0601

Combining the Benefits of Cooling and Phosphine Fumigation to Meet the Biosecurity Challenge Posed by Grain Insects

G. J. Daglish[1,2], H. Pavic[1,2], P. R. Burrill[1,2], J. C. Holloway[2,3] and C. R. Newman[3,4]

Abstract: Two of the best options for Australian farmers to manage insects in stored grain are aeration cooling and phosphine (PH_3) fumigation. Farmers are being encouraged to use aeration to preserve grain quality and to slow insect population growth. However, insects are surprisingly difficult to kill with cold and the temperatures achieved using aeration in Australia are unlikely to result in adequate control. Therefore, the best strategy would be to combine aeration and fumigation to meet market demands for grain free of live insects. This paper summarises laboratory and field research addressing the question of whether Australian farmers can successfully fumigate cool grain. Published data show that there are minimal differences between the most PH_3 – resistant Australian strains of the rice weevil (*Sitophilus oryzae*) and lesser grain borer (*Rhyzopertha dominica*) at 25℃, but we found that resistant rice weevils were much harder to control in cool grain. Loss of gaseous PH_3 through sorption into the grain kernels can reduce the amount of PH_3 to which insects are exposed, particularly in a highly sorptive grain such as sorghum. We found that sorption was lower in cool sorghum grain resulting in higher average concentrations. Sorghum was less sorptive the longer it was stored before being fumigated for the first time, also resulting in higher average concentrations. These trends were observed to a lesser extent in wheat. These laboratory results suggest that farmers would achieve the best results by cooling the grain first and fumigating later. Field trials have been conducted in silos of up to 158 m^3 capacity in three states, indicating that fumigating cool grain is a useful option for farmers who have sealable silos.

Key words: Phosphine, fumigation, resistance, temperature, sorption, silos

Introduction

Stored grain insects represent a biosecurity threat in Australia, because a zero tolerance for live insects exists for export grain and this standard often applies to grain being sold within Australia. Two of the best options for Australian farmers to manage insects in stored grain are aeration cooling and phosphine (PH_3) fumigation. Farmers are being encouraged to use aeration to preserve grain quality during storage and to slow insect population growth. In addition, natural cooling may result when grain is stored into the cooler months. However, insects are surprisingly difficult to kill with cold[1], and the temperatures achieved by Australian farmers using aeration are unlikely to result in adequate insect control. Therefore, the best strategy would be a combination of aeration and fumigation to meet market demands for grain free of live insects. A significant effect of lowering temperature, however, is that PH_3 efficacy is lower at lower temperatures[2]. Sorption, however, is likely to be lower cooler grain resulting in higher average PH_3 concentrations[3]. A study was undertaken, therefore, addressing the question of whether Australian farmers can successfully fu migate cool grain. This paper summarises the key findings from laboratory and field research undertaken during this study.

Materials and Methods

Efficacy Experiments

Efficacy experiments were conducted based on published methods[4,5]. Essentially, wheat containing mixed-age cultures (i. e. eggs, larvae, pupae and adults) were exposed to constant PH_3 concentrations at 15℃. Samples were taken at intervals during each fumigation, any live adults were recorded, and if no live adults were found the samples were incubated at 25℃ for 10 wk to allow any surviving eggs, larvae or

1. *Department of Primary Industries and Fisheries*, 80 *Meiers Rd*, *Indooroopilly*, *Queensland*, 4068, *Australia*
2. *Cooperative Research Centre for National Plant Biosecurity*, *LPO Box* 5012, *Bruce*, 2617, *Australian Capital Territory*, *Australia*
3. *Department of Primary Industries*, *Pine Gully Road*, *Wagga Wagga*, 2650, *New South Wales*, *Australia*
4. *Department of Agriculture and Food*, 100 *Bougainvillea Avenue*, *Forrestfield*, 6058, *Western Australia*, *Australia*

pupae to complete development. The two strains chosen were a strong resistant strain of *Rhyzopertha dominica* (F.) and a weak resistant strain of *Sitophilus oryzae* (L.). The resistance factors based on adult mortality were about 600 times at 48 h for *R. dominica*)[6], and about 10 times at 24 h for *S. oryzae*[4].

Sorption Experiments

The general methods used in the sorption experiments were based on those of Daglish and Pavic[7]. Glass flasks(2 L capacity) were filled to 95% of volumetric capacity were injected with PH_3 at a dose of 1.5 mg/L based on the volume of the empty flasks. This dose equates to an application rate of 1.5 tablets of aluminium phosphide(AlP) per cubic metre of empty silo volume. Phosphine concentration was measured at intervals during storage beginning 2 h after injection and ending 11 days after injection. Grain was collected either at harvest or after a period of storage in farm silos, and generally stored frozen until shortly before the experiments began.

