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COMBATING RESISTANCE TO PHOSPHINE IN AUSTRALIA

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ABSTRACT

Fumigation with phosphine (PH_3) is a key component in the management of insect pests in stored grain in Australia. However, the almost exclusive reliance on this chemical has resulted in the development of strong resistance in several major pest species. Since first detecting this problem about three years ago, we have developed a research strategy aimed at combating this resistance. The most important elements of this strategy include continued resistance monitoring, development of fumigation protocols effective against resistant insects at various temperatures, studies of the relative fitness of resistant insects, and the development of a rapid test for resistance using molecular biology techniques. Research has concentrated on characterising resistance in *Rhyzopertha dominica*, *Sitophilus oryzae* and the psocid, *Lipocelis bostrychophila*, with strongest resistance levels. We found that three of the five currently registered PH_3 application rates were not effective against resistant beetles and the higher doses could not control resistant *L. bostrychophila*. Experiments at 25ºC using a continuous flow application method revealed non-linear relationship between PH_3 concentration and exposure time with all species responding in a similar pattern, although there were differences between species. Protocols specifying minimum concentrations and exposure periods were developed experimentally and then verified with field trials. Phosphine was more effective at higher temperatures (30 and 35ºC) and less effective at 20ºC. A major factor in the survival strategy of psocids is the marked delay in egg hatch under PH_3 fumigation. There was no apparent fitness deficit associated with the resistance genotype. Two separate loci have been identified as responsible for strong resistance in *R. dominica* using genetic linkage mapping. Several DNA markers residing within 0.6 map units of the resistance loci have been identified.

INTRODUCTION

Trading grain internationally is a highly competitive business. Australia contributes only about 7% of the world trade but this represents about 80% of our total production. To maintain a competitive edge, the Australian grain industry

endeavours to market a predictably high quality product. An important aspect of high quality is freedom from insect infestation and damage. Initially, grain protectants were widely used to achieve freedom from insects in Australia (van Graver and Winks 1994) but with the increasing reluctance of markets to accept pesticide residues and the growing problem of resistance to protectants (Collins 1998), fumigants have been favoured as a more acceptable and effective alternative. The high health and safety risks and potential danger to the environment associated with many fumigants, however, disqualifies them for use on stored-products both in Australia and internationally (Korunic 1993). Other alternatives such as controlled atmospheres are currently too slow to act and too expensive to be widely implemented in our system. These limitations coupled with the restricted use and phasing out of methyl bromide, have inevitably led to a situation where PH_3 has become the sole chemical treatment for 80% of the Australian grain harvest. The obvious question is: how long can, this situation last before a resistance arises in target pests that will jeopardise the future use of phosphine? Of course, Australia is not alone in this predicament, as fumigation with PH_3 is a major component of most grain storage systems worldwide. There are a number of alternative fumigants being developed for stored grain, for example, carbonyl sulphide (Desmarchelier 1998; Tan Xianchang *et al*. 1999), ozone (Mason *et al*. 1999), hydrogen cyanide and ethyl formate (Haritos *et al*. 1999). However, none of these candidates possesses the combined benefits of PH_3: ease of use, low cost, safe application to most stored commodities, and world-wide acceptance as a residue free treatment. Phosphine is particularly important in warm temperate to tropical regions where cooling stored grain is a less economically attractive management technique.

Reports of strong resistance to PH_3 in several pest species from various locations in Asia (Price and Mills 1988; Sayaboc and Gibe 1997; Pike 1994a), with the implication that these resistances may also occur in Australia, has motivated a nation-wide resistance monitoring program to provide industry with early warning of impending problems (Emery these proceedings). Strong resistance to PH_3 was first detected in Australia in 1997 in the lesser grain borer, *Rhyzopertha dominica* and in the flat grain beetle, *Cryptolestes ferrugineus* (Collins 1998). Resistance in *R. dominica* has been detected both on farms and in central storages, and in regions thousands of kilometres apart (Wallbank and Farrell in press). These new resistances are significantly higher than resistance previously detected (Daglish and Collins 1999) which can be regarded as 'weak', that is, controllable in fumigations using registered application rates. Economically important resistance has also been reported in more than one psocid species but resistance is particularly strong in the ubiquitous *Liposcelis bostrychophila* (Nayak *et al*. in press). Psocids have emerged as major pests in Australia and Asia (Pike 1994b). Strategies to combat resistance development include continued monitoring, development of fumigation protocols effective against resistant insects, and studies of both the population and molecular genetics of resistance. The last of these is aimed at developing a rapid test for

