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# DOES UNDERDOSING SELECT FOR RESISTANCE TO PHOSPHINE?

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## ABSTRACT

Farmers in eastern Australia are increasingly reliant on phosphine to control insect pests. Many fumigations undertaken on farms and by produce merchants are done in unsealed storages of all types. These fumigations vary considerably in doses achieved and hence in efficacy. The aim of this work was to determine what effect current small-scale fumigation practices have on selection for resistance to phosphine. Five small-scale fumigations in a variety of situations were monitored to determine doses typically achieved in practice. Meanwhile, the responses to phosphine of adults, eggs and pupae of phosphine-resistant, heterozygous and susceptible *Sitophilus oryzae* and *Tribolium castaneum* were measured in the laboratory. A range of phosphine concentrations were assayed for exposure periods of 3 and 6 d. The responses to phosphine of each life stage and each species varied considerably, as did the dominance of the resistant phenotype. A wide range of concentration × time profiles was observed in the field fumigations, none of which, however, would have controlled all stages of all species. Analysis of the responses of resistant, susceptible and heterozygous individuals showed that any fumigation that does not achieve a dose sufficient to kill all life stages of resistant insects (underdosing) will select for resistance. The significance of this in the management of phosphine resistance must be considered.

## INTRODUCTION

Market pressure for reduced pesticide residues in Australian grain is increasingly being met through the use of phosphine ($PH_3$) fumigation. Pest control in most of Australia's grain crop is now achieved using this method. Heavy reliance on $PH_3$ for insect control, however, means that there is enormous selection pressure for insects to evolve resistance. Although the resistance to $PH_3$ so far detected in Australia is relatively weak, the possibility that a stronger resistance will develop, as has occurred in the Indian sub-continent (Taylor, 1989), is very real.

In eastern Australia small-scale fumigations, those undertaken on farms and by produce merchants, are carried out under a wide range of conditions. Sealed silos are

sometimes used, but fumigations are often undertaken in unsealed storages such as bag-stacks, covered with plastic sheets or tarpaulins, and metal bins. Although in Western Australia sealed silos are used on farms, many of them are poorly maintained (Newman, 1994).

Current small-scale fumigation practices are expected to vary both in their efficacy, with some practices giving incomplete control, and in the selection pressure they exert for resistance. Practices that select for $PH_3$ resistance should be discouraged to ensure the long-term efficacy of this fumigant.

The aim of this work was to determine what effect current small-scale fumigation practices have on selection for resistance to $PH_3$. To achieve this, a range of typical small-scale field fumigations was monitored and the data obtained compared with the laboratory responses of insects to a range of $PH_3$ concentrations and exposure times. The species tested were *Sitophilus oryzae* and *Tribolium castaneum*; tests were conducted on adults, eggs and pupae of resistant and susceptible strains and on insects heterozygous for resistance.

## MATERIALS AND METHODS

### Field fumigations

Fumigations on farms were monitored to determine what dosages were typically achieved in practice. The fumigations monitored included a standard above-ground galvanised-steel silo, aluminium phosphide $PH_3$-generating tablets placed near the fan of an aeration system, a farmer-built sealed silo, a commercially built sealed silo in which the seal had failed, and a plastic-sheeted bag-stack. All fumigations were undertaken by the usual operator in the usual manner.

A number of gas sampling lines (1.8-mm internal diameter nylon pressure tubing) were inserted at a range of points inside each fumigation enclosure before dosing with $PH_3$. During each fumigation a small amount of gas was pumped out of the enclosure and the concentration of $PH_3$ at each point was measured using $PH_3$-sensitive indicator tubes (Dräger).

### Laboratory assays

*Resistant and susceptible strains.* About 30 field strains each of *S. oryzae* and *T. castaneum* were collected from farms and grain merchants' premises in central and southern Queensland and assayed for resistance to $PH_3$ using the FAO 20-h exposure method (FAO, 1975). Those adults with the highest levels of $PH_3$ resistance were selected over several generations until there was no further increase in resistance. At this point the resistant strains were substantially homozygous for resistance.

