Effects of low oxygen phosphine fumigations on adult *Rhyzopertha dominica*

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Abstract

The effects of low oxygen atmospheres on the mortality of adult *Rhyzopertha dominica* in flow-through and single-dose phosphine fumigations are described. Flow-through fumigations were conducted using phosphine at 0.1 mg/L applied continuously to adult phosphine-susceptible and resistant *R. dominica* over a period of 30 hours in the presence of either 1, 2, 8 or 21% oxygen atmospheres. Fumigations were conducted using a phosphine concentration of either 0.003 mg/L or 0.007 mg/L to adult phosphine-susceptible *R. dominica* with 1, 8, 12, and 21 or 1, 6, 8, and 21% oxygen atmospheres for a period of 20 hours. The results of this study showed no synergistic effect of low oxygen on the toxicity of phosphine for either the flow-through or the single-dose fumigations.

Introduction

Fumigation with phosphine is an important method for controlling stored-product pests and in recent years there has been an interest in developing techniques which might lead to improved efficacy at lower dosages. Experiments (Bond 1989) have shown that modifications to the normal atmosphere can change the susceptibility of insects to the fumigant. In particular the effect of low oxygen on the efficacy of phosphine has been investigated by several researchers, though not all results have agreed. Studies by Liang Quan (1981, 1982) on the synergy of phosphine with oxygen and carbon dioxide against common stored-product beetles in the laboratory showed that a decrease in oxygen below 12% and/or an increase in carbon dioxide above 4% enhanced the effectiveness of phosphine. On the other hand, other researchers (Bond 1963; Bond et al. 1976; Price and Walter 1981; Kashi 1981a, b, 1982) have found that the lower the oxygen concentration during fumigation the lower the insect mortality. In fumigations containing no oxygen no deaths occurred even when the dosage was increased to more than a 1000 times that used in air. Post-fumigation exposure of phosphine-treated insects to either oxygen or nitrogen atmospheres showed that insects recovering in nitrogen had a higher survival rate than those recovering in air (Bond 1963).

Combined low oxygen and low phosphine atmospheres are currently being used in China. One of the techniques which uses this combination is called the 'double low' method of insect control (Tao and Wang 1993; Xu and Wang 1993). Freshly harvested grain is covered with PVC sheeting which is then hermetically sealed. The oxygen concentration in the grain store falls due to natural respiration. The grain is then fumigated with phosphine (0.05–0.5 g/m^3) from aluminium phosphide (AlP, 1–5 g/m^3). With use of the 'double low' fumigation method stored grain losses have been reduced to 0.05% as compared with 3.5% for conventional fumigations (Tao and Wang 1993). Techniques which have the potential to make phosphine more effective at lower application rates are of interest particularly with respect to cost, and environmental and safety considerations. For these reasons it was considered important to verify the role of low oxygen atmospheres in the use of phosphine. The work presented here reports on the effects of low oxygen on the mortality of adult *Rhyzopertha dominica* using phosphine flow-through and single-dose fumigations. The initial experiments were conducted using a flow-through fumigation method to ensure constant oxygen, carbon dioxide and phosphine levels. Subsequent experiments were carried out using conditions which duplicated as closely as possible those used by Liang Quan (1981).

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Materials and Methods

Flow-through fumigations

Three stains of *R. dominica*, differing in phosphine susceptibility were used for the flow-through fumigation trials [for culturing details see Winks 1975)]. The strains were cRD2, a phosphine-susceptible strain, cRD316, a mid-range susceptible strain and cRD 235p10, a selected phosphine-resistant strain. The adult *R. dominica* were subjected to a constant level of phosphine, 0.1 mg/L and oxygen levels of 1, 2, 8 and 21%. Insects were exposed to a particular phosphine/oxygen regime for periods between 15 minutes and 30 hours. The insects were then assessed for end-point mortality during a 28 day recovery period. The concentrations of oxygen and phosphine were maintained during the fumigation period using mass flow controllers. Before the addition of phosphine the gas mixture was conditioned to 60% relative humidity (r.h.) by passage through an appropriate aqueous glycerol solution. The fumigations were conducted at 25°C in modified glass desiccators (Fig. 1). During the fumigation the insects were placed inside mesh cages within the desiccators. For each fumigation two cages with 50 insects for each strain and exposure time were randomly assigned to the treatment and control desiccators. The concentrations of oxygen and phosphine were monitored throughout the fumigation period using gas chromatography (see Table 4). Two replicates were conducted at each oxygen concentration.