resistance as well as improving our knowledge of the toxic action of PH_3 and the mechanism of resistance (Schlipalius *et al*. in press; Ebert *et al*. in press).

In this report, we describe progress in the development of new concentration x exposure period protocols to control resistant *R. dominica* and *L. bostrychophila.* In addition, because of the wide range of temperatures at which grain is fumigated, the influence of this variable on PH_3 toxicology was investigated. We also compare the response of 'strong' resistant *R. dominica* with that of 'weak' resistant rice weevil, *Sitophilus oryzae*, a phenotype already relatively common in the northern region of the Australian grain belt. We also present progress in the identification of resistance genes and the development of a rapid molecular test for resistance. Finally, we present some studies of the fitness of PH_3 resistant genotypes relative to susceptibles.

MATERIALS AND METHODS

Insects

Rhyzopertha dominica: Strain QRD569 was collected from a central storage in Millmerran, Queensland, Australia in 1997. Initial studies revealed that resistance to PH_3 in this strain was significantly stronger than that shown previously in this species (Collins 1998; Daglish and Collins 1999) and is similar to high resistance reported from Asia (Daglish and Bengston 1998). This strain is homozygous for strong resistance to PH_3 (Schlipalius *et al*. in press).

Sitophilus oryzae: Strain QSO335 was collected from a farm near Millmerran in 1992. Initially diagnosed with 'weak' resistance to PH_3, it was selected to homozygosity in the laboratory. This strain is believed to be representative of the 'weak' resistance, which is common in this species (White and Lambkin 1990)

Liposcelis bostrychophila: Strain SLB3 was collected from a central storage at Karkoo, South Australia Queensland, Australia, in 1997. It has not been selected in the laboratory with PH_3. Previous studies had shown that strains of *L. bostrychophila* were significantly more tolerant to PH_3 than strains of either *L. entomophila* or *L. pearlecolor* (Nayak *et al*. in press). To date SLB3 is the most resistant strain of *L. bostrychophila* collected.

Time to population extinction assays

Response to PH_3 was measured by exposing mixed age cultures of insects from each strain to fixed concentrations of PH_3 using a continuous flow application of fumigant mixed with air essentially as described by Winks and Hyne (1997). Insect cultures were specially prepared so that they contained all life stages living in wheat (*R. dominica* and *S. oryzae*) or culture medium (*L. bostrychophila*, Nayak and Collins in press). These were placed into stainless-steel chambers and PH_3 and air were allowed to flow in and out in one direction controlled separately by mass flow

controllers. The experiments were undertaken in rooms that maintained a constant temperature ± 1ºC. Moisture content of the grain was maintained at 12% by passing the phosphine/air mixture through a water-bath set at an appropriate temperature.

Insects were removed from the fumigation after a pre-determined exposure period. In the beetle experiments, all adults, live and dead, were extracted and counted. The cultures were then incubated for 8 weeks and again examined for the presence of live adult insects. In the case of *L. bostrychophila*, the experimental concentrations always killed adults. Therefore, the criterion of population extinction for this insect was absence of nymphs when the cultures were inspected 4 weeks later. In this way, the time taken, in days, required to completely control all life stages was determined at each test concentration.

In addition to the mixed age culture experiments, a series of assays was carried out to test the observation that egg hatch was delayed by exposure to PH_3 in *L. bostrychophila*. Eggs, 1-3 days old, were exposed to three different concentrations of PH_3 for 6 days using the flow-through apparatus. Following the fumigation, the eggs were checked daily and the emergence of nymphs recorded.

