

# Phosphine fumigation of stored field peas for insect control

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## Abstract

The grain industry has on occasions experienced fumigation failures when fumigating stored field peas with phosphine to control pea weevil. In relation to this problem the present study has examined the susceptibility of the pea weevil to phosphine and the extent to which phosphine is sorbed and desorbed by field peas. Rates were not affected by changes in variety, condition or source of the peas. Fumigations with 1400 ppm phosphine in 80% filled containers showed that >200 ppm phosphine remained after 21 days. Studies of susceptibility of the developmental stages of pea weevil *Bruchus pisorum* (L.) established that relatively low concentrations of phosphine applied in sealed containers are sufficient to kill all stages of the beetle. No beetles survived in treatments with 30 ppm or more of phosphine. Absorbed phosphine desorbs readily from peas. In aired samples, phosphine levels were found to fall from above 12 ppb to less than 0.1 ppb in two days.

The results indicate that problems with commercial fumigations are most likely to arise from failure to maintain adequate gas tightness rather than excessive sorption of phosphine by peas or a tolerance of phosphine by pea weevils.

## Introduction

In spring, adult pea weevils, *Bruchus pisorum* (L.), fly into crops of field peas, *Pisum sativum* L., attracted by the flowers. They feed on pollen in the flowers before laying eggs on the pea pods. The larvae which hatch from the eggs bore through the pods into pea seeds where they develop to the adult stage. At harvest time in summer all stages of pea weevil can be found in the pods. Most adults emerge from the peas within the first 2 months of storage, but some adults can remain within undisturbed seed until the following spring (Comery and Chaffey 1987). The pea weevil does not reproduce in stored peas.

Control of the pea weevil requires effective treatment of both larvae and pupae within the peas as well as of the adult weevils. Small quantities of peas sometimes contaminate other grains and if live insects are present in the peas, export may be delayed. In addition, the adults are active fliers and can create problems at export terminals by flying from contami-

nated peas onto previously treated peas and other grains that are being loaded onto ships. Pea weevils must be controlled in peas for export from Australia in order to meet the nil tolerance for live insects in export grain required under the *Export (Grain) Regulations of the Customs Act 1901–1971*.

Contact insecticides are considered unsuitable for control of the weevils in stored grain because the insecticidal effects are largely restricted to the emerged adults. Some are so active that they will leave the grain before being killed. The grain industry relies on fumigation shortly after storages are filled to minimise damage by developing weevils and limit the dispersion of adults. Methyl bromide and phosphine are the only fumigants currently registered for this use in Australia. However, methyl bromide has been implicated as a potent ozone depletor and its use will be limited in accordance with the Montreal Protocol. Further controls over those already agreed to are anticipated.

There are differences in phosphine fumigation strategies employed for control of pea weevil. The recommended dosage, 2 g/t, may be applied as a single dose at the start of fumigation, or as two applications each of 1 g/t, the first at the start of the fumigation period and the second half-way through. The two-stage dosing attempts to counteract losses of phosphine (from sorption or leakage) and ensure that effective concentrations of the fumigant are still present at the end of the fumigation. Recommended minimum fumigation times vary from 21 to 28 days. In the 1980s shorter fumigation times were used but there is now evidence that this could permit survival of some weevils (Williams 1990; Waterford and Winks, these proceedings). An alternative method of treatment, SIROFLO<sup>®</sup>, in which low concentrations of phosphine are maintained by automatic gas introduction during the fumigation period (Winks 1993), is now sometimes used.

Reported failures of some phosphine fumigations are of concern to industry, particularly because the use of the alternative and effective fumigant methyl bromide is likely to be restricted in the future. High tolerance of phosphine by some life stages of the pea weevil and high sorption of phosphine by peas under some conditions have been considered to be possible causes of fumigation failures. This study is concerned with examining these factors.

