EFFECTS OF PHOSPHINE ON THE DEVELOPMENT OF STORAGE MYCOFLORA IN PADDY RICE.

A. D. HOCKING[1] and H. J. BANKS[2]

[1]CSIRO Division of Food Processing, North Ryde, NSW, 2113, Australia and [2]CSIRO Division of Entomology, Canberra, ACT, 2600, Australia

ABSTRACT

Freshly harvested paddy rice, equilibrated to 0.92 a_w (equivalent to 20.8% moisture content, wet basis), was inoculated with a mixture of *Aspergillus parasiticus* and *Eurotium chevalieri*. The inoculated samples, and uninoculated controls, were exposed to 0.1g m^{-3} phosphine or to air 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. The population of *A. parasiticus* rose substantially in the inoculated samples, but development was less rapid in the phosphine treated samples. Phosphine slowed the growth rate of most storage fungi, but *Penicillium* species appeared to be particularly resistant to phosphine. *Eurotium* species were rapidly overgrown by *A. parasiticus* in both the phosphine treated samples and the air controls. Considerable amounts of aflatoxins were formed in the inoculated samples, however, after 2 weeks exposure, aflatoxin levels in the phosphine treated rice were less than half those in the air controls. 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 0.1g m^{-3} may be necessary if moist rice is to be held for longer than a few days.

INTRODUCTION

During storage of grains considerable losses are frequently caused by fungal growth and mycotoxin production, particularly in high moisture content grains. Phosphine fumigation, which is well known as an effective method of control of insect pests in grain (Bond, 1984) may assist in prevention of mould growth. Results of published studies indicate that phosphine has only a minor influence on non-growing moulds (e.g. Raghanathan Muthu and Majumder, 1969; Sinha, Berck and Wallace, 1967) but may be of some use under storage conditions where moulds are active. Natarajan and Bagyaraj (1984) noted some reduction in fungi on pulses exposed to very high phosphine levels (100 g m^{-3}), particularly at 15% moisture content (m.c.), approximately 0.8 water activity (a_w), the highest value they tested. Hocking and Banks (unpublished) also demonstrated some control of the development of storage mycoflora in wheat exposed to 0.1 g m^{-3} at a_w values of 0.80 and 0.86. Inhibition of growth of a number of fungal species on culture media at high a_w in the presence of phosphine was noted by Bailly, Leitao and Cabrol-Telle (1985) and Leitao, de Saint-Blanquet and Bailly (1987).

This paper describes experiments carried out to determine to what extent phosphine can control mould growth in grain under conditions where storage moulds can actively grow (0.92 a_w and 28°C). At this a_w, *Penicillium* and *Aspergillus* species tend to dominate, and *Eurotium* species, xerophilic fungi which are often the primary invaders of stored grains (Pelhate, 1968;

Pitt and Hocking, 1985), are less competitive. Data are presented here on the effects of phosphine fumigation on the survival and growth of the grain storage fungi *Eurotium chevalieri* Mangin and *A. parasiticus* Speare, and on the development of other mycoflora in the grain.

MATERIALS AND METHODS

General experimental design.

Eight kg of freshly harvested paddy rice (initial a_w 0.96) was bruised by passage through a laboratory rice huller (Satake) with the rollers set to crack many of the hulls but not remove them. This process provides a much better substrate for mould growth than intact paddy. The bruised paddy was then equilibrated to 0.92 a_w. Eight samples of this rice were exposed to a flow of humidified air, and eight exposed to a humidified flow of 0.1 g m^{-3} phosphine in air. In each set of eight samples, four were uninoculated controls, and four were inoculated with a mixed inoculum of *E. chevalieri* and *A. parasiticus*. The gas flow was maintained at 28°C and 0.92 a_w for 28 days, with half of the samples being removed after 14 days.

Cultures and preparation of fungal inocula

A. parasiticus Speare FRR 2752 and *E. chevalieri* Mangin FRR 544 were obtained from the culture collection of the CSIRO Food Research Laboratory, North Ryde.

Fungal inocula, applied dry to avoid changing the a_w of the rice, were prepared as follows. Suspensions in 0.05% Tween 80 of conidia of *A. parasiticus* and ascospores of *E. chevalieri* containing approximately 2.5 x 10^7 conidia or ascospores per mL were prepared. The suspensions were filtered through 0.45 um Nucleopore membrane filters in 10 ml aliquots. The membranes were then dried at 30°C for 18 hours. Each membrane contained sufficient inoculum for 500 g rice.

