*Donahaye, E.J., Navarro, S., Bell, C., Jayas, D., Noyes, R., Phillips, T.W. [Eds.] (2007) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, Gold-Coast Australia **8-13th August 2004**. FTIC Ltd. Publishing. Israel pp. 275-282.*

INVESTIGATION INTO CONTROL OF PHOSPHINE-RESISTANT RHYZOPERTHA DOMINICA IN SEALED FARM SILOS FUMIGATED WITH PHOSPHINE.

C.R. NEWMAN[1], G.J. DAGLISH[2], B. E. WALLBANK[3], P.R. BURRILL[4]

[1]*Department of Agriculture Western Australia, Bougainvillea Ave., Forrestfield, WA, 6058, Australia, cnewman@agric.wa.gov.au*
[2] *Queensland Department of Primary Industries and Fisheries, 80 Meiers Rd, Indooroopilly, Qld, 4068, Australia*
3 New South Wales Agriculture, Wagga Wagga Agricultural Institute, Private Bag, Wagga Wagga, NSW, 2650, Australia
[4] *Queensland Department of Primary Industries and Fisheries, Hermitage Research Station, Warwick, Qld, 4370, Australia*

ABSTRACT

Australian farmers commonly use phosphine to fumigate insects in grain stored in silos. The lesser grain borer, *Rhyzopertha dominica* is one of the most important pests of stored grain in Australia, and phosphine resistance is common in this species. Fumigation success against this strain depends on adequate phosphine levels being achieved within the grain mass for long enough to kill the target insects. Sealed grain silos are designed to ensure high standards of gas-tightness by incorporating rubber seals on hatches and other external openings. The adoption of this type of grain storage is becoming more common across Australia, but high levels of gas-tightness can only be achieved if the rubber seals are maintained in good condition. This paper reports on the results of farm fumigation trials undertaken in Queensland, New South Wales and Western Australia, investigating the adequacy of the recommendations for controlling phosphine resistant insects in sealed farm silos. The level of gas-tightness varied widely with pressure half-life ranging from zero to 600 seconds. Phosphine concentrations and commodity temperatures were compared with published and unpublished data on the response of resistant *R. dominica* strains, to determine factors that might limit the effectiveness of fumigations in farm silos.

INTRODUCTION

Phosphine fumigation is commonly used by Australian farmers to control insects in grain stored in silos. Amongst the most damaging pests of stored grain is the lesser grain borer, *Rhyzopertha dominica* F. and phosphine resistance is common in Australian populations of this species (Collins *et al*, 2003). Two genetically distinct types of resistance can be found in Australian *R. dominica*, namely a weak resistance and a strong resistance (Collins *et al.,* 2002). As phosphine toxicity against insects depends on both concentration and exposure period (e.g.

Bell, 1979; Daglish *et al.,* 2002; Ho and Winks, 1995; Winks, 1984), fumigation success will depend on adequate phosphine levels being achieved within the grain mass for long enough to kill the target insects. Considerable progress has been made towards understanding the effects of concentration and exposure time on phosphine efficacy against strong resistant *R. dominica* from Australia (Collins *et al.,* 2005; P.J. Collins, unpublished). Although these laboratory studies are valuable, there is a need to determine whether fumigations in farm silos will control such insects. Sealed silos are becoming more common on Australian farms, and are designed to ensure high standards of gas-tightness by incorporating rubber seals on hatches and other external openings. However, high levels of gas-tightness can only be achieved if the rubber seals are maintained in good condition. This paper reports the range of phosphine concentrations achieved in 44 fumigations in sealed farm silos of varying ages in Queensland, New South Wales and Western Australia, and estimates the likelihood of full control of strong resistant *R. dominica* in these silos.

MATERIALS AND METHODS

Fumigations were performed in silos designed for high gas retention. The shape and volumetric capacity of the silos varied, as did the commodity stored and the proportion of each silo occupied by the commodity. The design of silo sealing plates varies between manufacturers but all were resealed using EPDM (Ethylene Propylene Diene Mixture) rubber strips and fixed in place with weatherproof contact. Before grain was added to each silo, gas sampling tubes (nylon, 1/8" OD) were attached to a steel cable tensioned along the vertical axis of the silo, so that phosphine samples could be taken at a least five depths along the vertical central axis of the silo. Thermocouple cable (Type K) was attached to measure temperature at one or two levels. The fumigant monitoring lines and thermocouple cables were passed through a small sealable conduit near the peak of the roof and tethered to the side of the silo down to base level for readily accessible monitoring. The external cable and tube were encased in polyethylene water pipe to prevent weathering and bird damage. Concentrations were recorded using Canary™ 'Silo Chek' phosphine monitors (with some cross reference to Drager indicator tubes and a Drager PACIII monitor) and temperatures were recorded with hand-held digital thermometers.