## Materials and Methods

Field peas cultivar (cv.) Dun were grown at the Wimmera Research Station, Doon, Victoria and left untreated with insecticide before harvest so that they could become heavily infested with pea weevils. Peas harvested from the outer edges of the planted area, where the weevils are most numerous (Comery and Chaffey 1987), were segregated for use in laboratory fumigation studies with immature pea weevils. Peas harvested from the centre of the crop were used for phosphine sorption studies. Samples of the Dun cultivar were collected from Walpeup, Doon, Murtoa, Woomelang and Donald in Victoria during the 1992 and/or 1993 harvests. Samples of the Dinkum cultivar were collected from Walpeup in 1993 for use in the sorption studies.

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### Pea weevil fumigations

Peas containing immature pea weevils at different stages of development were fumigated in sealed 4.5 L jars in the laboratory. Phosphine was injected with a syringe into the jars via rubber septa in the screwtop lids. Jar-to-lid joints were sealed with silicone rubber to ensure gastightness. In each jar the peas (250 g) were loaded into a heavy duty wire gauze cage mounted on a perforated plastic container attached to the base of the jar. Within the plastic container was a magnetic stirrer. Different concentrations of phosphine were achieved in the jars by injecting different volumes from a standard cylinder containing 10000 ppm (ca 14 g/m<sup>3</sup>) of phosphine in nitrogen. Before introducing a volume of phosphine into a jar a syringe was used to remove a similar volume of air from the jar. This avoided increasing the pressure within the jar to above that of the surrounding atmosphere. Immediately after introduction of phosphine the gas within a jar was mixed using the magnetic stirrer.

In each fumigation experiment a minimum of four replicate jars were used for the untreated controls and for each phosphine concentration. The latter ranged from 0.01 to 1.4 g/m<sup>3</sup> (10–1000 ppm). The jars were held in a dark room or incubator at a temperature of 23–25°C during the fumigation periods which ranged from 21 to 28 days.

After fumigation the jars were opened and ventilated under a fume hood. The open tops were covered with terylene voile held in place by elastic bands to prevent insects from leaving or entering jars during ventilation. After ventilation, pea samples, at least 100 peas per jar, were dissected and examined for pea weevils. Dead and live pea weevils were counted and discarded and the remaining peas were incubated at 27°C (60–70% r.h.) and examined for emerged adults at monthly intervals for three months.

### Phosphine sorption and desorption studies

Sorption of phosphine by field peas was studied using 100 mL glass vials fitted with Mininert<sup>®</sup> valve lids to provide gas sampling ports. Each vial was loaded with ca 62 g of peas; either whole peas or a mixture of whole and damaged peas were used. The filling ratio was ca 80%. The vial was sealed with the valve lid, the valve was opened and a syringe needle was inserted through the septum to allow air pressure within the vial to equilibrate with that outside. A gas syringe was used to remove 8 mL of air from the vial, then 8 mL of a phosphine–nitrogen mixture from a 10000 ppm (ca 14 g/m<sup>3</sup>) phosphine standard cylinder was injected into the vial (restoring the pressure equilibrium) and the valve was closed. Assuming a true density of 1.48 g/mL for the peas, the free air space was ca 58 mL and the addition of the phosphine should result in a concentration of ca 2 g/m<sup>3</sup> of phosphine (ca 1400 ppm) in the free air space within the vial. The true density of the peas was assumed to be 1.48 g/mL on the basis of studies of the true densities of grains, including field peas from Dooen, Victoria (J. Cassells and H.J. Banks, pers. comm., 1992).

Five replicate vials were set up for each batch of peas to be assessed for phosphine sorption. The peas were held at a constant temperature of 23°C with moisture contents at particular values in the range 9.8–12.2% w.b. for at least 2 weeks before being used in experiments.

Moisture content determinations were made using an air oven method in which a small sample of peas was ground and ca 20 g was placed in a moisture determination tin which was weighed before and after the sample was dried to constant weight in an oven at 105°C. Samples were placed in the oven overnight and the following morning were transferred to a

desiccator to cool before being weighed so that the moisture loss could be calculated.