Inoculation

The equilibrated rice was divided into eight 1 kg subsamples by passing through a Borner divider. Four of these samples were left uninoculated. Each of the remaining samples was inoculated by placing in a large jar, and adding two membranes of dried spores of each fungal species. Jars and contents wer shaken and tumbled thoroughly for approximately 15 min. These were then pooled, mixed by passing through the divider a further four times, then separated into eight 500 g subsamples.

Exposure of grain samples

Conditioned and inoculated rice samples were exposed to phosphine as follows. Sixteen fruit-preserving jars (1.9 L Fowler jars, diameter 10 cm), modified to allow gas flow through the grain samples were used. Gas (air or 0.1 g m^{-3} phosphine in air) was admitted at a rate of 83 mL min^{-1}, giving a face velocity through the grain of about 0.3 m h^{-1}. A vent tube was fitted to the lid of each jar to prevent back diffusion. Flows through each sample were checked at the end of the vent tube using a hot wire anemometer (Datametrics model LM-500). The input gas was conditioned to the correct a_w by passage through two 500 mL Dreschel bottles in series containing an appropriate glycerol-water mixture. The evaporated water from the humidifying solutions was replaced every two days during the exposure period by adjusting the solutions to their original volume with water.

At the start of each experiment, samples were exposed to air (83 mL min^{-1}) for 24 h to provide fine adjustment of the interstitial humidity. The test samples were then switched over to 0.1 g m^{-3} phosphine. After 14 d exposure was complete, eight samples (four inoculated and four uninoculated controls) were removed and ventilated with conditioned air at 83 mL min^{-1} for 30 min. The remaining eight samples were left for a further 14 days exposure

before being removed. All samples were analysed for mould within 24 h.

The required 0.1 g m^{-3} phosphine stream was obtained by 10:1 dilution of 1.0% phosphine in a compressed nitrogen source. The exact required dilution was set using variable-area flow meters (Gapmeter) adjusted to give 0.1 (± 0.01) g m^{-3} as analysed by gas chromatography against a gravimetric reference standard.

Detection of fungi

Total fungal counts were carried out in duplicate on all samples. Samples were homogenised using a Coleworth Stomacher, diluted in 0.1% peptone water, and surface-plated onto Dichloran Rose Bengal Chloramphenicol agar (DRBC) for total yeast and mould count, Aspergillus Flavus and Parasiticus Agar (AFPA) for enumeration of *Aspergillus parasiticus*, and Dichloran 18% Glycerol agar (DG18) for enumeration of *Eurotium* species (Pitt and Hocking, 1985).

Aflatoxin analyses

Aflatoxin assays were performed on inoculated samples, using the method of Pons and Franz (1978). The rice was ground and extracted with methanol-0.1N HCl (4:1), and the extract assayed using thin layer chromatography. Aflatoxins were quantified by dilution and visual comparison of UV fluorescence with standards.

RESULTS

Mycoflora of initial samples

At the beginning of the experiment the fungal counts, as determined by dilution plating, were as follows. In the uninoculated sample, counted on both DRBC and DG18, the total count was 5.0 x 10^5 cfu g^{-1}. The mycoflora comprised mostly pigmented and non-pigmented yeasts, with *Cladosporium*, 6.4 x 10^4, *Alternaria*, 1.1 x 10^4; *Penicillium*, 4.5 x 10^3 and *Fusarium* 2.5 x 10^3. By direct plating, the infection rate was 100%, with the dominant species being *Alternaria* (on 100% of grains), *Cladosporium* (on at least 41% of grains, but this was probably a gross underestimate, since the *Cladosporium* colonies were overgrown by *Alternaria*), *Fusarium* (9%), *Penicillium* (6%), *Phoma* (4%), *Nigrospora* (9%), *Epicoccum* (2%) and *A. niger* (1%).

In the inoculated sample, inoculum levels were close to the desired value of 1.0 x10^5 cfu g^{-1} of each of the two species. Actual values were *A. parasiticus* 1.0 x 10^5 cfu g^{-1} and *E. chevalieri* 1.2 x 10^5 cfu g^{-1}.

