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USING CARBON DIOXIDE TO ENHANCE THE EFFICACY OF PHOSPHINE FUMIGATION

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ABSTRACT

These experiments were undertaken in a large warehouse of the Tongliang Grain Depot. Fumigations with 6.6%, 5.0% and 2.6% CO_2+PH_3 were carried out on susceptible and resistant strains of *Sitophilus oryzae* (resistance level to phosphine: x183), *Tribolium castaneum* (resistance level to phosphine: x8), *Rhyzopertha dominica* (resistance level to phosphine: x184) using the adult and immature stage insects . The results showed that insects of different stages (adults, eggs, larvae and pupae) in the storehouse and test insects (susceptible and resistant) placed in the grain beforehand were all killed; though different CT-products were required to obtain 100% mortality. After fumigation the PH_3 residues were very low. In the storehouse fumigated with 5% CO_2+PH_3, PH_3 residues were not detected, while in the storehouse fumigated without CO_2 they were 0.0005mg/Kg. For grain that was fumigated with 2.6%CO_2+PH_3 and 6.6% CO_2+PH_3 for over 21 days with AlP, using a surface sealing with PVC sheeting in the summer season, the PH_3 concentration could be maintained and available PH_3 concentration could be ensured. The PH_3 concentrations in the three test storehouses were maintained at approximately 100~150 ppm for 21 days after application.

INTRODUCTION

Phosphine has been widely used in about 80% of stored grain in China, to protect the grains from insect infestation. Although commercial fumigations have been generally successful, some resistant strains have been revealed in several major pest species and resistance levels are increasing. In our resistance survey in 2002, resistant strains were detected in 42% in all surveyed samples. The most resistant *S. oryzae*

strain had a resistance level to PH_3 of x305, and for *R. dominica* it was x315. Due to the combined advantages of low cost, ease of use and acceptance as a residue-free treatment, the fumigant phosphine will remain the central component for insect pest management.

The current study was undertaken to investigate the addition of 6.6%, 5% and 2.6%CO_2 into the stored grain during commercial scale fumigations of bulk paddy rice with PH_3. The PH_3 concentrations were determined and the response of the insects to control the resistant insects and mixed-age insects (eggs, larvae and pupae) was monitored by bio-assays. At the end of the fumigation the grain samples were tested for PH_3 residues.

An earlier study reported on the question as to whether or not grain insects, and especially the resistant strains of *R. dominica*, *S. oryzae* and *T. castaneum* were controlled by PH_3 In addition to resistance in the target pests it became apparent that several other factors in addition to resistance were contributing to control failures with phosphine. It is evident that there is a need to improve fumigation practice to enhance the efficacy of phosphine, and the development of management strategies to control *R. dominica, S. oryzae, T. castaneum.* This study was conducted to improve the fumigation method, and management to control resistant insects, and improve safety aspects.

MATERIALS AND METHODS

Warehouse: The experiment was carried out in three same-capacity government-owned large-scale horizontal storages of 1,458 m^3 capacity of grain in the Tongliang Grain Storage, Chongqing. These storages had a concrete floor (18×18×10m) with 10 windows and a door above the grain surface. The grain surfaces were enveloped with PVC membranes (fumigation sheets) and their gas tightness levels were determined as having a half life decay time of negative pressure from -250 Pa to -125 Pa at over 50 seconds.

Grain Each storage contained 1,458 m^3 paddy rice in bulk. This grain had been purchased from the local grain storage.

Tested insect strains*:* Bio-assays were carried out with susceptible and resistant insects cultured in the laboratory. Details of the insect strains used are as follows:

Susceptible insect strains used in the bio-assays were: *S. zeamais* (Chengdu), *T. castaneum* (Chengdu), *R. dominica* (Chengdu);

Resistant strains were *: S. oryzae* (resistance level to PH_3 x183), *T. castaneum* (resistance level to PH_3 x8), *R. dominica* (resistance level to PH_3 x184).

Cultures of 250 g wheat were prepared in the laboratory and after the introduction of 100 test insects they were incubated for 42 days to obtain mixed-age populations. Consequently, each sample of wheat taken from these cultures contained each of the life stages of the test insects. Samples containing 10 g taken from this wheat culture were placed in PE bottles, each containing mixed-age insects plus 20 adults, and inserted into the grain bulk. There were three susceptible-insect test samples and others for resistant-insect samples, which were placed in the paddy rice at different depths.

Other materials were: 56% Aluminum phosphine tablets, cylindered CO_2 gas, a phosphine generator, recirculation equipments and a Bedfont EC80 PH_3 monitor.

