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THE USE OF PHOSPHINE FOR INHIBITION OF FUNGAL GROWTH IN STORED GRAINS

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ABSTRACT

Phosphine (PH_3) is in widespread use for the control of insects in stored grains. Phosphine may be applied as a single treatment to kill insects already present in grain, or at a low level over an extended period for the continuous control of insects in sealed grain storages (SIROFLO process). Because phosphine acts as a respiratory inhibitor, it can also control the growth of fungi in grain stored at marginal moisture contents, although it does not actually kill dormant fungal spores. The primary fungal invaders of stored grains are *Eurotium* species, *Aspergillus* series *Restricti* species, particularly *A. penicillioides* and *A. restrictus*, and *Wallemia sebi*. Phosphine has been shown to be useful in controlling the establishment of populations of these fungi in relatively moist grain over short storage periods of up to four weeks, by increasing the length of the lag period, and slowing the growth rate. This paper reviews the effects of phosphine fumigation on fungal succession and the development of storage fungi in grains, with reference to specific examples from the authors' own work, and from published literature. The practical implications of using phosphine to prevent fungal deterioration of stored grains are assessed.

INTRODUCTION

Phosphine fumigation is used throughout the world because it is relatively cheap and effective, and easy to apply. In normal fumigation practice, concentrations of phosphine up to about 3 $g \cdot m^{-3}$ are used against insects. Where long exposure times are possible, target maximum phosphine concentrations may be 0.5 $g \cdot m^{-3}$ or less. The lowest level that is effective against insects appears to be in the range of 0.02-0.1 $g \cdot m^{-3}$ (Winks, 1986). Phosphine is an inhibitor of respiratory enzymes (Nakakita *et al.*, 1971) and thus could be expected to have some effect on fungi also.

Although phosphine has been in widespread use for insect control for some decades, much less is known about the effects of phosphine fumigation on the development of storage fungi in commodities, particularly over extended periods. Most studies on the effect of phosphine on growth of moulds, either on natural substrates or in culture media, have been carried out over relatively short periods. These studies indicate that phosphine has only a minor influence on non-growing moulds (e.g. Hocking and Banks, 1991; Raghunathan *et al.*, 1969; Sinha *et al.*, 1967) but that it may have some useful effects under storage conditions where moulds are active.

REVIEW OF PUBLISHED STUDIES

Effects of phosphine on fungi in pure culture

Studies on the effects of phosphine on fungi growing in pure culture have reported varying results, with some species of fungi being more sensitive than others. Phosphine at a concentration of 0.3 $g \cdot m^{-3}$ reduced growth (measured as dry weight of mycelium) of pure cultures of various *Aspergillus* species grown on liquid medium (Leitao *et al.*, 1987), but the reduction varied from as little as 3.5% for one strain of *Eurotium rubrum* up to 86.5% for one strain of *A. flavus*. There was considerable strain-to-strain variation in the level of inhibition. Ren *et al.* (1983), using phosphine concentrations between 0.31 and 3.08 $g \cdot m^{-3}$ for 3, 5 and 10 days at 30°C, demonstrated that *E. amstelodami* was more sensitive to phosphine than *A. flavus*. Germination of *E. amstelodami* was inhibited by the lowest concentration of phosphine applied, but *A. flavus* germinated after 10 days in 0.97 $g \cdot m^{-3}$ phosphine. Once growth was established, the fungi were considerably more resistant to phosphine. Ren *et al.* (1983) also found that phosphine was more effective in preventing fungal growth if applied in low (<1.0%) oxygen atmospheres.

Bailly *et al.* (1985) demonstrated the inhibition of a number of fungal species held for 21 days at ambient temperature in an atmosphere of 1.6 to 2.0 $g \cdot m^{-3}$ phosphine. After phosphine treatment, cultures were incubated at 28°C for 15 days. Of the 100 strains tested, 41 were killed by the phosphine treatment, 41 were inhibited, but subsequently grew when removed from the phosphine fumigation chamber, and the remainder were unaffected. *Fusarium* species were the most resistant, and *Penicillium* strains the most sensitive to the treatment. Of the 46 strains of *Aspergillus* tested, nine were killed, 31 inhibited, and six unaffected by the phosphine treatment. Similar results were reported by Bailly *et al.* (1987) using higher concentrations of phosphine (4.3 to 4.9 $g \cdot m^{-3}$) over a longer time period, up to 90 days. By contrast, four *Fusarium* isolates and the single isolate of *Byssochlamys* tested survived exposure for 90 days.