Changes in mycoflora

After 2 weeks exposure to phosphine or air, considerable changes occurred in the mycoflora of both inoculated and uninoculated samples (Tables 1 and 2). Total mould counts rose in both air and phosphine treated samples, but were still ten-fold less in the phosphine treated samples than in the samples held in air. The major changes in the fungal populations of the rice after 2 weeks exposure to air or phosphine are illustrated in Fig. 1 (data from DRBC agar). There were four noticeable changes in the mycoflora of the samples.

E. chevalieri, originally present at 1.2x10^5 was no longer detectable in the rice exposed to humidified air, while *Eurotium* species were present at close to the original inoculum level in the phosphine treated rice, in both inoculated and uninoculated samples. However, *Eurotium* species were only detected at this level on DG18 agar (0.955 a_w, Table 2), while on DRBC agar (0.995 a_w) *Eurotium* was detected only in the uninoculated sample at 2.5x10^4 cfu g^{-1} (Table 1). This indicated that the *Eurotium* had probably been physiologically damaged by the phosphine treatment. *Eurotium* species are xerophilic, and can only grow on high a_w media such as DRBC if all other conditions are favourable.

Table 1. Fungi detected on DRBC agar in rice before and after treatment with phosphine.

Species present[a] Sample	Total	Eur.	A.par	Pen.	A.can	A.fla	Ysts	Clad	Alt.	Other
PRE-FUMIGATION COUNTS										
Uninoculated	$5.5x10^5$			$4.5x10^3$			$4.2x10^5$	$6.4x10^4$	$1.1x10^4$	$2.5x10^3$
Inoculated		$1.2x10^5$	$1.0x10^5$							
POST-FUMIGATION COUNTS: 2 WEEKS										
AIR CONTROLS										
Uninoculated	$2.4x10^7$	$2.5x10^5$		$1.8x10^7$	$5.0x10^5$	$2.5x10^5$		$2.5x10^5$		$5.0x10^{6b}$
Inoculated	$3.2x10^7$		$2.7x10^7$	$3.8x10^6$	$2.5x10^5$			$5.0x10^5$		$2.5x10^{5b}$
PHOSPHINE TREATED										
Uninoculated	$6.6x10^6$	$2.5x10^4$	$2.5x10^4$	$5.2x10^6$	$3.3x10^5$	$1.0x10^5$	$5.0x10^4$	$7.5x10^4$		$9.5x10^5$
Inoculated	$3.4x10^6$		$7.5x10^5$	$1.9x10^6$				$7.5x10^4$		$7.3x10^5$
POST-FUMIGATION COUNTS: 4 WEEKS										
AIR CONTROLS										
Uninoculated	$2.0x10^8$			$1.9x10^8$						
Inoculated	$1.9x10^8$		$1.2x10^8$	$5.5x10^7$		$1.0x10^5$				
PHOSPHINE TREATED										
Uninoculated	$5.2x10^7$			$5.0x10^7$						
Inoculated	$7.5x10^7$		$9.0x10^5$	$7.0x10^7$		$4.5x10^5$				

a: Total, total yeasts and moulds detected; Eur, *Eurotium* species; A.par, *Aspergillus parasiticus*; Pen, *Penicillium* species; A.can, *A. candidus*; A.fla, *A. flavus*; Ysts, yeasts; Clad, *Clasdosporium* species; Alt, *Alternaria* species; Other, other fungus species, not identified.
b: *Aspergillus* species, not identified

Table 2. Fungi detected on DG18 agar in rice before and after treatment with phosphine