The susceptible insects used were reference laboratory strains that had not been exposed to insecticide or fumigant selection for 20–30 years.

*Preparation of eggs, pupae and adults for assay.* Pupae and eggs of *S. oryzae* were fumigated in whole grain. Adults of both species, and immature stages of *T. castaneum*,

were sieved from the culture medium and placed in small containers in the fumigation chamber with 0.5 g of wholemeal flour or cracked grain.

Eggs and pupae of *S. oryzae* were obtained by placing 100 adults in small jars containing 68 g wheat for 2 d at 30°C. Adults were then removed and the lidless jars of grain were either placed in the fumigation chamber (desiccator) for treatment of eggs or returned to a 30°C room. Pupae of *S. oryzae* were fumigated for 27 d (R-strain) and 29 d (S-strain) after the 2-d oviposition period. Dissections of whole grains revealed that, at 30°C, about 90% of the insects had pupated within these times.

Eggs and pupae of *T. castaneum* were sieved from the flour after the adults were allowed a 24-h oviposition period at 30°C. Eggs were used at 1–2 d, and each assay of pupae contained a range of ages. Fifty eggs or pupae were placed ready for fumigation in each of four plastic, 50-ml open-topped measuring cups with 0.5 g flour. Adults of both species were assayed at 2–4 weeks after emergence.

*Obtaining $F_1$ progeny for assay.* Virgin adult *S. oryzae* were obtained by isolating individual infested grains in gelatine capsules. After emergence the adults were sexed according to the shape of the rostrum (Halstead, 1963). Reciprocal crosses of the resistant and susceptible parent strains were made by adding 50 males and 50 females of the appropriate strain to 68 g whole wheat.

The oviposition period was 2 d. $F_1$ eggs, pupae and adults were obtained from these cultures as described above.

Pupae of resistant and susceptible *T. castaneum* were sieved from flour and sexed (Halstead, 1963). Reciprocal crosses of parental strains were made by placing 100 male and 100 female pupae in flour. About 1 week after eclosion, the adults were removed from the culture medium and placed onto fresh flour for an oviposition period of 1 d. $F_1$ eggs, pupae and adults were removed from the flour as described above.

*Phosphine susceptibility tests.* Response to $PH_3$ was measured by exposing insects to a range of concentrations of fumigant at 25°C and 55% r.h. in desiccators as described in the FAO method (FAO, 1975). Exposure periods were either 3 or 6 d with a post-fumigation recovery period of 14 d for eggs and adults and 21 d for pupae.

Four batches of each assay were undertaken. Batches of adults consisted of 50 insects confined within glass rings on a filter paper base supplied with 0.5 g wholemeal flour or cracked grain. Numbers of eggs or pupae in each batch were as described above. The response of insects to $PH_3$ was subjected to probit analysis (Finney, 1971) where control insects could be counted directly, i.e. adults of all species and eggs of *T. castaneum*. Wadley's method (Finney, 1971) was used where controls were estimated, i.e. eggs and pupae of *S. oryzae*.

$PH_3$ was generated from a commercial aluminium phosphide formulation and collected over acidified water. A gas density balance chromatograph (Varian Aerograph Model 90-P) was used to determine the concentration. Dichlorofluoromethane was used as the carrier gas. $PH_3$ was injected using gas-tight syringes through a rubber septum in the lid of the desiccator to give the required concentration.

## RESULTS

### Phosphine fumigations in farm silos

Fumigation 1 was in an unsealed bolted galvanised-steel 1000-t silo fitted with aeration ducting. At the time of this fumigation, the silo was almost full of sorghum leaving a 3-m headspace. Grain temperature ranged from 27 to 41°C with headspace temperature from 37 to 75°C. $PH_3$ was applied by adding aluminium phosphide tablets to the grain as it was being augured back into the silo. The operater added the tablets at a rate of 3 tablets $t^{-1}$ so that the applied dose of $PH_3$ was 3 g $t^{-1}$, double the recommended rate. Concentrations of $PH_3$ were monitored inside the silo at three points on the surface of the grain and three additional points at a 3-m depth. The concentration of $PH_3$ at the surface did not exceed 60 ppm and was usually less than 30 ppm. $PH_3$ concentration at 3 m below the surface peaked at about 150 ppm after almost 2.5 d but declined rapidly over the following 3 d. Gas lines placed inside the aeration ducts and the auger boot indicated that gas was lost rapidly from the silo at these points.