Effects of phosphine on mycotoxin production

When freshly harvested paddy rice (0.92 water activity [a_w]) was inoculated with *Aspergillus parasiticus* and exposed to 0.1 g·m^{-3} phosphine at 28°C, Hocking and Banks (1990) reported the formation of considerable amounts of aflatoxins in the inoculated samples, both in air and in phosphine. However, after a 2-week exposure, aflatoxin levels in the phosphine-treated rice were less than half those in the air controls, and after 4 weeks, the phosphine-treated rice contained just over half the amount of aflatoxins detected in the air controls.

Exposure to phosphine for 5 days has been shown to decrease growth and aflatoxin production by *A. flavus* on potato dextrose agar (Dharmaputra *et al.*, 1991), with aflatoxin production decreasing with increasing concentrations (0.5-3.5 g·m^{-3}) of phosphine. Phosphine (150 ppm, equivalent to 0.15 g·m^{-3}, for 21 days) also inhibited the production of sterigmatocystin by *A. versicolor* and *A. nidulans* (Leitao *et al.*, 1990).

Effects of phosphine on growth of fungi in stored commodities

Phosphine treatment can affect fungal populations in stored commodities. Fungi were reduced on pulses exposed to very high phosphine levels (100 g·m^{-3}), particularly at 15% moisture content (m.c.) (approximately 0.80 a_w), the highest value tested (Natarajan and Bagyaraj, 1984). Hocking and Banks (1991) inoculated wheat of 0.80 and 0.86 a_w with a common storage fungus, *Eurotium chevalieri* and a mycotoxigenic species, either *Aspergillus flavus* or *A. parasiticus*. The wheat was then exposed to a stream of phosphine (0.1 g·m^{-3}) for 2 weeks at 28°C, with control samples exposed to air under the same conditions. Storage of the moist grain in phosphine rather than air led to a less rapid development of most storage fungi, but did not prevent growth completely (Fig. 1). The level of inhibition observed suggests that phosphine may be useful in retarding fungal spoilage during the short-term storage of high moisture grain (15-19% m.c. for wheat). Phosphine at 0.1 g·m^{-3} caused only a slight decrease in populations of fungi that were unable to grow at the a_w of the stored grain, and there was no elaboration of aflatoxins in the grains, as the a_w was just marginal for growth of the aflatoxigenic fungi (Wheeler *et al.*, 1988), and below the level at which toxin can be formed (Northolt *et al.*, 1976).

The effect of phosphine at higher a_w on aflatoxigenic fungi and aflatoxin production was studied by Hocking and Banks (1990) for freshly harvested paddy rice, equilibrated to a_w 0.92 (20.8% m.c.), inoculated with a mixture of *A. parasiticus* and *E. chevalieri*. The inoculated samples and uninoculated controls were exposed to 0.1 g·m^{-3} phosphine for 14 and 28 days at 28°C. There was extensive development of storage fungi, particularly *Penicillium* species, in both the inoculated and uninoculated samples held in air (Fig. 2). The population of *A. parasiticus* rose substantially in the

inoculated samples, but development was less rapid in the phosphine-treated samples. Storage of moist paddy rice in phosphine slowed the growth rate of most storage fungi but failed to prevent growth altogether. *Penicillium* species appeared to be particularly resistant to phosphine.

The use of phosphine to inhibit mould growth and mycotoxin formation appears promising as a method of short-term preservation of undried paddy. However, phosphine levels higher than 0.1 $g \cdot m^{-3}$ would be necessary if moist rice is to be held for longer than a few days. The experiments at a relatively high a_w indicate that at a_w values which are marginal for growth of common storage fungi, in the region of a_w 0.65-0.73, phosphine might be very effective in preventing the establishment of fungal populations.

PRESENT STUDIES

In a series of experiments completed recently in our laboratories, we studied the effects of long-term, low level phosphine exposure on the development of storage fungi in wheat at a_w values of 0.72-0.73, 0.70-0.71, and 0.68-0.69.