Species present[a] Sample	Total	Eur.	A.par	Pen.	A.can	A.fla	Ysts	Clad	Alt.	Other
PRE-FUMIGATION COUNTS										
Uninoculated	$5.5x10^5$			$4.5x10^3$			$4.2x10^5$	$6.4x10^4$	$1.1x10^4$	$2.5x10^3$
Inoculated		$1.2x10^5$	$1.0x10^5$							
POST-FUMIGATION COUNTS: 2 WEEKS										
AIR CONTROLS										
Uninoculated	$3.2x10^7$			$2.5x10^7$	$2.0x10^6$					$4.5x10^{6b}$
Inoculated	$3.0x10^7$		$2.2x10^7$	$3.8x10^6$	$2.5x10^5$			$1.0x10^6$		$5.0x10^5$
PHOSPHINE TREATED										
Uninoculated	$6.6x10^6$	$1.0x10^5$		$4.5x10^6$	$1.4x10^6$	$5.0x10^4$		$7.5x10^4$		$3.3x10^5$
Inoculated	$2.4x10^6$	$1.8x10^5$	$5.5x10^5$	$1.7x10^6$	$1.0x10^5$		$2.5x10^4$	$3.0x10^4$		$5.0x10^4$
POST-FUMIGATION COUNTS: 4 WEEKS										
AIR CONTROLS										
Uninoculated	$2.8x10^8$			$2.2x10^8$	$6.0x10^7$					
Inoculated	$2.5x10^8$		$1.6x10^8$	$5.0x10^7$	$5.0x10^6$					
PHOSPHINE TREATED										
Uninoculated	$1.0x10^8$			$6.8x10^7$	$2.5x10^7$					
Inoculated	$8.5x10^7$	$2.5x10^4$	$1.0x10^6$	$8.0x10^7$	$3.9x10^6$					

For explanatory footnotes, see Table 1.

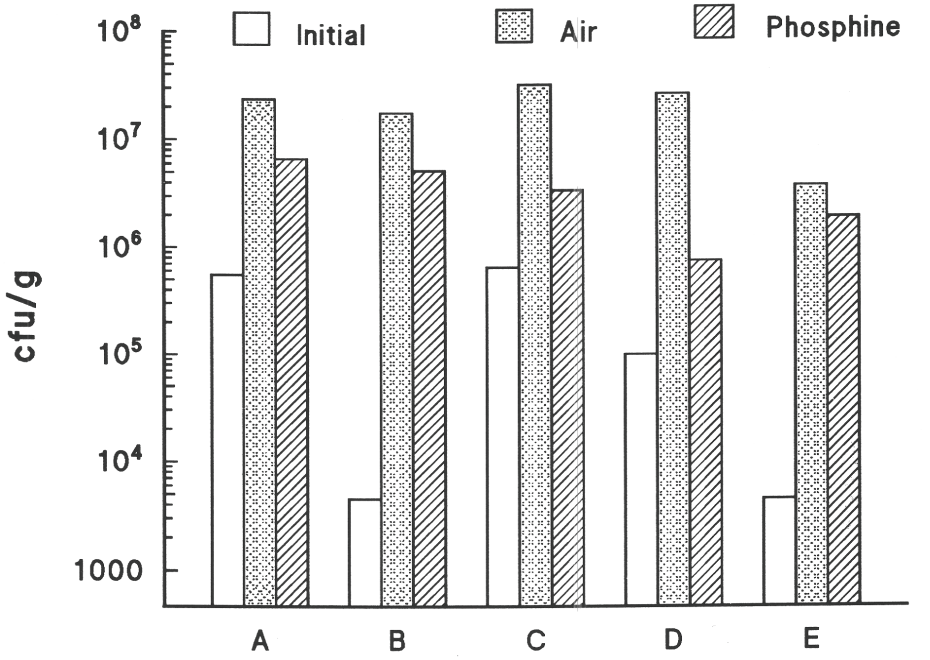

Fig. 1. Fungal counts on DRBC agar from rice, 0.92a_w, exposed to air or phosphine for 2 weeks. (A) total fungi in uninoculated rice; (B) Penicillium *in uninoculated rice; (C) total fungi in inoculated rice (D)* A. parasiticus *in inoculated rice; (E)* Penicillium *in inoculated rice.*