Most experiments were carried at 25ºC, however, the influence of fumigation temperature was examined at 20, 30 and 35ºC using the same methodology. Phosphine concentrations were monitored throughout these experiments using a gas chromatograph (Varian Star 3600X®) fitted with a pulsed-flame photometric detector.

The results from the beetle species were subjected to probit analysis, Wadley's method (Wadley 1949), using an in-house computer program (Anon. 1993) based on the analysis described by Finney (1971) and using a logarithmic transformation to obtain estimates of $LT_{99.9}$ values. $LT_{99.9}$ equals the time taken, in days, to control 99.9% of the population at a particular concentration of PH_3. Because of the difficulty of counting psocids, the results were recorded simply as the least number of whole days taken to achieve population extinction.

Field trials

Trials of both registered PH_3 doses (concentration x time) and experimental doses were undertaken in large scale, commercial grain storages belonging to GRAINCO Australia. Mixed age cultures of QRD569 and QSO335 were placed about 300 mm below the surface of the grain a few hours before the commencement of each trial. The insects were contained in 500 mL plastic jars with gauze across the top and bottom to allow the free flow of PH_3. Phosphine was applied to the storages either from cylinders or as aluminium phosphide (blankets) and recirculated. Gas concentrations were monitored and maintained as close as possible to nominated dose rates. Registered PH_3 concentrations and fumigation times tested were: 0.03 mg/L (21 days), 0.05 mg/L (7, 12, 15, 17 days), 0.3 mg/L (10 days) and 1.0 mg/L (4, 5, 6, 7 days). Other rates tested included: 0.1 mg/L (10, 14, 21 days) and 0.2 mg/L (14 days). Temperature of the grain was measured during the trials.

Fitness experiments

The relative fitness of phosphine-resistant *R. dominica*, *S. oryzae* and *T. castaneum* was studied in the laboratory using the population cage approach. For each species, two hybrid strains were produced by crossing a homozygous weak resistant strain with each of two homozygous susceptible strains (Table 4). Two replicated cultures were set up from each hybrid strain and maintained without selection. Changes in the frequency of resistance were monitored by testing samples of adults from each generation with a single dose of PH_3 that discriminated between susceptible and other genotypes.

In addition, homozygous resistant cultures of strong resistant *R. dominica*, QRD569, were mass crossed with a reference susceptible strain. The response to PH_3 of F_1 and F_5 generations was tested at a range of PH_3 concentrations in desiccators using the FAO method (Anon. 1975) but with a 48 h exposure period (Daglish and Collins 1999).

Molecular biology

Genetic linkage mapping of *R. dominica* was performed according to Schlipalius *et al*. (in press). Briefly, a segregating mapping population was obtained by crossing a homozygous resistant individual from line QRD569 with a homozygous susceptible individual from line QRD14. An intercross between a single pair of F_1 siblings produced a segregating F_2 population for genetic mapping. DNA was prepared by a rapid Chelex extraction procedure and used for DNA fingerprint analysis. Polymorphic markers were then ordered into a linkage map using the Map Manager QTX software program which determines linkage by exhaustive pairwise comparisons to predict the most likely order of markers along the chromosome (Manly and Olson 1999). Markers tightly linked to resistance loci were identified by linkage analysis performed on survivors from a subsequent F_5 generation that was selected for PH_3 resistance at 1.0mg/L for 48 hours.

Preliminary linkage mapping has also been performed on a backcross population from a weakly resistant individual from strain QRD369 crossed with an individual from susceptible strain QRD14. Progeny were selected at 0.015 mg/L and 0.04 mg/L PH_3 (48 h exposure of adults) for subsequent identification of markers tightly linked to resistance loci.