Following fumigation the insects were placed in recovery jars, containing whole wheat, at 25°C and 60% r.h. in air. Mortality was assessed after 24 hours recovery time, and then at 7, 14, 21, and 28 days. Probit analysis was used to assess the end-point mortality against time for each of the oxygen treatment results.

Single-dose fumigations

The strain of insect used in the single-dose fumigations was the phosphine-susceptible strain cRD2. The insects were exposed to a single dose concentration of 0.003 mg/L or 0.007 mg/L phosphine for 20 hours. During the fumigation the insects were placed in desiccators which were flushed with an

appropriate oxygen concentration to establish the required atmosphere. The desiccators were then sealed and dosed with phosphine to give an atmosphere with either 0.003 mg/L or 0.007 mg/L. The 0.003 mg/L fumigations were conducted at 1,8,12, and 21% oxygen and the 0.007 mg/L fumigations at 1, 6, 8, and 21% oxygen. The oxygen atmosphere was conditioned to 60% r.h. using glycerol solutions before entering the fumigation desiccators. The fumigations were conducted at 25°C. The conditions in to these experiments duplicated (as closely as possible) those used by Liang Quan (1981,1982, personal communication). The concentrations of the oxygen and phosphine within the single-dose fumigation were monitored at intervals throughout the 20-hour fumigation using gas chromatography (see Table 4). Insects were monitored until end-point mortality was reached. Data were assessed for significant differences between the treatments using Student's t test.

Results

The results for the probit analysis on the mortality of adult *R. dominica* to flow-through low oxygen/phosphine fumigations are tabulated in Table 1. Data are shown for the time to kill 50 and 99% of the insects (shown as lethal times, LT_{50}, LT_{99}). Student's t tests performed on the LT_{50}, LT_{99} and slopes of the probit lines for each insect strain indicate no significant difference between the toxicity of phosphine to insects exposed to low or normal atmospheric concentrations of oxygen.

The results for the single-dose phosphine fumigations conducted at 0.003 mg/L and 0.007 mg/L are given in Tables 2 and 3, respectively. Student's t tests showed that there were no significant differences between the mortalities in the low oxygen/phosphine treatment and those for insects exposed to normal atmospheric oxygen and phosphine. The results indicate that phosphine at 0.007 mg/L causes, on average, a 60% mortality in adult *R. dominica* but that this is due solely to phosphine rather than a synergistic effect of low oxygen on the toxicity of phosphine. The results in Table 2 indicate that phosphine at 0.003 mg/L does not cause mortality in adult *R. dominica* after a 20-hour exposure at any of the oxygen concentrations used.

Discussion

The relationship between oxygen and phosphine has been investigated by several workers (Bond 1963; Bond et al. 1976; Liang Quan 1981, 1982). In addition, many studies have been conducted on the action of oxygen on stored-product pests and other insects (e.g. Annis 1990; Bell 1984; Storey 1980). It was the results from these studies that prompted the present investigation. The study conducted by Liang Quan (1981) investigated the effect of low phosphine and low oxygen concentrations on several stored-grain pests. The results indicated an increase in phosphine toxicity when oxygen levels were held below 8%. The ability to extend the efficacy of phosphine would have obvious environmental, occupational health and safety and commercial benefits, but the present work has been unable to confirm a synergistic effect for low oxygen on the toxicity of phosphine against adult *R. dominica*.

Initially, fumigations were carried out using a continuous flow or flow-through process. The flow-through system has the advantage that gas concentrations and humidity can be held constant during the course of the fumigation. In contrast the single-dose procedure may incur problems associated with changes in experimental conditions due to insect respiration, changes in relative humidity and the decline in phosphine concentration during the fumigation. The results from the flow-through fumigation showed no synergistic effect of oxygen concentration on the efficacy of phosphine in any of the strains of *R. dominica* used. Subsequently, the work conducted by Liang Quan (1981, 1982) was replicated as closely as possible to test the increase in mortality in low oxygen atmospheres when using single dose fumigation (Liang Quan 1981). End-point mortalities assessed over 28 days showed no evidence of a synergistic effect for low oxygen atmospheres. Not all experimental details were duplicated exactly, and a compari-

Table 1. Probit analysis[a] of flow-through fumigation with 0.1 mg/L phosphine and oxygen treatments, 1, 2, 8 and 21%.

strain	% oxygen	slope	slope SE[b]	LT_{50}[c] (days)	LT_{99}[c] (days)
cRD2	1	1.46	0.38	1.06	2.66
cRD2	2	1.66	0.67	1.08	2.49
cRD2	8	6.62	3.87	0.33	0.68
cRD2	21	3.51	0.88	0.67	1.32
cRD316	1	0.91	0.18	2.30	4.85
cRD316	2	1.39	0.64	2.14	3.81
cRD316	8	1.51	0.52	2.09	3.63
cRD316	21	1.21	0.47	2.69	4.61
cRD235P10	1	1.29	0.33	3.64	5.43
cRD235P10	2	0.85	0.20	3.21	5.93
cRD235P10	8	0.91	0.09	3.27	5.83
cRD235P10	21	1.21	0.47	2.69	4.61