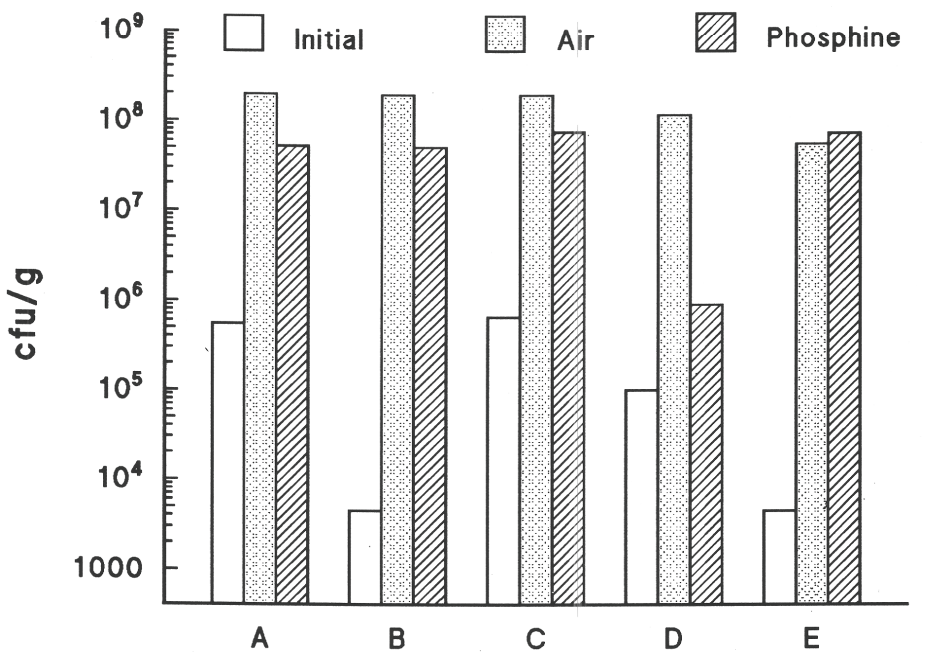

Fig. 2. Fungal counts on DRBC agar from rice, 0.92a_w, exposed to air or phosphine for 4 weeks. (A) total fungi in uninoculated rice; (B) Penicillium *in uninoculated rice; (C) total fungi in inoculated rice (D)* A. parasiticus *in inoculated rice; (E)* Penicillium *in inoculated rice.*

The levels of *A. parasiticus* detected in the rice rose substantially from the initial inoculum of $1.0x10^5$ to $2.7x10^7$ in the air controls, but the increase in the phosphine treated rice was less, with only $7.5x10^5$ cfu g^{-1} *A. parasiticus* detected on DRBC agar (Table 1).

Populations of *A. candidus*, most readily detected on DG18 agar (Table 2) developed in all samples. There was little difference between air and phosphine treatments, but there was about 10 times less *A. candidus* in the inoculated samples than the uninoculated samples, possibly due to competition from *A. parasiticus*.

Penicillium species increased by 3 to 4 orders of magnitude in both air and phosphine exposed rice (Tables 1 and 2), though the increases were slightly less in the phosphine treated samples. There was little difference in diversity of species between uninoculated samples exposed to either air or phosphine (Table 3), however, in the inoculated samples, *Penicillium* species were not identifiable in the air controls because of overgrowth by *A. parasiticus*. The dominant species present in most samples was *P. fellutanum*, a species in the sub-genus *Aspergilloides*, and not known to produce any mycotoxins. However, most of the other *Penicillium* species identified belonged to the sub-genus *Penicillium*, which contains many known mycotoxin producers.

After 4 weeks exposure to air or phosphine at 28°C and 0.92 a_w, the fungal populations in the rice samples were extremely high, with counts near 10^8 in all samples (Fig 2; Tables 1 and 2). However, in the samples exposed to air, counts of *A. parasiticus* were two orders of magnitude greater than for the phosphine treated samples (10^8 vs 10^6), with *Penicillium* species being the dominant mycoflora of the rice exposed to phosphine. *A. candidus* was also present in all samples, but was only detected on DG18 agar (Table 2). Levels of *A. candidus* were similar in both air and phosphine treated samples.

Effects of phosphine on aflatoxin production

Considerable amounts of aflatoxins were detected in all inoculated samples (Table 4 and Fig. 3). Aflatoxin G_1 was present in the greatest quantities, followed by aflatoxin B_1. B_2 and G_2 aflatoxins were present in much lower amounts (Table 4).