Methods

Pesticide-free, unfumigated samples of wheat were conditioned, by appropriate water addition, to the required a_w values. After conditioning, the grain samples were inoculated with *E. chevalieri* ascospores to give 10^3 colony forming units (cfu) g^{-1}, then subdivided by Boerner divider into 32 subsamples (100 g each). During the test, samples were exposed in duplicate at 28°C either to air or to 0.1 g or 1.0 g m^{-3} PH_3 at 5.0 mL min^{-1}, appropriately humidified through each subsample, plumbed in parallel, and held in jars equipped with a lower plenum and vent (to prevent back-diffusion), as described by Hocking and Banks (1991). Subsamples were removed at intervals of one, two, four, and six months.

Mycoflora was assessed by both direct and dilution plating on DG18 agar (Hocking and Pitt, 1980). This medium is designed for enumeration of xerophilic fungi, particularly *Eurotium* species and *Aspergillus* series *Restricti* species, in foods and commodities of reduced a_w. On DG18, it is possible to distinguish between different species of *Eurotium* by the production of coloured encrusting hyphae, enabling monitoring of the development of populations of the inoculum (*E. chevalieri*) and of other *Eurotium* species, as well as *A. restrictus* and related species.

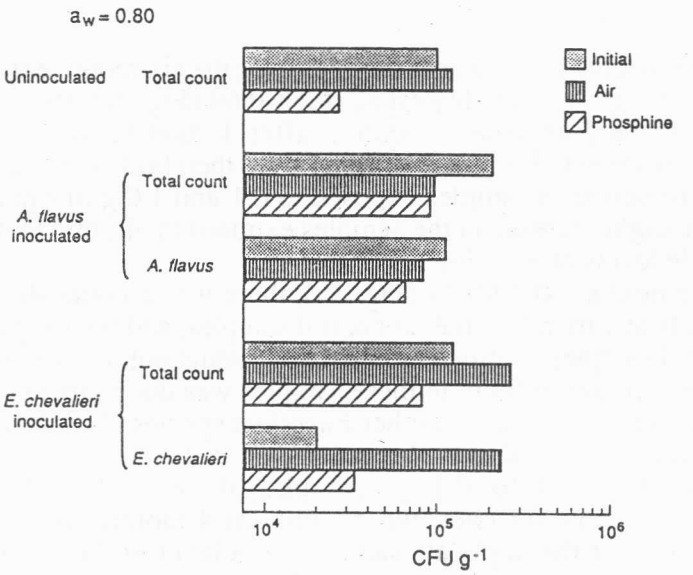

Fig. 1: Fungal counts (logarithmic scale, colony forming units g^{-1}) in wheat inoculated with *A. flavus* and *E. chevalieri*, and in uninoculated wheat, 0.80 a_W, held at 28°C for 14 days in air or 0.1 $g \cdot m^{-3}$ phosphine compared with initial counts The bar graph shows total fungal counts in a sample and counts of the inoculated species in that sample. *A. flavus* was enumerated on DRBC agar, and *E. chevalieri* enumerated on DG18 agar (Data of Hocking and Banks, 1991).

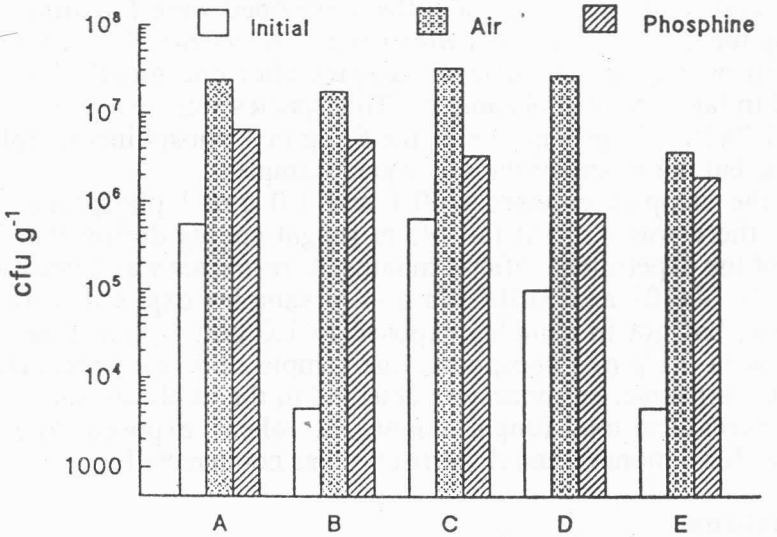

Fig. 2: Fungal counts (logarithmic scale, colony forming units g^{-1}) on DRBC agar, from paddy rice, 0.92 a_W, exposed at 28°C for two weeks to air or phosphine, 0.1 $g \cdot m^{-3}$. (A) total fungi in uninoculated rice; (B) *Penicillium* in uninoculated rice; (C) total fungi in rice inoculated with *E. chevalieri* and *A. parasiticus*; (D) *A. parasiticus* in inoculated rice; (E) *Penicillium* in inoculated rice (Data of Hocking and Banks, 1990).

