11 and 9 days and 2.0 mg L^{-1} (1438 ppm) for 10, 7 and 6 days at temperatures of 25, 30 and 35 °C, respectively. Similarly, fumigation periods of 5 and 7 days were required for 1.0 mg L^{-1} (719 ppm) of phosphine at 25 °C to attain population control of mixed-age populations of strongly resistant *R. dominica* from Australia and India.[37,38] Fumigation experiments in the current study were done at 25 °C and the phosphine concentration needed to eradicate strongly resistant individuals in 10 days held at 500 ppm, which was about half the concentration required for control of *C. ferrugineus* and similar to what was required to completely kill all strong-resistant *R. dominica* in India and Australia.

The approved application rates of phosphine in the USA range from a minimum of 200 ppm up to 3625 ppm under temperature conditions of 25 °C and minimum fumigation period of 48 h for non-fresh commodities.[32] From our study, the concentrations we generated are in line with these recommended rates, making phosphine still usable as a fumigant to manage resistant *R. dominica*. Information in the present study can provide the grain industry with some options in application of phosphine at a range of concentrations and exposure times for management of infestations of strongly resistant *R. dominica*. This type of flexibility allows grain managers to operate more effectively and economically, provided that they observe good fumigation practices such as proper sealing of bins and monitoring of gas during fumigation. Despite encouraging results from laboratory studies like these for controlling strongly resistant insects with high phosphine concentrations held for many days, the commercial application of such treatments to large bulks of stored grain may face practical challenges. Fumigators must be familiar with the level of resistance in a specific population needing control and then be prepared to apply sustained high concentrations of phosphine for long periods. Such high-dose treatments will likely do best using a cylinderized formulation of phosphine to apply gas from outside the bin and maintain the needed concentration by periodic addition of gas based on effective measurements of gas concentration.[1,20] However, continued application of phosphine at normal recommended rates against phosphine-resistant insects will likely result in continued selection for strongly resistant insects in the future.

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