Donahaye, E.J., Navarro, S. and Leesch J.G. [Eds.] (2001) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, Fresno, CA. 29 Oct. - 3 Nov. 2000, ***Executive Printing Services, Clovis, CA, U.S.A.*** *pp. 617-623*

THE TOXICITY OF PHOSPHINE IN COMBINATION WITH 5% AND 10% CARBON DIOXIDE AGAINST ALL STAGES OF A STRONGLY PHOSPHINE-RESISTANT STRAIN OF *RHYZOPERTHA DOMINICA* (COLEOPTERA: BOSTRYCHIDAE)

T.A LAMBKIN

Department of Primary Industries Queensland, Indooroopilly, Qld 4068, Australia
[e-mail: lambkit@dpi.qld.gov.au]

ABSTRACT

Carbon dioxide (5% and 10%) was trialed as a synergist for phosphine against all life stages of a highly phosphine-resistant strain of *Rhyzopertha dominica* and toxicity results were compared to the toxicity of phosphine alone. Results showed that the addition of 10% carbon dioxide to phosphine did not increase phosphine's toxicity, but adding 5% carbon dioxide decreased the toxicity of phosphine.

INTRODUCTION

Rapid and effective fumigation at import and export terminals is essential to facilitate the efficient movement of commodities. Delays can result in major logistic and economic costs. In the past, methyl bromide (MB) has been the fumigant of choice for this purpose. However, with the restricted use and planned phasing out of this material, industry is in need of a replacement fumigant for rapid fumigations. Currently however, there is no acceptable fumigant that can match the characteristics of MB, in particular its speed of action. Phosphine (PH_3) is the world's most popular fumigant in situations where rapid fumigations are not a priority. This material has many advantages: it is effective against most species on a very wide range of commodities, easy to use and apply, relatively inexpensive and most markets accept it as a residue free treatment.

Several authors have suggested that the exposure period required to control insect pests with PH_3 could be reduced by combining it with carbon dioxide (CO_2), (Athie *et al.,* 1998; Desmarchelier 1984; Kashi and Bond 1975; Liang Quan 1989; Mueller 1998; Rajendran 1989, and 1990; Rajendran and Muthu 1989; and Ren *et al.,* 1998). In general, these studies indicate that the toxicity of PH_3 can be increased by the addition of CO_2 at concentrations of 5% to 40%. These reports, however, provide only limited useful information because the investigations were restricted to very

short exposure periods, adult insects or PH_3 susceptible insects. In practice, fumigations need to be as short as possible but also effective against all life stages of resistant insects. A particular concern in Australia and many other regions is the occurrence of populations of *Rhyzopertha dominica* (F.) with strong resistance to PH_3 (Collins *et al.* in press; Daglish and Bengston, 1999). My aim, therefore, was to investigate the potential of CO_2 as a synergist for PH_3 against all life stages of a highly PH_3 resistant strain of *R. dominica*. In this paper, I present data on the potential of 5% and 10% CO_2 as a synergist for PH_3 at 1 mg/L.

MATERIALS AND METHODS

R. dominica is the most common and most quarantine significant stored grain pest in Australia, and for this reason, a strongly PH_3 resistant strain of this species was chosen for testing. This resistant population of *R. dominica* originated from southern Queensland (Collins 1998) and was used to set up mixed age cultures for testing as described by Winks and Hyne (1997). Because different life stages of most stored grain insect species have different tolerances to PH_3 (Barker 1969; Lindgren *et al.* 1958; Lindgren and Vincent 1966), the major advantage of testing mixed age cultures is that all life stages are exposed to the fumigant allowing a true population extinction to be determined.

The fumigation of these mixed age cultures was done in a specially constructed flow-through fumigation apparatus similar to the dosing apparatus described by Winks and Hyne (1997). This apparatus has stainless steel chambers that house the test cultures and allows a continuous flow of constant concentrations of PH_3, CO_2 and air through the chambers. Three gas mixtures were tested: 1 mg/L PH_3 in air, 1 mg/L PH_3 in air plus 5% CO_2 and 1 mg/L PH_3 in air plus 10% CO_2. Gas concentrations were controlled using separate mass flow controllers (Sierra®: Mass-Trak®). The advantages of using a flow-through system were that varying doses of CO_2 could be more easily set using gas flow controllers, and CO_2 emission from the test insects could not build up in the fumigation chambers. For these reasons this test method was chosen in preference to fumigating test insects in gas-tight desiccators.

Before the commencement of each test, Sierra® mass flow controllers were adjusted to deliver the required amounts of air, PH_3 and CO_2. This was done by bleeding the gas mix through the system without test insects, and then determining the concentration of the two gases from each chamber through two sampling ports. Mass flow controllers were then adjusted to deliver the desired gas concentrations. The gas concentrations were checked using a gas chromatograph fitted with a flame photometric detector for measuring PH_3 concentrations and a thermal conductivity detector for measuring CO_2 concentrations.