Each silo was pressure tested before fumigation to obtain a pressure halving time (P_, seconds) using the oil-filled pressure relief valve of the silo as a manometer. Positive pressure of up to 250 Pa (i.e. 25 mm difference in oil levels) was first achieved in the silo using an air compressor or the aeration fan. The time taken for the pressure to decay to half after sealing off the pressure source was then measured.

Efficacy of fumigations was estimated from the known response to phosphine of mixed-age cultures of a strong resistant strain of *R. dominica* in the laboratory (P.J. Collins, unpublished data). At 25 or 30ºC this strain required nine days exposure to phosphine at 0.3 mg/L (215 ppm) for population extinction. Higher temperatures or higher concentrations reduced this time. At 20ºC the strain required 11 days at this concentration. The response at lower concentrations was complex. Fumigations were therefore considered successful if the concentration at all monitored points in a bulk above 25ºC exceeded 200 ppm for nine days, or

11 days for bulks cooler than 25ºC. This criterion is expected to be conservative because higher concentrations will generally shorten the necessary fumigation period (Daglish *et al.*, 2003).

RESULTS

Forty-three fumigation trials are summarised in Tables 1-3. Pressure-halving times are used in Australia to indicate the approximate gas-tightness of a silo (Andrews *et al.*, 1994), and ranged from zero to 600 s, indicating a wide range of gas-tightness from minimal to excellent. Peak gas concentrations in better sealed silos (P_ >30 s) usually exceeded 400 ppm at the lowest point for more than 10 days, and the concentration eventually became uniform at all points. Poorer-sealed silos (P_ <30 s) usually failed to achieve uniform concentrations at any time, and also usually failed to reach 400 ppm at the deepest level in the grain. Silos with longer halving times tended to hold higher concentrations for longer at all points, thereby achieving high concentration-time (Ct) products. Some apparently leaky silos achieved higher Ct products than expected.

TABLE 1.
Summary of trials of phosphine fumigation of wheat in sealed farm silos.

Trial	Silo volume (m^3)	Fill (%)	Grain moisture content (% wb)	Grain temp. (ºC)	Pressure halving time (s)	Days >200 ppm PH_3	Success[1]
1	58	99	10.2	<15	600	34	Yes
2	90	99	8.6	>30	360	31	Yes
3	58	99	7.0	>30	315	23	Yes
4	58	99	10.5	>30	300	28	Yes
5	90	99	10.0	>30	180	18	Yes
6	90	98	8.7	>30	165	10	Yes
7	90	99	8.7	>30	120	15	Yes
8	76	99	10.0	25-30	30	0	No
9	32	60	8.9	25-30	20	12	Yes
10	70	50	10.2	20-25	15	1	No
11	76	90	10.8	>30	5	3	No
12	76	90	10.8	>30	5	0	No
13	210	95	9.1	>30	0	0	No

[1]A fumigation was classified as successful if the phosphine concentration exceeded 200 ppm for at least 9 days at all monitoring points.

Based on the criterion of a concentration of 200 ppm being maintained for at least 9 days at all levels in the bulk, 68% of 41 silos tested with a grain temperature of at least 25ºC would have been successful in controlling the most resistant strains of *R. dominica* known to exist in Australia. A further 9% would also have achieved the criterion if the fumigation had been allowed to continue to 14 days. Those silos which failed the criterion, apart from those which were terminated early, had P_- times of 0-30 seconds, but even these silos often achieved the criterion for success in upper layers of the bulk. The grain nearest the base of the silo was where the fumigation was most likely to fail.

DISCUSSION

The fumigations were unexpectedly successful over a range of half pressure times from about 30 seconds upwards. Some practical factors favoured this result: most silos were more than three quarters full, thereby lessening headspace pumping effects, and most but not all grains were recently harvested and stored at temperatures similar to maximum ambient temperatures. Apart from routine replacement of seals before fumigating, the normal recommended procedures for farm use were followed. Thus about three quarters of these fumigations in small sealed silos of various ages and condition on-farm in three states over a two-year period achieved a concentration-time profile considered sufficient to control the strongest resistant strain of *R. dominica* currently known in Australia.