Table 3. Development of Penicillium *species in rice after 2 and 4 weeks exposure to air or phosphine.*

	PH_3				AIR			
	Uninoculated		Inoculated		Uninoculated		Inoculated	
Species	2 wks	4 wks	2 wks	4wks	2wks	4wks	2wks[a]	4wks
P. fellutanum	$1.2x10^6$	$1.1x10^7$	$5.0x10^5$	$4.1x10^7$	$8.7x10^6$	$1.2x10^8$	-[b]	-
P. chrysogenum	$1.3x10^6$	$1.2x10^6$	-	-	-	-	-	-
P. aurantiogriseum	$5.5x10^5$	$1.7x10^6$	$3.0x10^4$	$1.0x10^5$	-	$1.5x10^6$	-	-
P. viridicatum	$2.5x10^5$	-	$1.0x10^5$	-	$3.0x10^6$	-	-	-
P. griseofulvum	$4.5x10^5$	$3.4x10^6$	$1.0x10^5$	$9.0x10^5$	$3.3x10^5$	$3.3x10^6$	-	$5.0x10^6$
P. brevicompactum	$3.5x10^5$	$2.8x10^5$	$1.3x10^5$	$2.2x10^6$	$6.7x10^5$	$2.5x10^6$	-	-
P. solitum	$2.0x10^5$	-	$5.4x10^4$	-	-	$1.8x10^7$	-	$1.3x10^7$
P. citrinum	$1.5x10^5$	-	$1.5x10^5$	-	-	-	-	-
P. commune	-	-	$3.0x10^5$	-	$3.7x10^6$	-	-	-
P. glandicola	-	-	-	$6.7x10^5$	-	-	-	-
P. islandicum	-	-	-	$2.0x10^5$	-	-	-	-
P. lividum	-	-	-	$5.0x10^5$	-	-	-	-
P. corylophilum	-	-	-	$1.0x10^5$	-	-	-	-
TOTALS (approx.)	$5.2x10^6$	$1.7x10^7$	$2.0x10^6$	$4.5x10^7$	$1.7x10^7$	$1.4x10^8$	-	$1.8x10^7$

a: *Penicillium* colonies not identifiable from these samples because of overgrowth by *A. parasiticus*
b: species not detected in sample

After 2 weeks exposure aflatoxin levels in the rice held in air were approximately three times higher than in the rice exposed to phosphine. After 4 weeks, aflatoxin levels in the phosphine treated rice were still lower than the air controls, but the differences were much less (Fig 3). Aflatoxin B_2 was not detected in the samples held in air after two weeks or four weeks exposure (Table 4).

Table 4. Aflatoxin content (ppm) of inoculated rice samples exposed to air or phosphine for two and four weeks

SAMPLE	AFLATOXIN: B_1	B_2	G_1	G_2
2 WEEKS				
Phosphine (a)	300-400	0.42-0.48	600-700	0.60-0.90
(b)	320-420	0.32-0.38	530-640	0.64-0.95
Air (a)	1260-1470	n.d.[a]	1680-1890	0.63-0.95
(b)	1060-1270	n.d.	1690-1908	0.64-0.95
4 WEEKS				
Phosphine (a)	1430-1670	2.14-2.86	2380-2860	2.14-2.86
(b)	1428-1666	2.14-2.86	2140-2620	2.14-2.86
Air (a)	1960-2450	n.d.	3430-3920	1.47-2.20
(b)	1630-1900	n.d.	3260-3810	1.63-2.45