Cages containing mixed age cultures of *R. dominica* were then placed in the chambers and the fumigation started. Fumigation starting time was nominated as when gas taps were opened, allowing the gas mix to flow through the system. During the fumigation period concentrations of both gases were monitored twice on

the first day and then once on subsequent days. Untreated mixed age cultures (controls) were kept next to the flow-through system.

At the end of each fumigation period, cultures were removed from the fumigation chambers and adult insects were removed from the test cultures and from the controls to prevent any post-fumigation oviposition. The mixed age cultures were then incubated for seven weeks at 30°C and 55% r.h. This incubation time allowed complete development of any surviving insects and accounted for any slow development that may be induced by PH_3 exposure (Winks & Hyne 1997). All progeny from the mixed age cultures, including control progeny were counted.

Percent progeny survival for each gas mixture at each exposure time was calculated by comparing progeny numbers with control progeny at each exposure time. The data were subjected to probit analyses using Wadley's method (Finney 1971) to obtain estimates of LT_{50} and $LT_{99.9}$ values using an in-house computer program (Anon. 1993), these values being the time taken, in days, to control 50 and 99.9% of the population when exposed to each gas mixture.

RESULTS AND DISCUSSION

For all experiments, with or without CO_2, progeny survival was inversely proportional to exposure time (Table 1). A full explanation of this summary data is presented in the Appendix, Tables 3-5. At three days there was little difference in percent survival between PH_3 and PH_3 plus 10% CO_2 (4.8% and 2.6% respectively) (Table 1). In contrast, at the same exposure time PH_3 plus 5% CO_2 achieved only 79% suppression of progeny. Population extinction was achieved in five days with PH_3 alone but there was still a small proportion of insects surviving at five days in the PH_3 plus 5% CO_2 treatment. The LT_{50} and $LT_{99.9}$ values indicate that there was no significant difference (based on overlap of fiducial limits) in efficacy between PH_3 alone and PH_3 plus 10% CO_2 (Table 2). In contrast, the $LT_{99.9}$ value for PH_3 plus 5% CO_2 was significantly higher.

The addition of 10% CO_2 to PH_3 produced no synergistic effect in reducing the minimum exposure time for the control ($LT_{99.9}$) of cultures containing all stages of QRD 569. However, exposure to 5% CO_2 plus PH_3 reduced the efficacy of PH_3 by extending the exposure time required to control all life stages.

Synergism of PH_3 using CO_2 has been reported a number of times (Athie *et al.* 1998; El-Lakwah *et al.* 1989; Kashi and Bond 1975; Mueller 1998; Rajendran 1989 and 1990; Rajendran and Muthu 1989; Ren *et al.* 1998). However, these reports have little relevance to field applications as their tests were only for short exposure periods (24-72 h), usually on adults only of PH_3 susceptible strains. Because of this no real comparison can be made between their results and mine.

Some comparison of the present study can be made with the research done by Desmarchelier (1984). He tested various stages of several species, including *R. dominica* for seven day exposures. The addition of 25% CO_2 to PH_3 increased its toxicity against all stages of *R. dominica* except eggs. My results indicate that no synergism of PH_3 occurs with the addition of 10% CO_2 or less.

TABLE 1
Summary of percent survival of adult progeny from mixed age cultures of *Rhyzopertha dominica* (QRD 569) exposed (7 weeks prior) to constant concentrations of PH_3 (1 mg/L) with CO_2 (0, 5 and 10%) for various exposure times T (days). Replicate numbers (n) per exposure are in parenthesis

Treatment	Exposure time T (in days)							
	0	1.0	1.5	2.0	2.5	3.0	4.0	5.0
PH_3 1mg/L	100% (8)	100% (2)	72% (2)	52.6% (2)	-	4.8% (11)	0.13% (11)	0 (2)
PH_3 1mg/L CO_2 10%	100% (8)	100% (5)	61.1% (2)	40.3% (7)	23.2% (2)	2.6% (2)	-	-
PH_3 1mg/L CO_2 5%	100% (6)	-	-	34.6% (3)	-	21.5% (14)	5.7% (14)	0.4% (11)

TABLE 2
Results of statistical analyses of percent survival of adult progeny from mixed age cultures of *Rhyzopertha dominica* (QRD 569) when exposed for a range of exposure times (T) to PH_3 (1 mg/L) with CO_2 (0, 5 & 10%)