All fumigations that failed had half pressure times of 30 sec or less. While we do not advocate any reduction in the current recommended standard of 180 sec for a full silo, it is clear that good quality fumigations can be achieved in some cases for most of a silo bulk with lower half pressure times than previously considered necessary. The lowest monitoring point in most cases was within 0.5m of the bottom hatch of the silo, but not in a position to monitor immediately adjacent to a presumed leak source at the hatch. This part of the bulk may represent less than about 1% of the total but may be at higher risk of infestation from external sources or residual insects present near the leak. Even for this reason alone, it is desirable to retain a high level criterion for gas-tightness to minimise this risk as much as possible. The effects of other factors likely to adversely affect the fumigation, such as sorption of higher moisture grain, and increased headspace heating/cooling in partially filled silos have not yet been studied adequately because of the limitations of the season and farm requirements.

The gas-tightness of a sealed farm silo is difficult to measure accurately. The recommended pressure-halving technique is imprecise in practice because it is subject to fluctuations in environmental conditions such as temperature and wind. Tests conducted during the morning when temperatures were rising may overestimate the level of gas-tightness, because the pressure from heating of enclosed silo air may reach or exceed the 250 Pa test pressure. This may explain why some silos with high P_- times failed to achieve concentrations consistent with other silos of similar P_- times. In addition P_- times measure total leakiness irrespective of position. Leaks only at the bottom hatch would cause loss of gas from low levels in the bulk, whereas leaks at both top and bottom hatches could result in a flowthrough effect resulting in lower concentrations at several depths.

TABLE 2
Summary of trials of phosphine fumigation of barley in sealed farm silos.

Trial	Silo volume (m^3)	Fill (%)	Grain moisture content (% wb)	Grain temp. (ºC)	Pressure halving time (s)	Days >200 ppm PH_3	Success[1]
14	78	90	11.4	25 - 30	180	17	Yes
15	158	90	11.4	25 - 30	180	8	Likely
16	78	90	11.4	25 - 30	180	16	Yes
17	58	65	7.5	>30	135	7	Likely
18	58	95	8.6	>30	120	9	Yes
19	78	95	11.5	25 - 30	90	20	Yes
20	32	40	8.9	25 - 30	90	9	Yes
21	90	99	9.7	>30	75	10	Yes
22	78	99	11.1	25 - 30	60	17	Yes
23	78	99	11.1	>30	60	18	Yes
24	158	90	11.4	25 - 30	45	8	Likely
25	78	90	11.6	25 - 30	45	19	Yes
26	78	95	8.9	25 - 30	40	16	Yes
27	158	90	12.3	25 - 30	35	7	Likely
28	78	98	11.4	25 - 30	30	22	Yes
29	57	25	8.6	>30	15	11	Yes
30	76	95	8.5	>30	15	5	No
31	57	75	8.6	>30	8	8	No
32	158	90	12.3	25 - 30	6	0	No
33	81	90	10.8	25 - 30	5	0	No
34	81	90	9.0	25 - 30	1	6	No

[1]A fumigation was classified as successful if the phosphine concentration exceeded 200 ppm for at least 9 days at all monitoring points

Ideally the test is best done when conditions are constant, such as under full cloud cover and no wind, or if this is not possible it should at least be repeated after the maximum heating of the day has passed. Despite these difficulties the test remains the simplest feasible tool available to farmers for assessing gas-tightness. Pressure-halving times are recommended to exceed three minutes for full silos, and five minutes for empty or partially filled silos in Australia (Andrews *et al.* 1994), and failure to achieve this standard should be investigated.

TABLE 3

Summary of trials of phosphine fumigation of field peas and oats in sealed farm silos.

Trial	Silo volume (m^3)	Fill (%)	Grain moisture content (% wb)	Grain temp. (ºC)	Pressure halving time (s)	Days >200 ppm PH_3	Success[1]
Field peas							
35	90	75	5.2	>30	420	24	Yes
36	90	100	5.3	>30	420	15	Yes
37	90	59	9.0	25 - 30	180	25	Yes
38	90	30	10.0	25 - 30	180	18	Yes
39	90	59	9.0	>30	180	17	Yes
40	49	90	10.0	15 - 20	120	20	Yes
Oats							
41	53	99	8.3	>30	440	15	Yes
42	68	90	7.2	>30	90	27	Yes
43	75	70	11.0	>30	70	12	Yes

[1]A fumigation was classified as successful if the phosphine concentration exceeded 200 ppm for at least 9 days at all monitoring points.