RESULTS

Time to population extinction at 25ºC

Plotting $LT_{99.9}$ values for strong resistant *R. dominica* (QRD 569) and weak resistant *S. oryzae* (QSO335) at each PH_3 concentration against time revealed a predictable relationship between PH_3 fumigation concentration and exposure time (Fig. 1) for both species. Although time taken to achieve 99.9% reduction in population ($LT_{99.9}$ values) decreased as PH_3 concentration increased with both strains, there were clear limits and differences between the responses of the two species. With QRD569, there was no significant decrease in $LT_{99.9}$ values with increases in PH_3 concentration

greater than 0.5 mg/L. In addition, time taken to achieve the $LT_{99.9}$ increased markedly at concentrations below 0.1 mg/L. Although there was some reduction in progeny production at concentrations less than 0.1 mg/L, these concentrations could not effect population extinction within 28 days. The results for QSO335 revealed a somewhat different pattern of response (Fig. 1). Concentrations of PH_3 less than 0.1 mg/L could still produce $LT_{99.9}$ values of less than 30 days. At higher concentrations of PH_3, however, this strain showed higher $LT_{99.9}$ values than QRD569 and increasing concentrations above 0.5 mg/L resulted in reducing time to 99.9% progeny reduction.

Experiments with *L. bostrychophila* were incomplete at the time of writing; however, some important observations could be made (Table 1). Time to population extinction was higher in this species at high concentrations of PH_3 than recorded for the two beetle strains. In contrast, the SLB3 strain of *L. bostrychophila* took less time to attain population extinction than the resistant two beetle strains at lower concentrations. In addition, the response of these insects to PH_3 followed the same pattern as the beetle species – a dramatic increase in exposure period needed for population extinction as PH_3 concentration is decreased and little increase in efficacy as concentrations increased.

TABLE 1
Time to population extinction at 25ºC of the SLB3 strain of *Liposcelis bostrychophila* exposed to a range of concentrations of phosphine

Phosphine concentration (mg/L)							
0.05	0.1	0.4	0.5	0.74	1.0	1.4	2.0
Time to population extinction (in days)							
16	11	> 7	> 7	> 7	> 7	> 6	6

Delayed egg hatch in *L. bostrychophila*

Hatching was delayed markedly in eggs exposed to PH_3 compared with controls (Table 2). Moreover, the delay in egg hatch increased with increases in PH_3 concentration. For example, hatching in eggs exposed to 0.05 mg/L for 6 days was retarded by 7 days compared with a 14-day delay in eggs exposed to 1.0 mg/L.

Field trials

A PH_3 application rate (time x concentration) was judged a success if no insects from the mixed age cultures of either strong resistant *R. dominica* (QRD569) or weak resistant *S. oryzae* (QSO335) in the experimental cages survived the fumigation. Four of the five currently registered minimum application rates were tested in these trials (the fifth, 0.02 mg/L for 28 days was not trialed). Of these, only the higher

concentrations at shorter exposure periods, 0.3 mg/L (10 days) and 1.0 mg/L (7 days), were successful against either test strain (Fig.1). The outcome of the field trials closely matched our laboratory results. The temperature of the grain in the trial storages was 25-27 ºC.

TABLE 2
Delay in hatching of eggs of the SLB3 strain of *Liposcelis bostrychophila* exposed to various concentrations of phosphine for 6 days

Phosphine (mg/L)	Time to egg hatch (days) including 6 day fumigation	Delay compared with control (days)
0 (Control)	6 - 9	-
0.05	13-16	7
0.5	17-20	11
1.0	20-23	14

TABLE 3
Time to population extinction (days) at 0.3 and 1.0 mg/L phosphine and 25, 30 and 35ºC

Phosphine concentration (mg/L)	Temperature ºC		
	25	30	35
Strong resistant lesser grain borer (QRD569)			
0.3	8	10	7
1.0	5	3	2
Weak resistant rice weevil (QSO335)			
0.3	10	<5	<5
1.0	5	2	2

The effect of fumigating at higher temperatures

The effect of temperature on phosphine toxicity was complex (Table 3). In general, time to population extinction decreased as temperature increased with the exception of strong resistant *R. dominica* at the lower temperature. With this species, time to population extinction was longer at 30ºC than at 25 or 35ºC at 0.3 mg/L. However at the higher concentration (1.0 mg/L), fumigation times required to completely control this strain were significantly reduced at 30 and 35ºC compared with 25ºC. In the case of QSO335, the weak resistant strain of *S. oryzae*, fumigation times required for complete control were more than halved at 30º and 35ºC compared with 25ºC at both 0.3 and 1.0 mg/L. Preliminary data showed that fumigation times increased significantly at 20ºC compared with those required at 25ºC in both strains.