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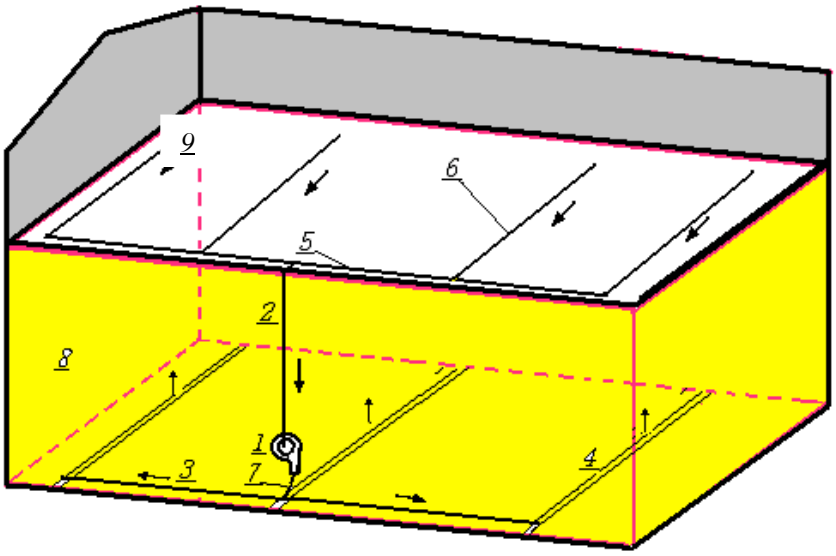

Figure 1: Recirculation-fumigation under the gas-tight sheeting

1. Recirculation fan 2. Main recirculation tube (duct) 3 Branch recirculation tube
4. Aeration holes 5. Main-tube under sheeting 6. Branch—tube under sheeting
7.Appliction-point of PH3 8. Grain 9. Membrane sealing surface of grain (fumigation sheeting

Recirculation tube set-up under the gas-tight membrane*:* A gas recirculation tube (diameter 110 mm) was pierced with holes, with the holes forming 13.4% of the surface area of the tube. This was connected to branch tubes at the floor level, and others positioned under the grain surface at a depth of 15 cm from the surface thus forming a circulation system as shown in Fig 1. . After this, the grain surface were leveled, and was then covered with the PVC sheeting and sealed

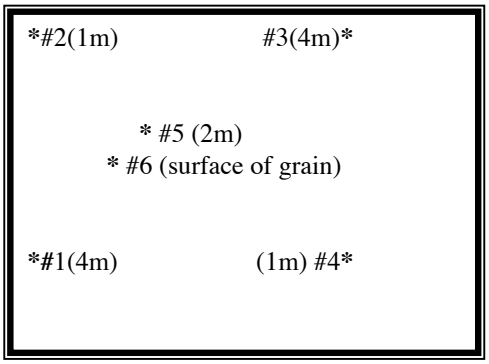

Figure 2. Position of test insects and gas sampling points in the grain

Positioning of insect cages in the paddy rice and setup of gas-sampling tubes*:* The depths of sampling tubes for monitoring the PH_3 concentrations and the depths of the insect cages were the same. The sampling equipment was inserted through the grain to different depths of 1, 2 and 4 m deep under the grain surface. There were 6 points for the bio-assay and gas sampling tubes for monitoring PH_3 concentration using a detector. These points are shown in figure 2.

Fumigation procedure: Before fumigation, air was sucked out of the grain-bulks until the negative pressure caused the PVC membrane to be drawn close to the grain surface. Then CO_2 was applied until the CO_2 concentrations (in the three test-storages) were measured at 2.6%, 5% and 6.6%, after which, introduction of CO_2 was stopped. The PH_3 generator was then used for fumigation of the paddy rice. A control storage unit was fumigated using the same dosage of $2g/m^3$ aluminium phosphide. The storage airspaces of the three test-storages were fumigated with aluminium phosphide tablets at $1.5g/m^3$. The tablets were placed in a basin containing a layer of wet sand about 2 cm high.

Ventilation with the recircualtaion fan: When the grain was fumigated, the fan was operated at same time and the air in the grain was recirculated, so that the PH_3 could reach the whole grain bulk.

Measurement of PH_3 concentration*:* The PH_3 concentrations were determined at 4, 8, and 12 h after the beginning the fumigation on the 1st day. After that, the PH_3 concentrations were monitored and recorded each day in the morning. with the EC-80 PH_3 monitor.

Insect mortality*:* After the storages had been fumigated for 21 days, the 6 groups of adult insects and mixed-age cultures were removed from the grain. The adults were separated and put into culture-bottles, each containing 10 g fresh food medium. These were cultured for 14 days, and then insect mortality was counted and recorded. The mortality rate was corrected using "Abbott"s formula. Fifteen g of fresh medium were added to the food media in the fumigated test bottles and these were held in a culture-room for 42 days. Then each bottle was checked, and the number of emerging adults was recorded (F1). This was done to ascertain whether fumigation had effectively controlled the eggs, larvae, and pupae.