The phosphine sorbed by the peas in the vials was monitored by analysing the phosphine concentrations in samples of gas from the vials during 21 days. Each vial was shaken to mix the gas within immediately before a gas sample was taken for analysis.

An hour after phosphine was injected into a set of replicate vials of peas, a 10 L sample from each vial was injected into a gas chromatograph (GC) to analyse phosphine concentrations. The GC was fitted with a flame photometric detector and a J&W Scientific DB-5 fused silica column 16 m 0.25 mm i.d. with a film of 5% phenyl methyl polysiloxane of 0.25 µm thickness. Operating temperatures were: oven 50°C, injection port 150°C and detector 200°C. Samples were then taken at 2-hourly intervals for the first 8 hours and thereafter at 1–3-day intervals. When the vials were not being sampled they were kept in dark rooms or incubators at 23°C.

Phosphine desorption from fumigated peas was monitored by successive measurements of phosphine concentrations in the peas using the Brockwell (1978) method of phosphine residue analysis. This method, which was described for the analysis of phosphine in wheat, uses grinding to release sorbed phosphine into an enclosed headspace. The released phosphine is then determined by gas chromatographic analysis. The method was tested for peas by placing a known weight of peas in the analytical apparatus and introducing a known amount of phosphine. The peas were then ground and the phosphine gas in the headspace determined. This process was repeated for different amounts of phosphine. Phosphine recovery was 75% and linear in the range 0.1–40 ppb. Undamaged peas (cv. Dun from Donald, 250 g) were fumigated by treatment with 1.5 g/m<sup>3</sup> of phosphine in an air stirred container for 24 hours. After this time the phosphine was removed and the peas divided into 30 g samples in petri dishes and placed in a fume hood. Individual samples were analysed for phosphine by the Brockwell method at various times up to 28 hours. Peas were also treated with a constant flow of phosphine at 0.1 g/m<sup>3</sup> for 48 hours and then analysed as above.

Freshly fumigated (1.5 g/m<sup>3</sup>) peas were allowed to stand in air for 0.5 hours and then 30 g samples (solid volume, 20 mL) were placed in glass bottles (120 mL) sealed with Mininert<sup>®</sup> valves. After 95 hours the headspace atmospheres were analysed for phosphine gas (by GC), and the vessels were opened and the peas analysed for residual phosphine by the Brockwell method. For the purposes of comparison both results were expressed as parts per billion with respect to the weight of peas in the vessel.

## Results and Discussion

### Pea weevil fumigations

About 6% of the peas used in the fumigation experiments were infested, ca 97 peas in each fumigation jar. The distribution of developmental stages in these peas was 21% larvae, 5% pupae and 74% adults. Most of the pupae and adults were alive at the start of the fumigations, but up to 85% of the larvae were dead. First and second instar larvae accounted for most of the dead. In fumigations in which dosages of phosphine have ranged from 0.04 to 1.4 g/m<sup>3</sup> (30–1000 ppm) all fumigated pea weevils were killed, whereas most insects in unfumigated control jars remained alive. The dead insects in the fumigated jars included many newly developed adults in the process of emerging from within the peas. The duration of the fumigations may have been sufficient to allow pupae, which are

believed to be the most phosphine tolerant life stage, to develop into adults, which are more susceptible. Consequently, some emerged and emerging adults may have been pupae at the start of a fumigation. At concentrations of phosphine below  $0.04 \text{ g/m}^3$  some weevils survived. Further experiments at phosphine levels below  $0.04 \text{ g/m}^3$  are currently in progress.