Results

Fungal counts in the wheat samples over the six month exposure period are shown in Fig. 3. At the lowest a_w, 0.68-0.69 (Fig. 3a), there was a slight reduction in the *Eurotium* inoculum after 1 month, irrespective of the fumigation treatment. The levels of fungi were then fairly constant throughout the exposure period in samples exposed to 0.1 and 1.0 $g \cdot m^{-3}$ phosphine, but there was a slight increase in the samples exposed to air, due to development of detectable levels of *A. restrictus* (Table 1).

At the next a_w, 0.70-0.71 (Fig. 3b), there was a noticeable increase in fungal counts at 1 month in the air control samples, and the fungal population in the control samples continued to increase throughout the 6 month exposure period. The increase in fungi in these samples was due to increased numbers of the inoculum, *E. chevalieri*, other *Eurotium* species (*E. rubrum, E. repens* and *E. amstelodami*), and *A. restrictus*.

Wheat exposed to 0.1 $g \cdot m^{-3}$ phosphine at 0.70-0.71 a_w was mycologically stable for over two months. At 4 months, *A. restrictus* was detected in one of the duplicate samples, at a level of $7x10^3$ cfu g^{-1}. After 6 months, both samples had detectable levels of *A. restrictus* ($7x10^3$ and $5.8x10^3$ cfu g^{-1} respectively). At this a_w, the wheat samples treated with 1.0 $g \cdot m^{-3}$ phosphine showed no increase in fungal counts over the 6 month exposure period (Fig. 3b).

At the highest a_w, 0.72-0.73 (Fig. 3c), there was a steady increase in fungal counts in the air control samples over the 6 month period. As with the samples at 0.70-0.71 a_w, the fungi that developed were *Eurotium* species, including the inoculum (*E. chevalieri*) and *A. restrictus*. *Wallemia sebi* was present in both of the air control samples after one month, but was not detected in later air control samples. This species was also detected at low levels ($1.7x10^3$ cfu g^{-1}) in one of the 0.1 $g \cdot m^{-3}$ phosphine samples after 2 months, but not in any of the subsequent samples.

In the samples exposed to 0.1 and 1.0 $g \cdot m^{-3}$ phosphine at 0.72-0.73 a_w, there was a slight fall off in fungal counts during the first two months of the experiment. After 4 months, *A. restrictus* was detected at very low levels ($4x10^2$ and $5x10^1$ cfu g^{-1}) in samples exposed to 0.1 $g \cdot m^{-3}$ phosphine, but not in samples exposed to 1.0 $g \cdot m^{-3}$ phosphine. After 6 months, with 0.1 $g \cdot m^{-3}$ phosphine, one sample of wheat contained $3x10^4$ cfu g^{-1} *A. restrictus*, but none was detected in the duplicate sample. There was no increase in total fungal counts in wheat exposed to 1.0 $g \cdot m^{-3}$ phosphine for 6 months, and *A. restrictus* was not detected.

Conclusions

The effect of long term phosphine fumigation on patterns of development of storage fungi is well illustrated by considering the counts of *A. restrictus* (Table 1). This shows that at very low a_w, below about 0.67, grain is stable in storage for up to 6 months with little development of storage