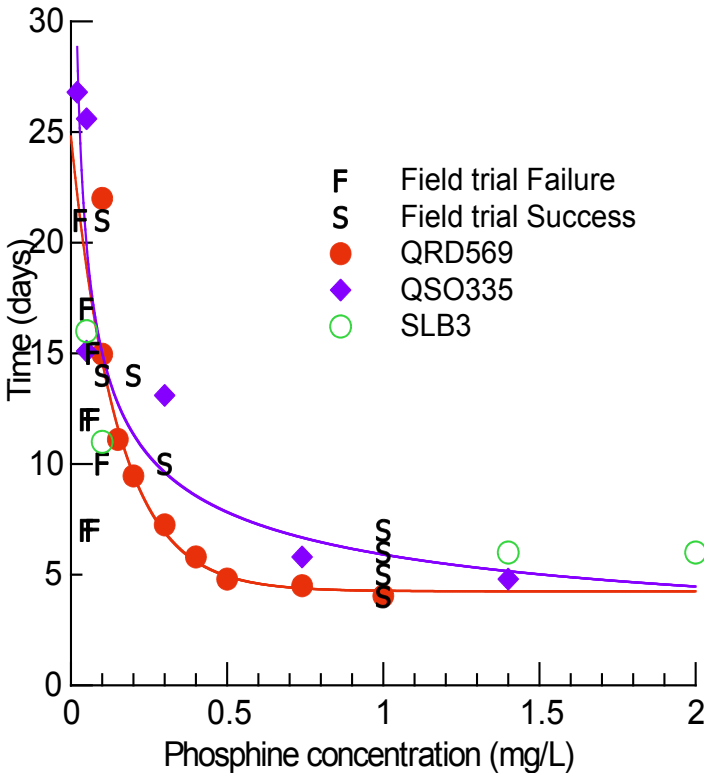

Fig. 1. Time to population extinction ($LT_{99.9}$) of mixed age cultures of strong resistant *Rhyzopertha dominica* (strain QRD569), weak resistant *Sitophilus oryzae* (strain QSO335) and resistant *Liposcelis bostrychophila* (SLB3) at a range of constant concentrations of phosphine.

Fitness

In crosses between weak resistant and susceptible strains of *R. dominica*, *S. oryzae* and *T. castaneum* mortality tended to be high in the F_1 hybrids, but was lower and relatively constant in the six subsequent generations (Table 4). There was no significant difference (measured as the overlap of fiducial limits at the LD_{50} and LD_{99}) in the response of the F_2, F_6 and F_{10} generations from the cross of strongly resistant *R. dominica* (QRD569) with a PH_3 susceptible strain.

These results show that there was no observable difference in fitness between resistant and susceptible phenotypes. We cannot, therefore, expect fitness-related changes in the frequency of resistant insects in field populations if selection pressure is relaxed.

TABLE 4
Change of frequency of susceptible insects over time in the absence of selection pressure

Species	Strain	Percentage of population susceptible			
		F_1	F_2	F_7	Mean F_2-F_7
S. oryzae	QSO335 x LS2	99.5	87.3	86.0	87.2
	QSO335 x CSO231	82.6	71.6	69.5	69.7
R. dominica	QRD369 x QRD14	99.4	78.1	83.0	81.5
	QRD369 x QRD63	97.1	73.1	74.5	77.2
T. castaneum	QTC300 x QTC4	100	94.9	94.1	93.0
	QTC300 x QTC285	100	88.2	86.9	84.1

Molecular biology

Genetic linkage mapping using arbitrary DNA markers and resistance screening has resulted in the identification of two separate loci that are responsible for strong resistance in *R. dominica*. DNA markers have been identified that reside within 0.6 genetic map units of each gene, making map-based gene cloning a possibility. Subsequent mapping of resistance loci in strain QRD369 has shown that one of the two resistance genes from the highly resistant line is at least partially responsible for resistance in the weakly resistant line as well.