### Phosphine sorption and desorption studies

Sorption of phosphine by field peas is neither rapid nor extensive when compared with wheat and many other commodities (Banks 1993). At a constant temperature ( $23^\circ\text{C}$ ) and after an initial 8 hours, when sorption is more rapid, the amount of phosphine ( $C$ ) absorbed with time ( $t$ ) can be represented by the relationship  $\log C = a + kt$ , where  $a$  and  $k$  are constants. A comparison of data for fumigations carried out at constant temperature (Table 1) showed that there was little variation in the value of the slope ( $k$ ) and also that there were no obvious effects arising from changes in the condition or source of the peas. This is further illustrated in Figure 1, in which results of phosphine sorption experiments with whole field peas of cv. Dinkum (12.09% m.c.) from Walpeup and whole and damaged field peas of cv. Dun (9.90% m.c.) from Dooen are shown. Over 21 days the phosphine concentration in the headspace was found to fall by less than one order of magnitude. Sealed containers, filled to 80% capacity with peas and fumigated with  $2 \text{ g/m}^3$  phosphine, were found to contain better than  $0.28 \text{ g/m}^3$  (200 ppm) phosphine after 21 days. This is well in excess of the minimum initial dose of  $0.04 \text{ g/m}^3$  (30 ppm) required for complete control.

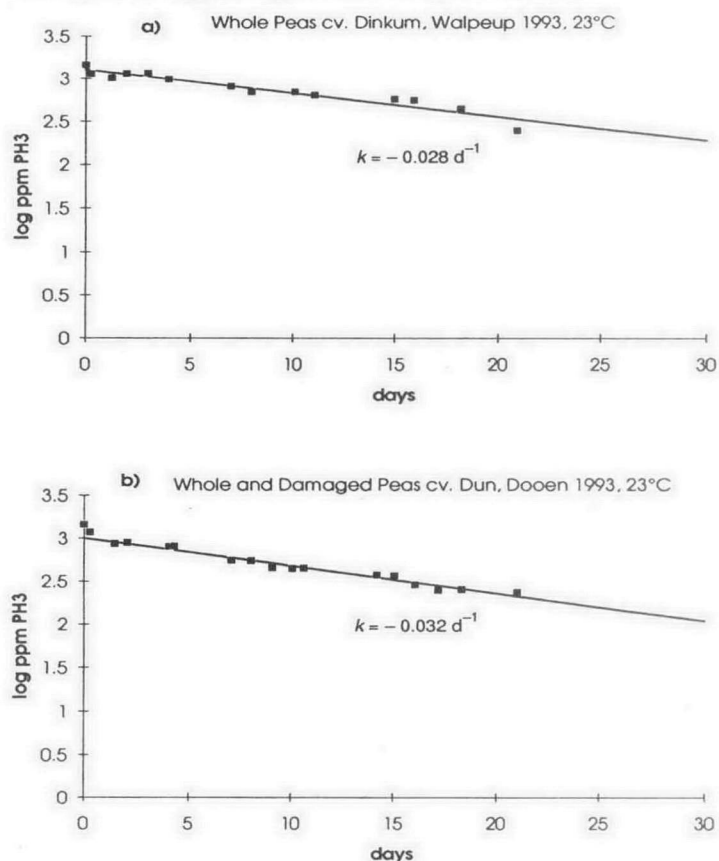


Fig. 1. Phosphine sorption by a) whole field peas cv. Dinkum (12.09% m.c.) from Walpeup and b) mixed whole and damaged peas cv. Dun (9.90% m.c.) from Dooen. Sorption measured by phosphine loss from headspace.

Figure 2 shows the release of sorbed phosphine from peas after fumigation at  $1.5 \text{ g/m}^3$ . The greater part of the phosphine desorbs within 8 hours of commencing aeration and after about 2 days the amount of phosphine in the peas is below detectable limits (0.1 ppb). A similar result was obtained for peas which had been fumigated at  $0.1 \text{ g/m}^3$ . However, in this case the initial amount of sorbed phosphine in the peas was only 2.5 ppb. When freshly fumigated peas containing 15 ppb of sorbed phosphine were placed in a sealed container and allowed to stand, the phosphine equilibrated between the headspace and the peas. Where the volume of the headspace was 6 times that of the peas and after 95 hours standing, the equivalent of 14 ppb phosphine was detected in the headspace of the container but no phosphine could be detected in the peas. These results indicate that when sorbed phosphine equilibrates between the atmosphere and the peas, the desorption of the phosphine is rapid and, after several air exchanges, the amount of sorbed phosphine in the peas can be expected to be below detectable limits ( $<0.1 \text{ ppb}$ ).