Treatment	LT_{50} (95% limits)	$LT_{99.9}$ (95% limits)	Slope
PH_3 1mg/L	1.98 (1.76-2.16)	4.40 (3.83-5.54)	8.93±1.23
PH_3 1 mg/L CO_2 5%	1.77 (1.32-2.11)	8.75 (6.69-14.29)	4.46±0.71
PH_3 1 mg/L CO_2 10%	1.8 (1.53-1.99)	5.14 (4.26-7.26)	6.79 (±1.07)

In contrast, bulk grain is regularly fumigated in China for long exposure periods with low PH_3 concentrations and using only 4% CO_2 as a synergist (Jin Zuxun 1998). The important feature of these fumigations is that during the exposure period the O_2 level is reduced to less than 12% (referred to as 'the double low technique'). An explanation of these contrasting results may be that synergism of PH_3 also may occur when O_2 levels are lowered in the presence of CO_2. The apparent synergism of PH_3 using 25% CO_2 (Desmarchelier 1984) may be in reality due to the combined effects of a lowered O_2 concentration in the test chambers in addition to a toxic effect of CO_2.

The toxic effect of PH_3 used in combination with CO_2 and varying O_2 levels is poorly understood. Further studies will include controlled laboratory trials of combinations of low concentrations of these three gases against mixed age insect cultures.

ACKNOWLEDGEMENTS

The author thanks Dr Patrick Collins for valuable guidance during the development of the project, and Megan C Cameron, Tina M Lambkin and Linda Bond for technical support during the conduction of this research. Grainco Australia Ltd provided financial support for purchases of the flow through systems, while Queensland Department of Primary Industries (Farming Systems Institute) provided financial and base facility support.

REFERENCES

Anon. (1993) Wadley Program Version 4.1. Queensland Department of Primary Industries manual, 29 June 1993. 9 pp.

Athie, I., Gomes, R.A.R., Bolonhezi, S., Valentini, S.R.T. and De Castro, M.F.P.M. (1998) Effects of carbon dioxide and phosphine mixtures on resistant populations of stored-grain insects. *J. stored Prod. Res.,* **1,** 27-32.

Barker, P.S. (1969) Susceptibility of eggs and young adults of *Cryptolestes ferrugineus* and *C. turcicus* to hydrogen phosphide. *J. Econ. Entomol.,* **62,** 363-365.

Collins, P.J. (1998) Resistance to grain protectants and fumigants in insect pests of stored products in Australia. In: *Stored Grain in Australia. Proceedings of the Australian Postharvest Technical Conference*, (Edited by Banks, H.J, Wright, E.J and Damcevski, K.A.), Canberra 26-29 May 1998, 55-57.

Collins, P.J., Daglish, G.J., Pavic, H., Lambkin, T.M., and Kopittke, R. (2001) Combating strong resistance to phosphine in Australia. In: *Proc. Australian Postharvest Technical Conf.,* 1-4 August 2000, Adelaide, Australia, 1-4 Aug. 2000. Stored Grain Research Laboratory, Canberra, (in press).

Daglish, G. J. and Bengston M. (1999) Phosphine resistance in Asia. In: *Stored Grain in Australia. Proceedings of the Australian Postharvest Technical Conference*, (Edited by Banks, H.J, Wright, E.J and Damcevski, K.A.), Canberra 26-29 May 1998, 58-60.

Desmarchelier, J.M. (1984) Effect of carbon dioxide on the efficacy of phosphine against different stored product insects. Mitteilungen aus der Biolodischen Bundesanstalt fur Land-und Forstwirtschaft, Berlin-Dahlam. 57 pp.

El-Lakwah, F.A., Wohlgemuth, R. and Khattab, M.M. (1989) Efficiency of phosphine with carbon dioxide against Khapra Beetle larvae *Trogoderma granarium* Everts. (Col., Dermestidae). *Anz. Schadlingskde., Pflanzenschutz, Umweltschutz,* **62,** 85-88.

Finney, D.J. (1971) Probit analysis, 3rd edition, Cambridge University Press, London.

Kashi, K.P. and Bond, E.J. (1975) The toxic action of phosphine: role of carbon dioxide on the toxicity of phosphine to *Sitophilus granarius* (L.) and *Tribolium confusum* DuVal. *J. stored Prod. Res.,* **11,** 9-15.

Liang Quan, (1989) The current status of fumigation and controlled atmosphere storage technologies in China. In: *Fumigation and Controlled Atmosphere Storage of Grain: Proc. Int. Conf.* (Edited by Champ, B.R., Highley, E., and Banks, H.J.) Singapore, 14-18 Feb. 1989 ACIAR Proceedings No. 25. 1989, 166-173.

Lindgren, D.L. and Vincent, L.E. (1966) Relative toxicity of hydrogen phosphide to various stored-product insects. *J. stored Prod. Res.,* **2,** 141-146.