[a]Raw data corrected with Abbot's correction factor
[b]SE, standard error
[c]LT, lethal time

Table 2. End-point insect mortality (%) from single-dose fumigations with 0.003 mg/L phosphine and oxygen atmospheres of 1, 8, 12 and 21% for a 20-hour fumigation period.

Oxygen percent	without phosphine (n=6)		with phosphine (n=6)	
	mean mortality[a]	SD[b]	mean mortality[a]	SD[b]
1	2.06	0.66	0.23	0.46
8	3.09	1.90	0.65	0.71
12	2.07	0.51	0.49	0.68
21	1.88	0.77	0.46	0.74

[a] Abbot's corrected figures.
[b]SD, standard deviation

Table 3. End-point insect mortality (%) from single-dose fumigations with 0.007 mg/L phosphine and oxygen atmospheres of 1, 6, 8, and 21% for a 20-hour fumigation period.

Oxygen percent	without phosphine (n=6)		with phosphine (n=6)	
	mean mortality[b]	SD[a]	mean mortality[b]	SD[a]
1	2.81	0.90	46.9	9.75
6	1.89	0.69	51.7	9.61
8	3.66	0.96	52.3	2.48
21	3.11	1.36	47.1	7.61

[a]SD, standard deviation
[b] Abbot's corrected figures

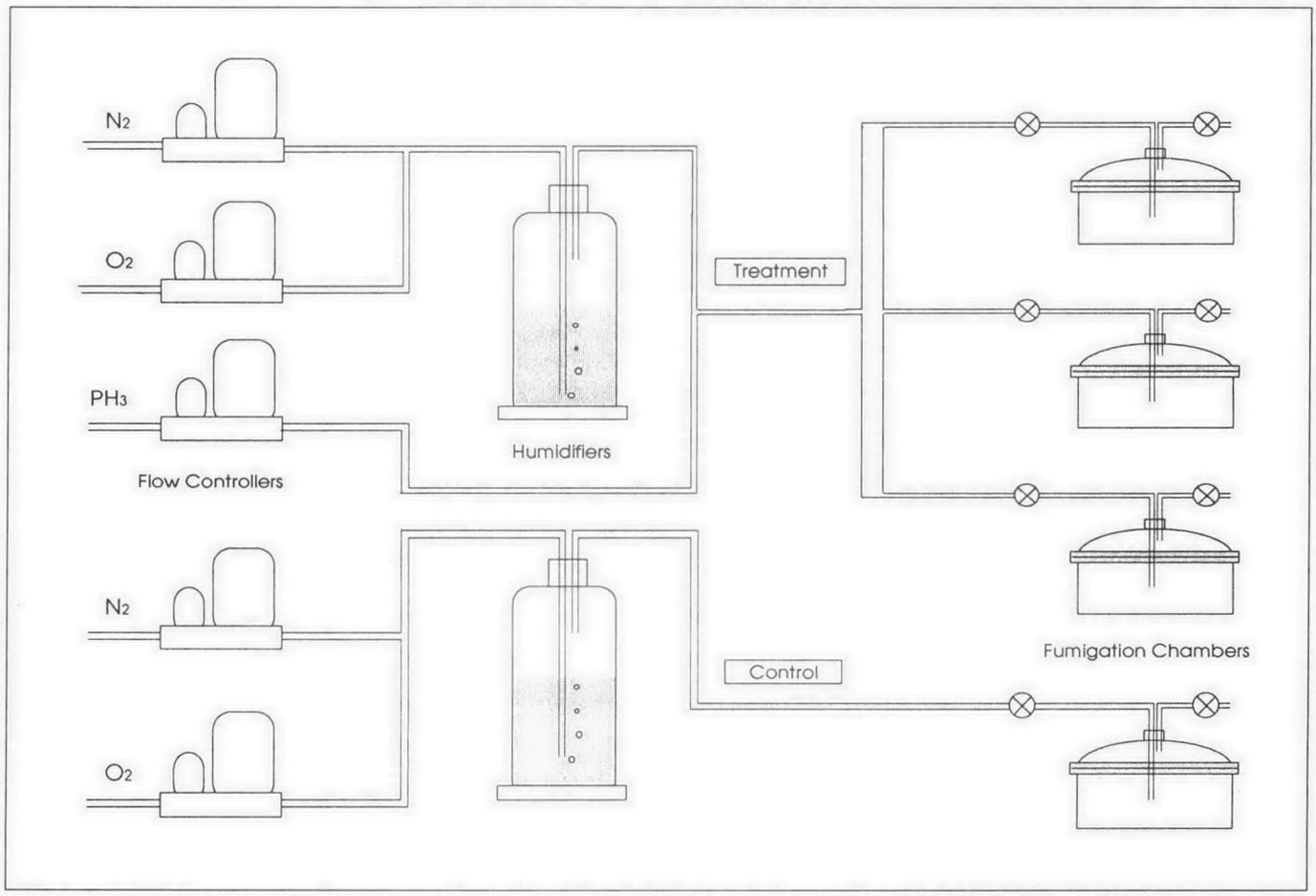

Fig. 1. Apparatus used to fumigate insects with phosphine in low oxygen atmospheres.

son of the work by Liang Quan (1981) and that conducted here (Table 4) shows some possible sources of experimental variation.

Although different results were found in the present study compared with that of Liang Quan the reasons have not been ascertained. Many environmental factors may influence the toxic action and effectiveness of fumigants. Among these, temperature, ambient pressure, humidity, and oxygen tension are the most important. Physiological factors such as digestion of food, age, sex, activity and excitability of the insects all effect the metabolism of the insect and thus susceptibility to a given fumigant (Bond and Monro 1967). Although the use of different strains could have a bearing on the observed response of the insect to the given fumigation conditions the present work was unable to demonstrate any differences between three insect strains with respect to the effects of low oxygen. On the other hand, differences in experimental procedure may offer a simpler explanation. In the study of Liang Quan (1981) a recovery period of 21 days was used, while in the experiments conducted here 28 days was used. In one experiment carried out during our investigation, the mortalities measured at 14 days indicated a synergistic effect for low oxygen, but this effect was no longer apparent when end-point mortalities were assessed. It is possible that different oxygen concentrations may affect the speed of action of phosphine without altering the end-point mortality. That is, insects in one oxygen concentration may die faster than those fumigated in a different oxygen atmosphere. Thus, mortalities assessed before the end-point may show an apparent synergistic effect for low oxygen on phosphine toxicity not observed when the final or end-point mortalities are assessed. Further work would be required to investigate this possibility.