### Conclusions

The laboratory experiments in which pea weevil larvae, pupae and adults were exposed to phosphine in sealed containers showed that relatively low initial dosages of ca  $0.04 \text{ g m}^{-3}$  could kill the weevils given an adequate exposure period of 21 days at  $23\text{--}25^\circ\text{C}$ . The sorption studies showed that field peas do not sorb excessive amounts of phosphine, and the desorption experiments demonstrated that the sorbed phosphine is readily desorbed leaving no detectable phosphine residue in the peas.

The pea weevil does not breed in stored peas and if insects that survive a fumigation are to breed, they must leave the storage and fly to fields where pea crops are to be planted. There they mix with other pea weevils which have remained in the fields and have never been exposed to phosphine. The opportunity for concentration of resistant genes and selection of highly resistant populations is thus much reduced compared with that for insects that breed continuously in stored commodities where populations may be exposed many times without dilution by susceptible individuals.

These results indicate that there should be no problems in controlling pea weevils in stored peas using a single dose of phosphine applied at the currently recommended rate of  $1.5 \text{ g/m}^3$  (2 g/t) and a minimum fumigation time of 21 days at

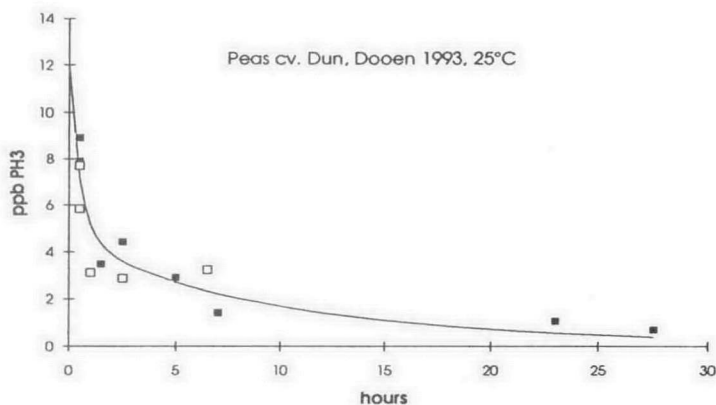


Fig. 2. Phosphine levels measured in peas at various times after fumigation at  $1.5 \text{ g/m}^3$  for 24 hours. Data from two experiments fitted to a curve expected for a diffusion controlled loss from a spherical particle (Banks 1986).

**Table 1.** Comparison of slopes from regression of log concentration against time for phosphine sorption by various samples of field peas fumigated with 2 g/m<sup>3</sup> at 23°C over 21 days.

Year	Location	Cultivar	Condition	Moisture content (% w.b.)	Slope (d <sup>-1</sup> )
1993	Dooen	Dun	whole and damaged	9.90	-0.032
1993	Dooen	Dun	whole	9.84	-0.029
1992	Donald	mixed	whole and damaged	11.01	-0.028
1992	Woomelang	mixed	whole and damaged	11.32	-0.029
1992	Murtoa	Dun	whole and damaged	9.87	-0.029
1993	Walpeup	Dun	whole	12.15	-0.031
1993	Walpeup	Dinkum	whole	12.09	-0.028

the storage temperatures normally experienced after harvest (usually 20–30°C). There is no record of phosphine resistant strains of pea weevil occurring, and the development of strains capable of withstanding a well conducted fumigation is considered unlikely. It appears that failing to ensure or maintain the necessary gastightness in storages under fumigation (Banks and Annis 1984) is the most likely cause of fumigation failures.

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