Fumigation practice on Australian farms has been cited as a cause for concern for many years. Poor techniques are believed to be responsible for the escalating incidence of resistance to phosphine around the country. Common practices such as fumigating in unsealed structures, or repeated fumigations of the same infested bulk, have the potential to expose insects to sub lethal doses of phosphine. The increased selection pressure resulting from such practices are much less likely to be significant in well-maintained sealed silos. The absence to date of any identified strong resistant strain of *R. dominica* in Western Australia (Collins *et al.,* 2003) where unsealed silos have not been available for purchase for over 20 years, may be significant. In eastern states most farm silos are unsealed, and strong resistance has been found on farms since 1997 (Wallbank and Farrell, 2002; Collins *et al.*, 2003). Prevalent strains of *R. dominica* in Australia have weak resistance to phosphine, but still require six days exposure to 200 ppm phosphine for population extinction at 25ºC (P.J. Collins, pers comm.). The practice of shortening the exposure time because of marketing constraints is also likely to result in further selection of resistant strains. Immature stages are likely to survive despite adults being killed, giving only short-lived cosmetic control.

This trial has demonstrated that many silos present on Australian farms, which were installed as sealed units, have fallen into disrepair and are unable to retain gas to original specification. A high degree of confidence can be placed in fumigations conducted in a silo with pressure halving times above 180 seconds. It is of concern that many of the silos did not achieve this standard despite a basic resealing exercise. Attention to the resealing process is critical, and a more stringent maintenance check may be needed if the silo remained below specification despite re-sealing of hatch seals.

The farmer can ensure the stored grain value is maintained and with correct procedure in the application of phosphine, will ensure there is minimal selection for resistance. In most cases where strong resistance has developed, phosphine was not be able to be used effectively, and alternative protection technologies were needed. Treatments such as protectants, if available, may not be suitable for the intended market and may cause a loss of value as well as delay in delivery.

ACKNOWLEDGMENTS

This research was supported by the Grains Research and Development Corporation. We are grateful to the many farmer families who made available their grain and silos for our trials.

REFERENCES

Andrews, A.S, Annis, P.C., Newman, C.R., (1994) Sealed storage technology on Australian farms. In: Stored Product Protection, *Proceedings of 6th International Working Conference on Stored-product Protection*. Canberra, Australia, 17-23 April 1994, (Edited by Highley, E., Wright, E.J. Banks, H.J., and Champ B.R) pp. 27 - 36.

Bell, C.H., (1979) The efficiency of phosphine against diapausing larvae of *Ephestia elutella* (Lepidoptera) over a wide range of concentrations and exposure times. *Journal of Stored Products Research* **15,** 53-58.

Collins, P.J., Daglish, G.J., Bengston, M., Lambkin, T.M. and Pavic, H., (2002) Genetics of resistance to phosphine in *Rhyzopertha dominica* (Coleoptera: Bostrichidae). *Journal of Economic Entomology* **95,** 862-869.

Collins, P.J., Daglish, G.J., Pavic, H. and Kopittke, K.A., (2005) Response of mixed-age cultures of phosphine-resistant and susceptible strains of the lesser grain borer, *Rhyzopertha dominica*, to phosphine at a range of concentrations and exposure periods. *Journal of Stored Products Research* **41,** 373-385.

Collins, P.J., Emery, R.N. and Wallbank, B.E. (2003) Two decades of monitoring and managing phosphine resistance in Australia. In: *Proceedings of the Eighth International Working Conference on Stored-Product Protection*, (Edited by Credland, P.F., Armitage, D.M., Bell, C.H. Cogan, P.M. and Highley, E.) York, UK, 22-26 July 2002, pp. 570-575.

Daglish, G.J., Collins, P.J., Pavic, H. and Kopittke, K.A., (2002) Effects of time and concentration on mortality of phosphine-resistant *Sitophilus oryzae* (L.) fumigated with phosphine. *Pest Management Science* **58,** 1015-1021.

Daglish, G.J., Collins, P.J. and Pavic, H., (2003) Prospects for predicting insect mortality in relation to changing phosphine concentrations. In: *Proceedings of the Eighth International Working Conference on Stored-Product Protection, York, UK, 22-26 July 2002* (Edited by Credland, P.F., Armitage, D.M., Bell, C.H. Cogan, P.M. and Highley, E), pp. 668-670.

Ho, S.H. and Winks, R.G., (1995) The response of *Liposcelis bostrichophila* Badonnel and *L. entomophila* (Enderlein) (Psocoptera) to phosphine. *Journal of Stored Products Research* **31,** 191-197.

Wallbank, B.E. and Farrell, J.F. (2002) Development of phosphine resistance in *Rhyzopertha dominica* in New South Wales, and some strategies for containment. In: *Proceedings of the Australian Postharvest Technical Conference on Stored Grain in Australia Adelaide, 1-4 August 2000*. (Edited by Wright, E.J., Banks, H.J. and Highley, E.) 105-108.

Winks, R.G., (1984) The toxicity of phosphine to adults of *Tribolium castaneum* (Herbst): time as a dosage factor. *Journal of Stored Products Research* **20,** 45-56.