Fumigation 2 was designed to test the efficacy of applying $PH_3$ through the aeraton fan — a fairly common practice among farmers. Six sampling points were set up before harvest inside a 100-t steel above-ground silo. After the silo had been filled with wheat, the farmer placed 90 aluminium phosphide tablets, equal to 90 g $PH_3$, in an open-topped steel can (about 500 ml in capacity) that was suspended at the outside opening of the aeration fan. Aerator suction was recorded as 7.2 m/sec while wind velocity was 0.8 m/sec. Grain temperature was 35°C. All the $PH_3$ generated from the tablets was lost from the silo within 48 h, and the concentration did not exceed 4 ppm.

In fumigation 3, stacks of bagged seed (about 10 t) were stored inside a raised wooden shed with a floor area of about 7 × 5 m. Before fumigating, gas lines were installed at three points in the bag-stack and at two points above the position where the tarpaulin was to be placed. The farmer then spread 200 aluminium phosphide tablets on the bag-stack and covered the area with a plastic sheet. Windy conditions prevailed throughout the time of the fumigation with wind speed varying between 1.2 and 1.6 m/sec. Little or no $PH_3$ was detectable after 24 h. At the centre of the bag-stack the $PH_3$-concentration peaked at 380 ppm after 9 h, but the gas was rapidly lost. The $PH_3$ concentration at two other points monitored under the tarp did not exceed 50 ppm.

Fumigation 4 was in a farmer-built, sealable, bolted-steel silo of 25-t storage capacity (for wheat). There was no pressure relief valve. Before harvest eight gas monitoring lines were placed inside the silo at various points. The farmer added the recommended rate of 50 aluminium phosphide tablets (50 g $PH_3$) to sorghum as it was augured into the silo. Gas concentration reached a peak at about 2 d and then decreased rapidly. However, even at the sample point showing the lowest range of concentrations, $PH_3$ concentration remained above 100 ppm for 6 d, i.e. from day 1 to day 7.

Fumigation 5 was in a commercially sealed 1,700 bushels (46.32 t) silo. Although this silo had a pressure release valve, it could not be used to test gastightness. The silo was filled with sorghum and three gas sample lines were inserted into the grain from

the top hatch. Samples were taken at the grain surface and at 2 and 3 m below the surface. Fifty aluminium phosphide tablets were added by lifting the hatch and spreading them across the surface of the sorghum. This dose rate is slightly more than half that currently recommended. $PH_3$ concentrations rose to a peak of about 600 ppm in 2 d and then rapidly declined. At the surface of the grain the gas concentration fell to below 100 ppm at about 6 d, while at the 2- and 3-m points the concentration reached 100 ppm at about 8 d. Gas could be smelt near the hatch during the fumigation, so the hatch seal was probably leaking. This would explain the lower gas concentration at the surface.

### Laboratory assays

*S. oryzae.* At 3 d the most tolerant life stage of both the R- and S-strains was the pupa, with $LD_{99.9}$ values of 2,160 ppm (3.0 mg/L) and 144 ppm (0.2 mg/L), respectively. In addition, one strain (LS2), which showed resistance in the pupal stage but not as eggs or adults, had a $LD_{99.9}$ of greater than 3,000 ppm (4.25 mg/L). At 6 d eggs were the most tolerant stage giving $LD_{99.9}$ values of 625.5 ppm (0.9 mg/L) and 66.2 ppm (0.09 mg/L) for the R- and S-strains, respectively. Resistance to $PH_3$ in *S. oryzae* was almost completely recessive in the eggs at both the 3 and 6 d exposures.