Farm Silo Trials

The approach used in the fumigation trials was based on that of Newman et al[8]. Essentially, silos containing grain were pressure-tested and then fumigated following the Australian label for AlP, i.e. 1.5 tablets(= 1.5 g PH_3) per cubic metre of silo capacity, and PH_3 concentrations were measured within the grain bulk during the fumigations. In all fumigations, concentrations were measured at approximately 3 – 5 points long the vertical axis of the silo, and in some cases concentrations were measured at other locations around silo. Concentrations were measured by attaching nylon sample tubing to Canary™ Silo Chek' monitors, and temperatures measured using a thermocouple or Tinytag™ data-logger. Although a total of 19 trials have been in three states (Queensland, New South Wales and Western Australia), only the results of one fumigation trial in New South Wales are reported in detail in this paper.

Two silos each of 58 m^3 capacity were used. One was 50% full with barley(11% mc) and served as the control silo, and the one that was fumigated was 40% full with wheat(11% mc) and the pressure halving time for this silo was 300 seconds. The grain in each silo had cooled naturally and had not been aerated. Mixed-age cultures (eggs, larvae, pupae and adults) of each insect species were placed into separate cages. A strong resistant strain of *R. dominica* and a susceptible strain of *S. oryzae* were used. Each cage was probed into grain in a sealed silo to a depth of 1 – 2 m. The wheat was then fumigated, by applying 100 tablets spread out on a tray in the headspace, and PH_3 concentrations and grain temperatures monitored daily from 15 – 27 August 2007. On completion of the fumigation, insect cages were removed and the grain within checked for live adults. All adults (live and dead) were removed and the remainder of the grain placed in jars, with some culture medium, were stored for 10 wk at 25℃, 65% rh. At this time the grain was sieved and checked for live adult insects to determine whether eggs, larvae or pupae survived the fumigation. To ensure that insects did not die from cold alone, cages of insects were placed into the control silo and treated exactly the same as the fumigated insects except that they were not fumigated.

Results and Discussion

Efficacy Experiments

We believe that the strong resistant *R. dominica* strain and the weak resistant *S. oryzae* strain used in this study reflect the strongest PH_3 resistances present in these species in Australia. Published data show that there are minimal differences between these two strains when they are fumigated at 25℃[4,5], but we found that the weak resistant strain of *S. oryzae* was much harder to control at 15℃. This shows that the relevant importance of resistant strains from different species depends on temperature.

Table 1. Results of phosphine fumigation of mixed-age populations of *Rhyzopertha*

Days elapsed	ive adults(Mean ± SD, n = 2) recovered from wheat after 8 wk incubation at 25℃.		
	R. dominica(Strong resistant)	*S. oryzae*(Weak resistant)	*C. ferrugineus*(Susceptible)
Concentration = 0.3 mg/L(210 ppm)			
0	783.0 ± 335.2a	4016.0 ± 340.8a	280.5 ± 87.0a
6	6.0 ± 1.4b	652.0 ± 5.7b	5.0 ± 0.0b
7	0.5 ± 0.7c	351.5 ± 2.1b	1.0 ± 1.4c
8	0.0 ± 0.0c	311.5 ± 94.0b	0.0 ± 0.0c

Days elapsed	ive adults (Mean ± SD, n = 2) recovered from wheat after 8 wk incubation at 25℃.		
	R. dominica (Strong resistant)	*S. oryzae* (Weak resistant)	*C. ferrugineus* (Susceptible)
9	0.0 ± 0.0c	96.0 ± 87.7c	0.0 ± 0.0c
10	0.0 ± 0.0c	97.5 ± 41.7c	0.0 ± 0.0c
11	0.0 ± 0.0c	7.5 ± 3.5d	0.0 ± 0.0c
Concentration = 1 mg/L (700 ppm)			
0	1048.0 ± 306.9a	4325.0 ± 312.5a	317.0 ± 142.8
8	82.0 ± 5.7b	618.0 ± 281.4a	0.0 ± 0.0
9	1.0 ± 1.4c	456.0 ± 176.8ab	0.0 ± 0.0
10	0.0 ± 0.0c	14.5 ± 2.1bc	0.0 ± 0.0
11	0.0 ± 0.0c	70.0 ± 97.6bc	0.0 ± 0.0
12	0.0 ± 0.0c	47.0 ± 63.6bc	0.0 ± 0.0
13	0.0 ± 0.0c	0.5 ± 0.7c	0.0 ± 0.0

Within each dose and species, means in columns followed by different letters are significantly different ($P < 0.05$) based on analysis of transformed data.

Sorption Experiments

Sorption by grain can reduce the amount of PH_3 to which insects are exposed. Table 2 shows the results of some of the sorption fumigations completed during the study. Percentage daily sorption was lower at 15℃ than at 25℃ in wheat and sorghum, meaning that cooler grain will have higher average concentrations than warmer grain, countering to some extent the problem of lower PH_3 efficacy at cooler temperature. Percentage daily sorption tended to decrease with age of grain meaning that delaying fumigation of grain may yield higher average concentrations. Table 2 shows that sorghum was much more sorptive than wheat, but the sorghum was also moister than wheat and rate of sorption is related to moisture content[3]. However, sorghum which had been stored for 3.5 months in a farm silo and was 12% mc was still more sorptive than wheat.