The successful use of low oxygen and low phosphine atmospheres for insect control in China (the 'double low' fumigation technique) may arise from the use of gastight sealing practices to enclose grain maintaining low oxygen and phosphine over a long period, thereby extending the time the insects are exposed to phosphine. Moreover, the conditions produced using the 'double low' fumigation may involve not only the decrease in oxygen but also the increase in carbon dioxide levels. Carbon dioxide above 35% is toxic to insects and between 15 and 30% synergises the toxicity of phosphine (Y.L. Ren et al., these proceedings). Both low oxygen and high carbon dioxide concentrations have been used to control insect populations in stored grain. The results of Navarro et al. (1981) indicate that insects may seek to avoid low oxygen, high carbon dioxide atmospheres. Thus the protection of grain afforded by the 'double low' technique may be due to a combination of a number of these factors.

Conclusions

The single-dose fumigations conducted in the experiments reported here closely followed the work carried out by Liang Quan (1981). While experimental conditions such as temperature, humidity and basic equipment design followed those described by Liang Quan, the procedure varied with respect to fumigation technique, fumigation equipment, insect strain and post-exposure treatment of the test insects.

The results from both flow-through and single-dose phosphine fumigation trials showed no synergistic effect of low oxygen on phosphine toxicity against adult *R. dominica*.

Table 4. Experimental conditions compared with those used by Liang Quan (1981).

Experimental condition	Present work	Liang Quan
Insect species	*Rhyzopertha dominica*	*R. dominica*
Insect strain	cRD2	unknown, phosphine-susceptible
Insect age	adults- 4–8 weeks mixed	peak of emergence
Insect rearing technique	8 weeks at 60% r.h., 25°C	unknown
Insect recovery conditions	recovery bottles, 25°C, 60% r.h., food supplied (whole wheat)	rearing bottles, 28°C, 60–80% r.h
Criterion of insect death	only slight appendage motion	loss of normal climbing ability
Time to death period	28 days	21 days
Phosphine source	gas cylinder of known concentration	evolution from AlP using sulphuric acid with dilution
Phosphine application	gas-tight syringe	gas-tight syringe
Phosphine concentration	0.1 mg/L (flow-through); 0.003 mg/L (single dose); 0.007 mg/L (single dose)	0.007 mg/L
Fumigation equipment	mass flow controllers, sealed glass desiccators,	mass flow controllers, testing bottles
Fumigation conditions	flow through, single dose	single dose
Fumigation temperature	25–26°C	20–35°C
Fumigation relative humidity	60%	50–70%; 80–90%
Fumigation exposure time	30 hours (flow- through); 20 hours (single dose)	20 hours
Oxygen concentration check	Fisher GC with TCD and Porapak Q column	Orsat gas analyser
Phosphine concentration check	Tracor GC equipped with FPD and Porapak Q column	not stated
number of insects exposed	100/test	50/cage
oxygen range tested	1–21%	1–21%
Experimental result	no synergistic effect of low oxygen on phosphine at concentration tested	synergistic effect of oxygen below 8% on phosphine at 0.007 mg/L