DISCUSSION

Our strategy to combat strong resistance to PH_3 in insect pests includes continued monitoring and development of more rapid and more accurate monitoring tools, obtaining a better insight into the genetics of resistance, developing new and effective concentration x time protocols, and understanding the effect of fumigation temperature on PH_3 toxicity.

We have found that high resistance to PH_3 in the three species investigated can be controlled by either increasing concentration or increasing exposure period (Fig. 1). However, there are definite limits to this generalisation. At concentrations of PH_3 less than about 0.1 mg/L, extinction of *R. dominica* populations with strong resistance (QRD569 type) cannot be achieved in reasonable fumigation times (< 28 days). Furthermore, increases in concentration above about 0.5 mg/L will not achieve a reduction in exposure periods less than 4-5 days at 25ºC. A similar general pattern of response to PH_3 was seen in the other two test strains QSO335 and SLB3. However, both these strains were more susceptible than QRD569 to PH_3 at low concentrations and more tolerant at higher concentrations. For example, the $LT_{99.9}$ for *S. oryzae* (QSO335) was about 27 days at 0.02 mg/L. In laboratory experiments, this concentration produced no effect on QRD569 over an exposure period of 20 days. Similarly, resistant *L. bostrychophila* (strain SLB3) could be completely controlled in 16 days at 0.05 and in 11 days at 0.1 mg/L (Table 1). In contrast to the strong resistant strain of *R. dominica*, there was some benefit in increasing PH_3

concentrations past 1.0 mg/L in these species. Thus, at 2.0 mg/L the time to population extinction of SLB3 was 6 days compared with > 7 days at 1.0 mg/L. With QSO335, the $LT_{99.9}$ values were 5.8 days at 0.74 mg/L and 4.8 days at 1.4 mg/L (Fig. 1).

Our laboratory studies and field trials provide evidence that PH_3 doses (minimum concentrations and exposure periods) currently registered in Australia require revision. The close agreement between the results for the field trials and those obtained using the flow-through apparatus in the laboratory attest to the value of the latter to predict minimum effective concentrations and exposure periods for use in commercial practice. Our results have been used to develop provisional application rates for minimum fumigation periods over a wide range of PH_3 concentrations (Table 4). These will be tested in the near future using the field trial methodologies developed in these studies. These recommendations are the minimum number of whole days required to give complete control of all life stages of each insect strain. These exposure periods do not include time for the gas to penetrate all parts of the grain mass.

The majority of our experimental work was carried out at 25ºC. Wheat and barley, however, are harvested in Australia at the beginning of summer with the result that these cereals will often be brought into storage at temperatures of 30ºC or more (Bengston *et al*. 1983). We needed therefore to know how PH_3 is likely to perform at these higher temperatures. Except for strong resistant *R.□dominica* at the lower concentration (0.3 mg/L), our results suggest that fumigations at 30 and 35ºC will be highly effective. The difference in response to phosphine at 30ºC between *R. dominica* and the other species investigated could possibly be explained by the higher demographic performance of the former at this temperature (Beckett *et al*. 1994). In addition, it is likely that the insect's metabolic processes would also be optimal at 30ºC. The lower concentration and longer exposure period provided a greater opportunity for these processes to combat the toxic effects of PH_3. In general, however, we can conclude that fumigations should take place before grain is cooled for storage to ensure complete suppression of pest populations. Further research will be required to determine if PH_3 concentrations as well as exposure periods can be reduced at higher temperatures. Fumigations also take place at temperatures below 25ºC. Early indications are that PH_3 is less effective across a broad range of species at 20º than at 25ºC.

Only two loci have been identified as determining the level of resistance in the highly resistant line of *R. dominica*. This suggests that not much stands in the way of acquisition of resistance, but also that monitoring resistance will be simpler than would have been the case if many more resistance genes had been found. The identification of one of these genes as important to the resistance phenotype of a previously isolated weak resistant type is consistent with the stepwise acquisition of high level resistance. We have yet to determine the relative contributions of known alleles to the resistance phenotype and whether the same genes are responsible for resistance in completely independent outbreaks of resistance.