*T. castaneum.* At the 3-d exposure period eggs were about 10 times more tolerant than either pupae or adults for both the R- and S-strains. $LD_{99.9}$ values for eggs were 733 ppm (1.02 mg/L) for the R-strain and 63 ppm (0.088 mg/L) for the S-strain. Similarly, at 6 d the eggs were the most tolerant stage with $LD_{99.9}$ values of 220 (0.3 mg/L) and 50 ppm (0.07 mg/L) for the R- and S-strains, respectively. Resistance was almost completely recessive in adults and pupae at both the 3 and 6 d exposure periods. In eggs resistance was expressed as incompletely recessive at 3 d and semi-dominant at 6 d.

## DISCUSSION

### Efficacy of current phosphine fumigation practices in small-scale storages

Fumigations in the best unsealed storage produced enough $PH_3$ to kill some stages of susceptible insects, i.e. adults of *S. oryzae* and adults and pupae of *T. castaneum*. The highest doses achieved in this silo would also have resulted in 100% mortality of resistant *T. castaneum* adults and up to 80% kill of adult, resistant *S. oryzae*, giving the impression of a successful fumigation. However, about 90% of *S. oryzae* and 100% of *T. castaneum* eggs, and about 80% of *S. oryzae* and 25% of *T. castaneum* pupae, would have survived the fumigation.

The fumigations using the aeration fan and the bag-stack covered with plastic sheeting produced concentrations of $PH_3$ that would kill at least a proportion of susceptible adult insects, again giving the appearance of an effective fumigation. However, $PH_3$ doses would not kill resistant adults and were not sufficient to kill either eggs or pupae of even susceptible insects.

Dosages achieved in the sealed silos were significantly higher than those in the best unsealed silo. Even the silo that received only half the recommended number of $PH_3$ tablets gave a result superior to that in the unsealed silo. The underdosed sealed silo held enough $PH_3$ over a 6 d period to kill all stages of susceptible strains of both species, adults and pupae of resistant insects and the resistant pupae of the LS2 strain of *S. oryzae*. In the farmer-sealed silo, which received the correct number of $PH_3$ tablets, the lowest measured concentrations of $PH_3$ reached were still sufficient to kill all stages of susceptible insects and all stages of resistant insects except eggs and LS2 pupae of *S. oryzae*.

To achieve a successful fumigation, all life stages of the most tolerant species must be killed during the exposure period. The concentrations of $PH_3$ necessary to give 99.9% mortality of the eggs and pupae of the resistant strain of *S. oryzae*, the most tolerant species, in 6 d at 25°C are illustrated in Figs. 1 and 2 against the concentrations achieved in the best unsealed silo and the two sealed silos. Although resistant pupae were killed in sealed silos (Fig. 2), the concentrations of $PH_3$ for the required exposure time were not sufficient to control eggs (Fig. 1). The minimum concentration achieved in the unsealed silo, barely adequate to control all adults, would be ineffective against eggs and pupae.

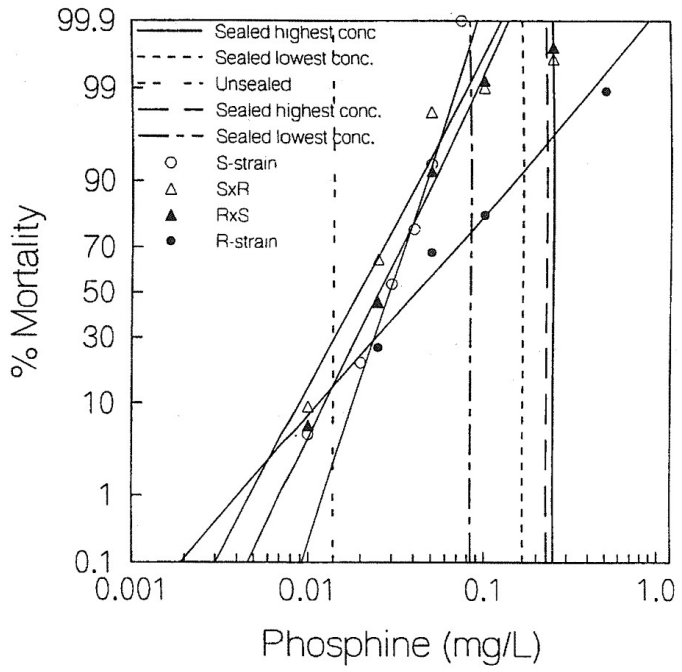


Fig. 1. Response to phosphine of susceptible, resistant and heterozygous eggs of *Sitophilus oryzae* compared with the maximum and minimum concentrations of phosphine that occurred at 6 d in two sealed silos and the maximum concentration in the best unsealed silo.