a: n.d. = not detected

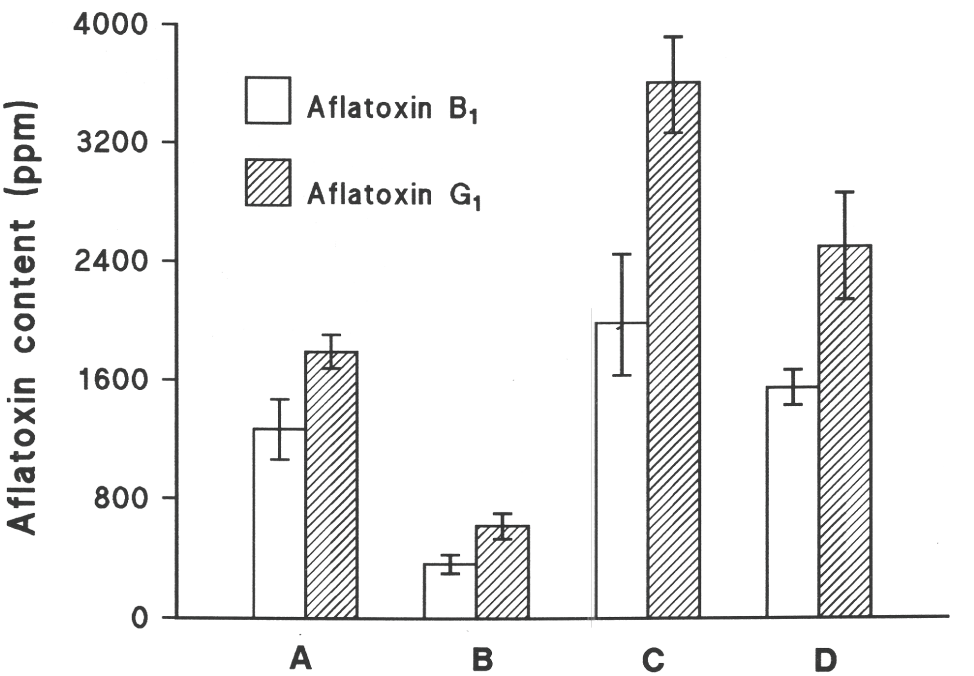

Fig. 3. Aflatoxin content of inoculated rice exposed to air or phosphine after 2 and 4 weeks. Bars show range of aflatoxin in duplicate samples. (A) 2 weeks in air; (B) 2 weeks in phosphine; (C) 4 weeks in air; (D) 4 weeks in phosphine.

DISCUSSION

Results of previous experiments with wheat at 0.80 and 0.86 a_w exposed to phosphine for 2 weeks (Hocking and Banks, unpublished) demonstrated good control of the aflatoxigenic fungi *Aspergillus flavus* and *A. parasiticus*. At these a_w values, storage mycoflora, particularly *Eurotium* species developed in the grain exposed to air, but there was much less growth in the phosphine treated grain. *Penicillium* species developed in grain held in air and in phosphine at 0.86 a_w, in both inoculated and uninoculated samples, and the levels were similar for both treatments. The species present were not identified. Aflatoxin analyses were not carried out on the wheat samples.

In the experiments described here, the a_w conditions under which the grain was held were much more favourable for fungal growth and mycotoxin formation. Even though fungal populations developed to very high levels, phosphine treatment slowed the development of some fungi, particularly *A. parasiticus*. After 4 weeks exposure, the *A. parasiticus* population was more than 2 orders of magnitude greater in the grain held in air than in the phosphine treated grain. Aflatoxin levels were also lower in the phosphine treated samples, but the differences were small after 4 weeks exposure. However, in the experiment reported here, the inoculum level of *A. parasiticus* (10^5 cfu g^{-1}) was much higher than would be expected from natural contamination in the field situation. Leitao *et al* (1987) showed that phosphine at a concentration of 0.3 g m^{-3} reduced aflatoxin production by a factor of 10 to 100 in pure cultures of *A. flavus* and *A. parasiticus* grown in liquid medium.

Eurotium did not develop in the rice samples, and the inoculated species *E. chevalieri* was not detectable after 2 weeks in the air controls, and after 4 weeks in all samples. This was probably because the higher a_w conditions were so favourable for growth of *A. parasiticus* and *Penicillium* species, that the slower growing *Eurotium* species could not compete.

Penicillium species appeared to be particularly resistant to phosphine. In the uninoculated rice, they became the dominant mycoflora, reaching very high levels of up to 2.0×10^8. High populations of *Penicillium* also developed in the inoculated rice exposed to phosphine, indicating that *Penicillium* competed favourably with *A. parasiticus* under the conditions of the experiment. This is contrary to results reported by Bailly *et al* (1985; 1987), who found that of the fungi they tested in pure culture, *Penicillium* were the most sensitive. Some of the species present in the rice were potentially mycotoxigenic, and although the a_w was probably high enough for toxin elaboration, analyses for their toxins were not carried out. A similar development of *Penicillium* was noted in the previous experiments with wheat at 0.86 a_w, but not at 0.80 a_w, which is too low for growth of most *Penicillium* species in two weeks.

It would appear that, even under conditions which are extremely conducive to mould growth, phosphine can have some inhibitory effect on development of storage fungi and formation of mycotoxins. For moist grain, the levels of phosphine applied in these experiments is insufficient to effectively control fungal growth and mycotoxin production over an extended period. However, for shorter periods of a few days, phosphine treatment, perhaps at a higher concentration, may be useful in holding freshly harvested moist grain until drying capacity becomes available. Further work is needed using a graded series of phosphine concentrations at various time intervals to determine the optimum parameters for short-term storage of freshly harvested paddy.