Our results indicate that there is no apparent fitness deficit associated with either the weak or strong resistance phenotype. This suggests that resistance gene frequencies will remain in equilibrium in populations containing resistant insects. Only gene flow from a predominantly susceptible population would reduce the frequency of resistance in a resistant population. The significance of this result is that tactics, such as the rotation of chemicals, to delay resistance development will not succeed because they rely on a decline in resistance gene frequency during the use of alternative products. Furthermore, it is likely that the enormous selection pressure from the almost universal use of PH_3 will result in resistant individuals becoming the normal genotype.

The proposed changes to registered application rates (Table 5) include increases in PH_3 concentrations. The inevitable question is will these rates select for even higher resistance? This question is impossible to answer unequivocally with the knowledge we have currently of PH_3 resistance mechanisms (Ebert *et al*. in press, Schlipalius *et al*. in press). The best we can do is to compare our results with studies of other highly resistant strains of the same species. Strong resistance to PH_3 has been reported from several countries (Taylor and Halliday 1986). Although much of the data published are difficult to reconcile because of differences in experimental methods and presentation, some of the information given by Price and Mills (1988) is comparable. They report assays of a resistant strain of *R. dominica* from Bangladesh. At PH_3 concentrations of 0.47 and 1.03 mg/L with Ct products (concentration x time) close to those in our experiments Price and Mills (1988) report percent mortalities of 79 and 99%, respectively. Allowing for differences in technique, there is not a large difference between these results and our results for QRD569 of 99 and 100%, respectively, at similar concentrations of 0.5 and 1.0 mg/L. In addition, our results are similar to those from assays of resistant strains of *R. dominica* undertaken in China (Qin Zhanggui, personal communication). Of greater concern for Australian grain industry, and more likely, is the potential for development of stronger resistance in *S. oryzae*. The strain of *S. oryzae* used in these experiments is already more tolerant at higher concentrations of PH_3 than QRD569. Moreover, we have undertaken limited experiments on a strain from China, which required a 6-day continuous exposure to 1.0 mg/L PH_3 to achieve population extinction (Daglish and Collins 1999).

TABLE 5
Minimum concentrations and exposure periods for phosphine currently registered in Australia and rates proposed for trial use

Phosphine concentration		Exposure period	Efficacy against known highly resistant types in Australia		
ppm	mg/L		*Rhyzopertha dominica*	*Sitophilus oryzae*	*Liposcelis bostrychophila*
Current registrations					
700	1.0	7	✓	✓	✖
200	0.3	10	✓	✓	✓
35	0.05	14	✖	✖	✖
20	0.03	21	✖	✖	?
15	0.02	28	✖	✖	?
Proposed minimum effective rates for trial					
1400	2.0	7	✓	✓	✓
700	1.0	7	✓	✓	✖
200	0.3	10	✓	✓	✓
120	0.17	14	✓	✓	✓
72	0.1	28	✓	✓	✓

CONCLUSION

Our experiments and field trials show that PH_3 resistant insects can be controlled with PH_3 by either increasing concentration or extending the exposure period (Table 5, Fig. 1). As PH_3 is more effective against resistant strains at higher temperatures (Table 3), fumigating grain before cooling or even warming grain before fumigating appears to be a useful strategy. Evidence that resistant insects are just as fit as susceptibles, limits the long term effectiveness of other popular tactics such as rotating treatments. Comparison of the data for the response of Australian insects to PH_3 with the limited published material indicates that resistance in *R. dominica* may not strengthen further, at least in the near future. However, we are expecting that stronger resistance in *S. oryzae* will become a serious problem. Progress in the development of a DNA based resistance monitoring methodology has been very encouraging. This technology has the potential to provide a test that will be rapid, accurate and be able to characterise the type of resistance present. Benefits from this research will include a much improved understanding of both the mode of action of phosphine and the mechanisms responsible for resistance.

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