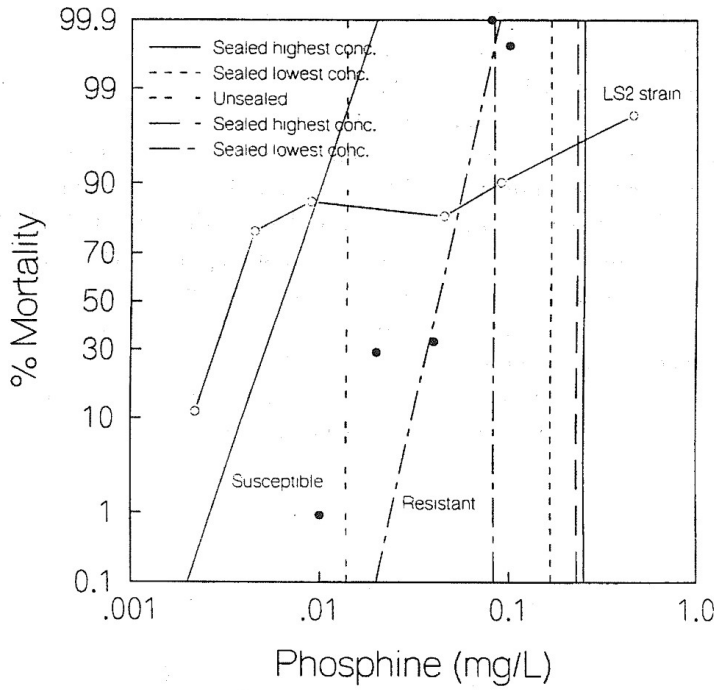


Fig. 2. Response to phosphine of susceptible and resistant pupae of *Sitophilus oryzae* compared with the maximum and minimum concentrations of phosphine that occurred at 6 d in two sealed silos and the maximum concentration in the best unsealed silo.

**Selection for resistance to $PH_3$ under current fumigation practices**

Selection for any trait requires that individuals possessing that trait are favoured; i.e. they produce more progeny than individuals not possessing the particular trait. To select for resistance to a toxicant such as $PH_3$ the chemical must be used in such a way that insects possessing the resistance gene(s) can survive and multiply while insects not possessing the resistance gene(s) will be killed or produce fewer progeny. This means that doses applied must be sufficient to kill all resistant insects. Lower doses must necessarily favour resistant insects by killing most of the susceptibles and allowing a high proportion of resistant individuals to survive. A caveat to this argument is that high doses may readily select for any new, stronger resistance that may be present in the population.

We have shown that even very poor fumigations producing low gas concentrations for short periods, such as those using an aeration fan or performed under a loose cover, will select for resistance by killing only susceptible adult insects (and probably larvae). On the other hand, these fumigations only select for resistance in a proportion of the population, allowing eggs and pupae of both resistant and susceptible insects to survive so there is no

selection effect on these life stages. Selection for resistance is also likely to occur in the most effective fumigations, i.e. the two sealed silos and the unsealed silo. The selective effect of these fumigations on the eggs and pupae of *T. castaneum* are illustrated in Figs. 3 and 4, respectively. In the first example (Fig. 3) a proportion of both homozygous resistant and heterozygous (R × S, S × R) *T. castaneum* eggs would survive fumigations in the sealed silos, but susceptibles would not survive. In this situation, development of resistance would be quicker in the sealed silos than in the unsealed silo because the unsealed silo also allowed survival of about 80% of susceptibles. These would dilute the resistance frequency in succeeding generations. In the second example, susceptible and heterozygous pupae of *T. castaneum* were controlled by fumigations in sealed silos (Fig. 4). However, the concentration of $PH_3$ reached over the 6 d fumigation in the unsealed silo was ideal for selecting for resistance in this species. It killed all susceptibles and heterozygotes (R × S, S × R) and allowed only homozygous resistant insects to survive.