Lindgren, D.L., Vincent, L.E. and Strong, R.G. (1958) Studies on hydrogen phosphide as a fumigant. *J. Econ. Entomol.,* **51,** 900-903.

Mueller, D.K. (1998) A new method of using low levels of phosphine in combination with heat and carbon dioxide. In: *Stored Grain in Australia. Proceedings of the Australian Postharvest Technical Conference*, (Edited by Banks, H.J, Wright, E.J and Damcevski, K.A.), Canberra 26-29 May 1998. 123-125.

Rajendran, S. (1989) The toxic action of phosphine, methyl bromide, methyl chloroform, and carbon dioxide, alone and as mixtures, against the pupae of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). In: *Fumigation and Controlled Atmosphere Storage of Grain: Proc. Int. Conf.* (Edited by Champ, B.R., Highley, E., and Banks, H.J.) Singapore, 14-18 Feb. 1989 ACIAR Proceedings No. 25. 1989, 273-274.

Rajendran, S. (1990) The toxicity of phosphine, methyl bromide, 1,1,1-trichloroethane and carbon dioxide alone and as mixtures to the pupae of red flour beetle, *Tribolium castaneum* Herbst. *Pesticide Science* **29,** 75-83.

Rajendran, S. and Muthu, M. (1989) The toxic action of phosphine in combination with some alkyl halide fumigants and carbon dioxide against the eggs of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). *J. stored Prod. Res.,* **25,** 225-230.

Ren, Y.L., O'Brien, I.G. and Whittle, C.P. (1998) Studies on the effect of carbon dioxide in insect treatment with phosphine. In: *Stored Grain in Australia. Proceedings of the Australian Postharvest Technical Conference*, (Edited by Banks, H.J, Wright, E.J and Damcevski, K.A.), Canberra 26-29 May 1998. 173-177.

Winks, R.G. and Hyne, E.A. (1997) The use of mixed-age cultures in the measurement of response to phosphine. In: *Proc. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products*, (Edited by Donahaye, E.J., Navarro, S. and Varnava, A.), 21-26 April 1996, Printco Ltd., Nicosia, Cyprus, 3-15.

APPENDIX

TABLE 3
Percent survival of QRD 569 progeny after exposure to 1 mg/L PH_3 for various exposure times (derived from comparing progeny numbers from treatments, with control progeny for each exposure time)

Exposure time T (days)	Control		PH_3		% survival
	Progeny	Mean progeny	Progeny	Mean progeny	
1.0	1187, 929	1058	1908, 824	1366	100
1.5	1187, 929	1058	845, 680	762	72.0
2.0	1187, 929, 454	756	491, 592, 231, 276	398	52.6
3.0	500, 454, 1187, 929, 506, 420, 417	615	23, 53, 13, 3, 62, 4, 36, 13, 24, 53, 68	29.5	4.8
4.0	500, 454, 506, 420, 417, 639	510	0, 0, 2, 2, 0, 0, 1, 0, 0, 1, 1	0.65	0.13
5.0	639, 500, 506, 420, 417	496	0, 0, 0, 0, 0, 0, 0, 0, 0	0	0

TABLE 4
Percent survival of QRD 569 progeny after exposure to 1 mg/L PH_3 plus 10% CO_2 for various exposure times (derived from comparing progeny numbers from treatments, with control progeny for each exposure time)

Exposure time T (days)	Control		PH_3		% survival
	Progeny	Mean progeny	Progeny	Mean progeny	
1.0	868, 620	744	1137, 1440, 1002, 1276, 1548	1302	100
1.5	846, 1019, 1120	995	469, 746	608	61.1
2.0	982, 868, 846, 1019, 1120	948	393, 443, 415, 322, 342, 323, 411	382	40.3
2.5	846, 1019, 1120	995	304, 157	231	23.2
3.0	982	982	30, 22	26	2.6

TABLE 5
Percent survival of QRD 569 progeny after exposure to 1 mg/L PH_3 plus 5% CO_2 for various exposure times (derived from comparing progeny numbers from treatments, with control progeny for each exposure time)

Exposure time T (days)	Control		PH_3 plus 5% CO_2		% survival
	Progeny	Mean progeny	Progeny	Mean progeny	
2.0	824, 949, 706	826	212, 330, 315	286	34.6
3.0	1050, 756, 698, 824, 949, 706	831	131, 120, 118, 97, 115, 185, 162, 136, 258, 212, 185, 321, 264, 197	179	21.5
4.0	1050, 756, 698, 824, 949, 706	831	20, 22, 36, 15, 14, 49, 135, 190, 37, 27, 31, 23, 25, 33	47	5.7
5.0	1050, 756, 698	835	5, 7, 4, 4, 1, 6, 0, 0, 4, 3, 0	3.1	0.4