Acknowledgements - The authors wish to thank Ms J.A. Gorman and Mr N.J. Charley for technical assistance, and Ms M. Ng for performing the aflatoxin assays.

REFERENCES

Bailly, J.R., Leitao, J. and Cabrol-Telle, A.-M. (1985) Effet antifongique du phosphure d'hydrogène sur diverses moisissures isolées de produits alimentaires. *Sci. Aliments* 5, Series V, 251-256.

Bailly, J.R., Leitao, J., Paillas, C. and de Saint Blanquet, G. (1987) Modification de la flora fongique de produits alimentaires par l'emploi d'un fumigant: le PH_3. Problèmes toxicologiques éventuels. *Sci. Aliments* 7, Series VIII, 323-328.

Bond, E. J. (1984) A manual of fumigation for insect control. FAO Plant Production and

Protection Paper No. 54. FAO, Rome. 432 pp.
Leitao, J., de Saint-Blanquet, G. and Bailly, J.R. (1987) Action of phosphine on production of aflatoxins by various *Aspergillus* strains isolated from foodstuffs. *Appl. environ. Microbiol.* **53**, 2383-2331.
Natarajan, T. and Bagyaraj, D.J. (1984) Fumigation effect on microflora and viability of blackgram and fieldbean seeds. *Pesticides* **18**, 40-42.
Pelhate, J. (1968) Inventaire de la mycoflore des blés de conservation. *Bull. trimest. Soc. mycol. Fr.* **84**, 147-143.
Pitt, J.I. and Hocking A.D. (1985) *Fungi and Food Spoilage.* Academic Press, Sydney.
Pons W.A. and Franz, A.O. (1978) High pressure liquid chromatographic determination of aflatoxins in peanut products. *J. Assoc. off. anal. Chem.* **61**, 793-800.
Raghunathan, A.N., Muthu, M. and Majumder, S.K. (1969) Control of internal fungi of sorghum by fumigation. *J. stored Prod. Res.* **5**, 389-392.
Sinha, R.N., Berck, D. and Wallace, H.A.H. (1967) Effect of phosphine on mites, insects and microorganisms. *J. econ. Entomol.* **60**, 125-132.

LES EFFETS DE LA PHOSPHINE SUR LE DEVELOPPEMENT DE LA MYCOFLORE DES STOCKS DE RIZ PADDY

A.D. HOCKING (1) et H.J. BANKS (2)

(1) CSIRO Division of Food Processing,
North Ryde, NSW, 2113, Australia
(2) CSIRO Division of Entomology,
Canberra, ACT, 2600, Australia

RESUME

On a ensemencé du riz paddy fraîchement récolté, ayant une activité de l'eau de 0,86 (équivalant à un degré d'humidité de 19,4 %) avec un mélange d' *Aspergillus parasiticus* et d'*Eurotium chevalieri*. Les échantillons et les témoins ont été exposés à 100 ppm de phosphine pendant 14 et 28 jours à 28° C. Les échantillons témoins ont été exposés à l'air dans les mêmes conditions. La flore mycélienne à été identifiée avant et après traitement, par la méthode d'ensemencement sur milieu de culture. La mycoflore s'est développée à la fois dans les échantillons ensemencés et dans ceux placés à l'air. La population de l'espèce mycotoxinogène *Aspergillus parasiticus* s'est nettement accrue dans les échantillons ensemencés. Le riz paddy humidifie, stocké avec de la phosphine, a vu décroître le développement d'une partie de sa mycoflore de stockage mais pas de l'ensemble. Le comptage de l'espèce *Eurotium* a donné un chiffre plus élevé, mais pas pour *A. parasiticus*. Le traitement à la phosphine a engendré une légère baisse de viabilité de la mycoflore qui n'a pu se développer à 0,68 a_w, ce qui indiquerait que la phosphine est plus efficace contre la mycoflore métaboliquement active et n'a que peu d'effet sur le mycélium et les conidies dormants. L'utilisation de la phosphine pour inhiber la croissance des moisissures et, par conséquent, le moisissement dû au chauffage et à la formation de mycotoxines, apparaît prometteuse en tant que méthode de préservation à court terme du riz non encore sec.