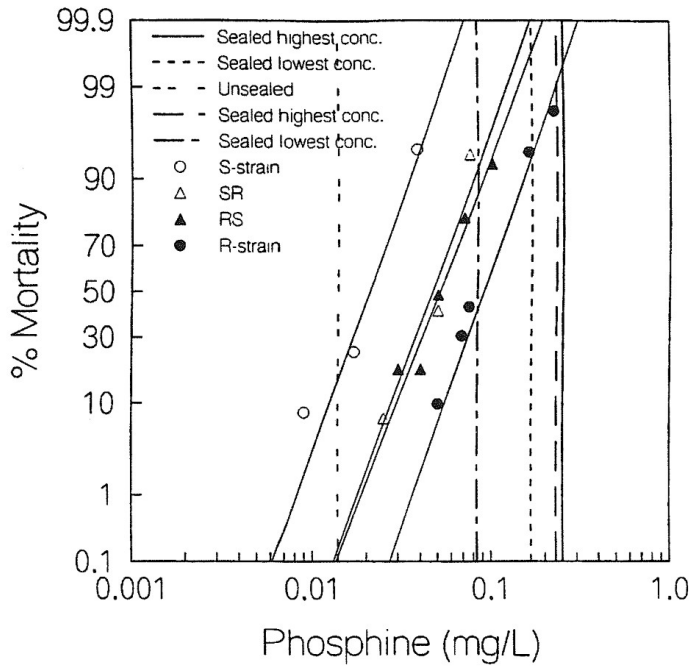


Fig. 3. Response to phosphine of susceptible, resistant and heterozygous eggs of *Tribolium castaneum* compared with the maximum and minimum concentrations of phosphine that occurred at 6 d in two sealed silos and the maximum concentration in the best unsealed silo.

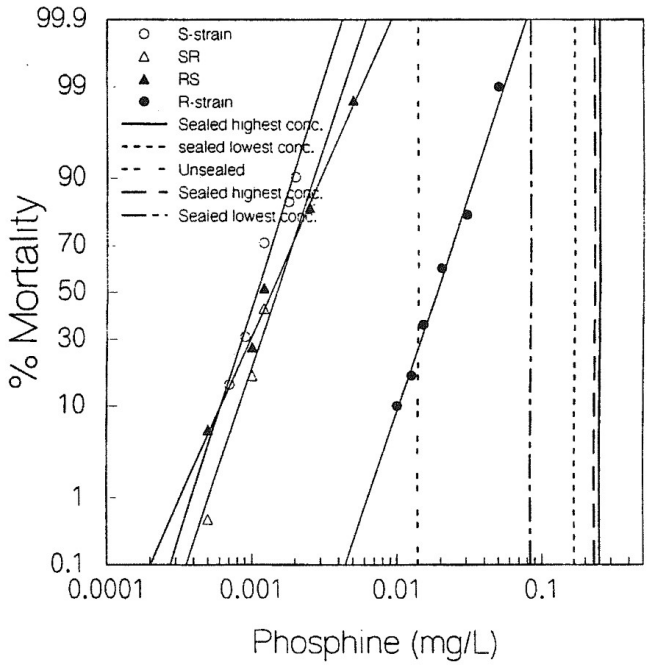


Fig. 4. Response to phosphine of susceptible, resistant and heterozygous pupae of *Tribolium castaneum* compared with the maximum and minimum concentrations of phosphine that occurred at 6 d in two sealed silos and the maximum concentration in the best unsealed silo.

## CONCLUSION

The two examples cited above and the results for *S. oryzae* (Figs. 1 and 2) illustrate that selection for resistance will vary from species to species and among life stages, depending on the expression of resistance and the dosage of $PH_3$ encountered. In general, however, a very poor fumigation will not select for resistance as quickly as a fumigation that is close to successful. Nevertheless, any fumigation that achieves less than the recommended dose level (concentration × time) is likely to select for resistance in at least one of the insect pest species involved.

## ACKNOWLEDGEMENT

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