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References

Annis, P.C. 1990. Requirements for fumigation and controlled atmospheres as options for pest and quality control in stored grain. In: Champ, B.R., Highley E. and Banks, H.J. ed., Fumigation and controlled atmosphere storage in grain: proceedings of an international conference, Singapore, 14–18 February 1989, ACIAR Proceedings, No. 25, 70–82.

Bell, C.H. 1984. Effects of oxygen on the toxicity of carbon dioxide to storage insects. In: Ripp, B.E., Banks H.J., Bond, E.J., Calverley, D. J., Jay, E.G. and Navarro, S., ed., Practical aspects of controlled atmosphere and fumigation in grain storages. Proceedings of an international symposium, Perth, Western Australia, 1983, 67–74.

Bond, E.J. 1963. The action of fumigants on insects — the effects of oxygen on the toxicity of fumigants to insects. Canadian Journal of Biochemical Physiology, 41,993–1114.

Bond, E.J. 1989. Current scope and usage of fumigation and controlled atmospheres for pest control in stored products. In: Champ, B.R., Highley E. and Banks, H.J. ed., Fumigation and controlled atmosphere storage in grain: proceedings of an international conference, Singapore, 14–18 February 1989. ACIAR Proceedings, No. 25, 20–29.

Bond, E.J. and Monro, H.A.U. 1967. The role of oxygen in the toxicity of fumigants to insects. Journal of Stored Products Research, 3, 295–310.

Bond, E.J., Monro, H.A.U. and Buckland, C.T. 1976. The influence of oxygen on the toxicity of fumigants to *Sitophilus granarius*. Journal of Stored Products Research, 3, 289–294.

Kashi, K.P. 1981a. Responses of five species of stored product insects to phosphine in oxygen deficient atmospheres. Pesticide Science, 12, 111–115.

Kashi, K.P. 1981b. Toxicity of phosphine to five species of stored product insects in atmospheres of air and nitrogen. Pesticide Science, 12, 116–122.

Kashi, K.P. 1982. Dose mortality responses of five species of stored products insects to phosphine. International Pest Control, 24 (2), 46–48.

Liang Quan. 1981. Controlled atmosphere and the control of stored grain insects. Grain Storage, 1, 20–27.

Liang Quan. 1982. Studies on the synergy of phosphine activity in control of stored grain insects by controlled atmospheres. Grain Storage, 1, 1–11.

Navarro, S., Amos, T.G. and Williams, P. 1981. The effect of oxygen and carbon dioxide gradients on the vertical dispersion of grain insects in wheat. Journal of Stored Products Research, 17, 101–107.

Price, N.R. and Walker, C.M. 1987. A comparison of some effects of phosphine, hydrogen cyanide and anoxia in the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Comparative Biochemistry and Physiology, 86C, 33–36.

Storey, C.L. 1980. Mortality of various stored product insects in low oxygen atmospheres produced by an exothermic inert atmosphere generator. Journal of Economic Entomology, 68, 316–318.

Tao, M-C. and Wang, Y-N. 1993. 'Double low': A synthesis of controlled atmosphere and fumigation techniques for stored grain conservation. In: Navarro, S. and Donahaye, E. ed. Proceedings of an International Conference on Controlled Atmosphere and Fumigation in Grain Storages, Winnipeg, Canada, 1992, 511–514.

Winks, R. G. 1975. Laboratory culturing of some storage pests. International training course on the Preservation of Stored Cereals. Australian Development Assistance Bureau 1, 206–219.

Xu, H. and Wang, N. 1993. Present and prospective state of the 'triple low' grain storage technique. In: Navarro, S. and Donahaye, E. ed., Proceedings of an International Conference on Controlled Atmosphere and Fumigation in Grain Storages, Winnipeg, Canada, 1992, 29–35.