Table 2. Effect of storage at two temperatures on sorption in wheat and sorghum fumigated at 1.5 mg/L of flask volume. Mean PH_3 concentration after 2 h was 2.77 (SD = 0.05) mg/L for wheat and 2.49 (SD = 0.08) mg/L for sorghum.

Grain	Temperature (℃)	Approximate age of grain* (m)	Moisture content (%)	Daily sorption (%)
Wheat	15	0.5	12	4.2
		1	12	3.5
		2	12	3.9
		4	12	4.4
	25	0.5	12	9.4
		1	12	6.9
		2	12	6.4
		4	12	5.2
Sorghum	15	0.5	15	22.4
		1	15	15.6
		2	15	13.3
		4	14	11.9
	25	0.5	15	37.5
		1	15	27.9
		2	14	20.7
		4	14	16.1

* Ignoring time stored at −15℃

Farm Silo Trials

Nineteen trials were completed in sealable farm silos and the results cannot be given in detail here. As with earlier research on farm silos of this size (≤158 m^3 capacity)[7], we found that PH_3 concentrations tended to be lower deeper in the grain mass. In most cases we have assessed the fumigation success by comparing concentration x time profiles achieved in silos with the known responses of resistant strains of *R. dominica* and *S. oryzae* to PH_3 under laboratory conditions. However, information on efficacy against such strains is only available down to 15℃ and grain temperature in some of our trials was < 15℃. In the fumigation described in the Materials and Methods, cages of mixed − age cultures were actually inserted into the grain mass to confirm fumigation success in grain at < 15℃. Mean grain temperature measured at 2

m from the grain surface was very stable with 10.1℃ (SD = 0.3). The corresponding result for the control silo was 11.0℃ (SD = 0.1). Moisture content of the grain in both of these silos was 11.4%. Concentrations measured at three depths along the central axis are shown in Table 2. The lowest readings were measured at 5 m depth and these ranged from 35 to 888 ppm. There were no live adults of either the strong resistant strain of *R. dominica* or the susceptible strain of *S. oryzae* in the assessment made 10 wk after termination of the fumigation, even though there were on average 60.5 (SD = 17.7) and 38.5 (SD = 13.4) live adults were recovered from the corresponding controls.

Table 3. Phosphine concentrations (ppm) during fumigation of a 58 m^3 silo 40% filled with wheat (11.4% mc).

Days elapsed	Depth from top of silo (m)			
	5.0	3.0	1.0 (headspace)	Mean ± SD
1	35	125	128	96 ± 53
2	107	278	277	221 ± 98
3	208	413	408	343 ± 117
4	312	530	520	454 ± 123
5	436	669	658	588 ± 132
6	564	755	745	688 ± 108
7	634	803	796	744 ± 96
8	710	864	850	808 ± 85
9	789	918	900	869 ± 70
10	853	946	927	909 ± 49
11	836	943	926	902 ± 58
12	888	945	927	920 ± 29

Conclusion

We conclude that PH_3 fumigation of cool grain, i. e. grain has cooled naturally or has been cooled using aeration, is a useful option for Australian farmers who have sealable silos. Insect population growth will be zero or negligible in the cool grain, and fumigation can be used to control whatever insects are present at low densities.

References

[1] Fields, P. G. The control of stored-product insects and mites with extreme temperatures. Journal of Stored Products Research, 1992; 28, 89 – 118

[2] Hole, B., Bell C. H., Mills, K. A. and Goodship, G. The toxicity of phosphine to all developmental stages of thirteen species of stored product beetles. Journal of Stored Products Research, 1976, 12: 235 – 244

[3] Banks H. J. Uptake and release of fumigants by grain: sorption/desorption phenomena, in Proceedings of International Conference on Controlled Atmosphere and Fumigation in Grain Storages, Winnipeg, Canada, ed. by Navarro S and Donahaye E. Caspit Press Ltd, Jerusalem, 1993. 241 – 260 (1993)

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

[5] Collins, P. J., Daglish, G. J., Pavic, H. and Kopittke, K. A. 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, 2005, 41: 373 – 385

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

[7] Daglish, G. J. and Pavic, H. Effect of phosphine dose on sorption in wheat. Pest Management Science, 2008, 64: 513 – 518

[8] Newman, C. R., Daglish, G. J., Wallbank, B. E. and Burrill, P. R. Investigation into control of phosphine-resistant *Rhyzopertha dominica* in sealed farm silos fumigated with phosphine. Proceedings of an International Conference on Controlled Atmosphere and Fumigation in Stored Products, Broadbeach, Australia, 8 – 13 August 2004, 2007, pp 275 – 282