Determination of PH_3 residues in the paddy rice: PH_3 residue analysis of the grain was conducted by a GC/FPD method, using a GC-14B gas chromatograph and flame photometric detector. Sub-samples (50 g) from each sampling point were transferred to a 500 mL flask, filled with 150 mL water, sealed with a stopper and injected with 5 mL HCL from an attaching syringes sampling adapter (stopper with silicone septum). The flasks were put in an ultrasonic wave-cleaner and shaken for 5 min, and then allowed to stand for 30 min. The headspace analysis was done using GLC.

RESULTS AND DISCUSSION

PH_3 concentration in warehouse

The averages of *PH_3* concentration of six points in tested warehouses are shown in table 1.

TABLE 1
The average of PH_3 concentration of six points in different tested warehouses

Days (d)	The average of PH_3 concentration in different tested warehouse (ppm)			
	2.6% CO_2	5%CO_2	6% CO_2	Without CO_2
1	489	556	570	386
2	412	542	564	355
3	383	500	549	327
4	379	466	483	277
5	349	423	471	262
6	348	361	383	232
7	346	347	371	206
8	324	324	368	200
9	297	304	383	182
10	277	243	302	164
11	271	226	262	153
12	262	214	247	146
13	205	197	238	126
14	156	173	210	113
15	/	166	/	107
16	/	159	/	100
17	/	135	/	84
18	/	126	/	83
19	/	117	/	82
20	/	104	/	75
21	100	101	146	69

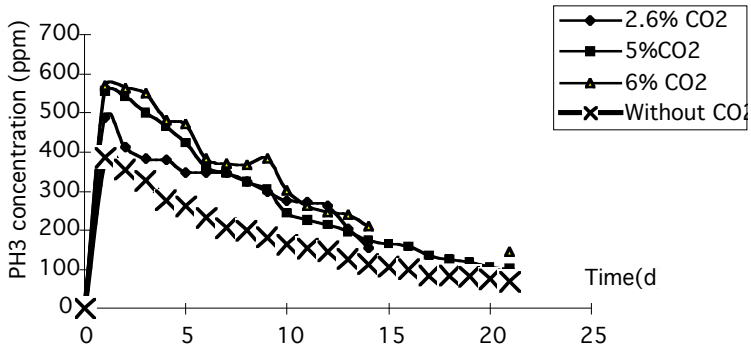

Figure 3. Phosphine concentrations (ppm) in the three trial storages and control storage during 21 days fumigation

From the table it can be seen that by fumigation with 2.6% CO_2, 5% CO_2 and 6.6% CO_2 and 2g/m^3 AlP under the sealed grain surface using PVC membrane in summer season, the PH3 concentration in the three tested stored-houses was maintained at approximately 100~150 ppm for 21 days. The rapid and uniform distribution of phosphine throughout the paddy rice was attributed to the re-circulation set-up.

The effectiveness of the fumigation

All 6 species of test insects exposed in the grain-bulks of the different stored-houses were killed by the treatments. However, resistant adult and immature stages of *S. oryzae*, *T. castaneum*, *R. dominica* required high CT-products and long exposures to obtain 100% kill.

The phosphine residues in the paddy rice

The phosphine residues in the paddy rice were determined at 14, 21 and 28 days after fumigation. The result are shown in table 2.

TABLE 2
The phosphine residues in the paddy rice fumigated with different CO_2 concentration

Fumigated time (d)	PH_3 residues (mg/kg)				
	2.6% CO_2+PH_3	5% CO_2+PH_3	6.6% CO_2+PH_3	Without CO_2	National Standard
14	0.0000	0.0000	0.0000	0.0004	<0.05
21	0.0015	0.0000	0.0002	0.0004	<0.05
28	0.0009	0.0000	0.0000	0.0005	<0.05

CONCLUSIONS

In three tested stored-houses, fumigation with 2.6% CO_2, 5% CO_2 and 6.6% CO_2 and 2g/m^3 AlP under the sealed grain surface using a PVC membrane cover in summer enabled PH_3 concentrations to be maintained at approximately 100~150 ppm for 21 days. The tested insects (susceptible and resistant strains) that were placed beforehand in the grain, were all killed, and the various stages (adults, eggs, larvae and pupae) were all killed, giving effective control. Phosphine fumigation with 2.6%CO_2, 5% CO_2 and 6.6% CO_2 may reduce the quantity of AlP required since the normal dosage for PH_3 fumigations is 3 g/m^3. After fumigation with 2.6%CO_2, 5% CO_2 and 6.6% CO_2 the PH_3 residues were reduced. However, in the three stored-houses storehouse fumigated without CO_2 the residue was 0.0005 mg/kg after 28

days. Consequently, in commercial scale fumigations, when CO_2 is added, the PH_3 concentration remains longer PH_3, and PH_3 residues in grain are less.

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