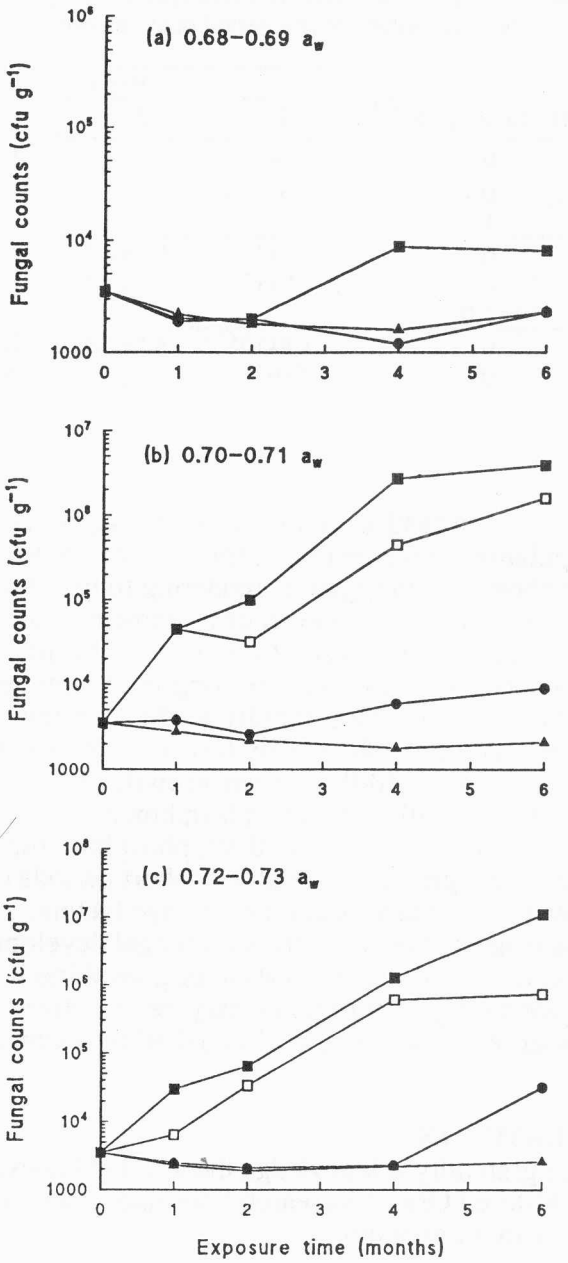

Fig. 3: Changes in fungal counts (logarithmic scale, colony forming units g^{-1}) in wheat inoculated with *E. chevalieri*, held at three different a_w values at 28°C for 6 months, exposed to air (❑, ■), 0.1 $g \cdot m^{-3}$ phosphine (●), or 1.0 $g \cdot m^{-3}$ phosphine (▲). Open and closed symbols represent duplicate samples differing from one another.

Table 1. Development of *Aspergillus* Series *Restricti* species (cfu g^{-1}) in wheat fumigated with phosphine over a period of six months.

		Exposure time (months)			
a_w	PH_3 Conc.(g m^{-3})	1	2	4	6
0.68-0.69	0	-[a]	-	$3.6x10^3$	$6.0x10^3$
	0.1	-	-	-	-
	1.0	-	-	-	-
0.70-0.71	0	$2.0x10^4$	$6.0x10^4$	$9.3x10^5$	$2.2x10^6$
	0.1	$5.0x10^2$	$2.0x10^2$	$3.5x10^3$	$6.4x10^3$
	1.0	-	-	-	-
0.72-0.73	0	$1.4x10^4$	$4.5x10^4$	$2.2x10^5$	$1.8x10^6$
	0.1	$5.0x10^1$	-	$4.0x10^2$	$3.0x10^4$
	1.0	-	-	-	-

-[a], not detected

fungi, irrespective of fumigation treatment. As the a_w is raised to about 0.70, *A. restrictus* populations develop more rapidly, but can be controlled by low level (0.1 $g \cdot m^{-3}$) phosphine fumigation, rendering fungal development similar to that in grain at a lower a_w. However, development of storage fungi at this a_w can be prevented by higher levels (1.0 $g \cdot m^{-3}$) of phosphine.

If the a_w is above the safe limit for long-term storage, e.g., a_w 0.72 or above, storage fungi will develop rapidly in the first few months, and this development is retarded only slightly by low level phosphine fumigation. If the a_w is marginal, e.g., 0.70-0.72, then growth of storage fungi can be inhibited by fumigation with 1.0 $g \cdot m^{-3}$ phosphine, but this level is too high for long-term use. At a_w values above 0.80, phosphine fumigation can only be used to retard fungal growth for relatively short periods of a few days to a few weeks, depending on the a_w and other storage factors.

More work is needed on the effects on fungal development of mixtures of phosphine and CO_2, and the application of phosphine in low (<1.0%) O_2 atmospheres. Such fumigation regimes may be effective for the control of growth in grains stored between a_w 0.72 and 0.80 for extended periods.

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